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Assessment of the Hypothalamic-Pituitary-Testicular Function in Male Patients with Wilson's Disease

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ABSTRACT: Sixteen male patients with Wilson's disease were studied to detect potential endocrine dysfunctions. There was no clinical evidence of feminization in any of the patients, and the patient group spanned most pubertal stages. Gonadotropin, testosterone, sex hormone binding globulin (SHBG), dehydroepiandrosterone sulphate, androstenedione, estradiol, prolactin, cortisol, thyrotropin, and free thyroxine levels were determined. Low or borderline luteinizing hormone (LH) levels were present in most of the patients. In six of the adult patients, a standard gonadotropin-releasing hormone (GnRH) test was performed. Five of the six patients had blunted LH and follicle-stimulating hormone (FSH) responses to GnRH. Increased androgen levels were found in eight of the patients. Sex hormone binding globulin was normal in eight of nine tested patients. Three single-dose human chorionic gonadotropin (hCG) stimulation tests of six adult patients showed normal responses. Three other patients who had elevated baseline levels responded with modest increases. Since liver disease is usually associated with decreased androgen levels, it is difficult to account for the elevated androgen levels. Both increased androgen levels and copper accumulation in the hypophysis could be responsible for the blunted GnRH response.

Key words: Liver disease, gonadotropins, gonadotropin-releasing hormone (GnRH) test, human chorionic gonadotropin (hCG) test, testosterone.

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The primary abnormality in Wilson's disease, an inherited disorder of copper metabolism, has not yet been identified, but the disease gene has been mapped to chromosome 13 (Frydman, 1990). The disease is characterized by a gross reduction in the incorporation of copper into ceruloplasmin, a substantial decrease in biliary excretion of copper, and accumulation of copper in the liver, brain, cornea, and other organs. The clinical presentation reflects tissue damage caused by copper deposition. Typically, it may include acute or chronic liver disease, an extrapyramidal neurologic syndrome, renal tubular disease, hemolytic anemia, osteoarthropathy, and other abnormalities (Danks, 1989). Until the introduction of chelating therapy, the disease was invariably fatal (Walshe, 1973).

Sporadic cases of endocrine disturbances in patients with Wilson's disease have been reported. These include diabetes mellitus (Sulochana and Viswanathan, 1982), hypoparathyroidism (Carpenter et al, 1983), and hypothalamic-pituitary lesions (Chihara and Fujita, 1984; Monfort et al, 1978). Possible interference in ovarian-follicular aromatase activity has been suggested in women with primary or secondary amenorrhea (Kauschansky et al, 1987). Other than a comment by Sherlock (1981) that feminization is present in men with Wilson's disease, the endocrine status of such patients has gone unmentioned. In this study, we assessed the hypothalamic-pituitary-testicular axis in patients with noncirrhotic Wilson's disease.

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Materials and Methods

Unprovoked endocrine studies were performed on 16 male patients with Wilson's disease who comprised most of the living men on our Wilson's disease registry. The patients ranged from 5 to 30 years of age. The age, clinical manifestations, and duration of penicillamine therapy are indicated in Table 1. Only patient 16 was married, and he had fathered two children. None of the patients had any symptoms or signs of endocrine dysfunction. Penile and testicular size and secondary sex characteristics were within the normal range, and none had gynecomastia. All the adult patients had a timely onset of puberty. The severity of the liver and neuropsychiatric manifestations was scored ad hoc. The scoring for liver disease was as follows: 0 = normal, 1 = hepatomegaly, 2 = enzymopathy, 3 = abnormal synthetic or excretory function, and 4 = cirrhosis. Neuropsychiatric manifestations were scored as follows: 0 = asymptomatic, 1 = mild (apparent only on thorough examination), 2 = moderate (insignificant interference with daily tasks), and 3 = severe (incapacitating rigidity or involuntary movements).

TABLE 1. *Clinical data*

Patient	Age* (years)	Tanner stage	Clinical manifestations†		Duration of treatment
			Liver	Neurologic	
1	5	1	1, 2	0	None
2	7	1	1, 2	0	None
3	10	1	1, 2	0	24 months
4	10	1	1, 2, 3	0	10 days
5	12	1	1, 2, 3	0	1 month
6	15	3	1, 2	0	24 months
7	13 (15)	3 (5)	1, 2	0	None‡
8	16	4	1, 2	2	2 months
9	16	5	1, 2, 3	0	12 months
10	17	4	0	0	10 years
11	20	5	0	2	1 month
12	15 (15)	5	1, 2, 3	0	None
13	16 (17)	5	1, 2	0	6 months
14	18 (20)	5	0	2	12 months
15	19 (19)	5	1, 2	0	None
16	27 (30)	5	0	1	30 months

* Age at time of baseline studies. Age at stimulation tests is listed in parentheses.
† See Materials and Methods for scoring definitions.
‡ Stimulation tests after 24 months of therapy.

Plasma samples were collected when the patients entered the registry and were stored at −20°C until use. Only patients undergoing stimulation tests had early morning cortisol determination.

Plasma luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were determined by a double-antibody radioimmunoassay (RIA) method according to Midgley (1966). For LH and FSH, LER-907 was used for iodination and the reference preparation was that of Laron et al (1975). Other hormone levels were determined by standard RIA commercial kits: prolactin (PRL) by Maia-Clone (Serono Diagnostics), thyrotropin (TSH) by Irma-count (DPC, Los Angeles, CA), androstenedione (Δ_4A) by Buhlmann Lab. Co. (Switzerland), total testosterone (T), dehydroepiandrosterone sulphate (DHEA-S), cortisol, free thyroxine (FT_4), and 17 β-estradiol (E_2) by Coat-a-Count (DPC). Sensitivity, coefficients of variation, and cross-reactivity of the various assays are presented in Table 2. Sex hormone binding globulin (SHBG) was determined by the method of Stumpf et al (1981).

Six of the patients (Table 1, patients 7 and 12 through 16), all at Tanner stage 5, consented to undergo further stimulation studies. Two of these patients had predominant neurologic symptoms and four had pathologic or biochemical evidence of noncirrhotic liver disease. Patients 12 and 15, who had active liver disease, were studied on enrollment and before the initiation of treatment. For the other four patients, penicillamine therapy was withheld for the test (Table 1). In these latter patients (7, 13, 14, and 16), stimulation tests were performed 1 to 3 years after the unprovoked studies reported in Table 3.

Stimulation tests were performed with gonadotropin-releasing hormone (GnRH; 50 μg/m^2; Serono) in one intravenous bolus and with a single intramuscular dose of human chorionic gonadotropin (hCG; 5,000 U/1.7m^2; Pregnyl, Organon). Plasma LH and FSH levels were measured before the test and every 15 minutes for 90 minutes after GnRH stimulation (Table 4). Plasma testosterone concentration was measured before and on day 5 after the hCG injection.

TABLE 2. *Manufacturer's specifications of sensitivity, coefficient of variation, and cross-reactivity of RIA kits*

	Sensitivity	Coefficient of variation (%)		Cross-reactivity (%)
		Intraassay	Interassay	
PRL	0.4 μg/L	1.4–5.4	5.5–8.6	0
T	0.13 nmol/L	5–10	9.2–12.9	0
DHEA-S	0.54 nmol/L	4.1–5.3	4.6–7.0	0.008–0.12
Δ_4A	0.1 nmol/L	2.0–3.1	1.8–6.2	0
E_2	2.9 pmol/L	4–7	4.2–8.1	1.1
Cortisol	5.5 nmol/L	3–8	4.5–6.3	0.001–3.1
FT_4	0.12 pmol/L	5	7–9	ND–0.03
TSH	0.03 μU/L	1–2.3	1.8–4.2	ND

ND = no data.

Results

Basal FT_4 ranged between 10 and 23 pmol/L (normal, 10 to 31 pmol/L), TSH between 0.7 and 5.7 mU/L (normal, <7 mU/L), PRL between 3 and 15.4 μg/L (normal, <20 μg/L), and cortisol between 140 and 550 nmol/L (8 AM normal, 138 to 635 nmol/L). Basal levels of other hormones are summarized in Table 3. Most of the patients, who were in all Tanner stages, had low or borderline LH levels, and patient 10 had low FSH levels as well. Patient 14 had elevated LH at age 18, but normal levels when restudied at age 20. Patient 4 (Tanner 1) had elevated FSH and T levels, possibly reflecting initiation of puberty.

Blunted LH and FSH responses were found in five of the six patients who underwent GnRH stimulation (Figs 1 and

TABLE 3. *Summary of unprovoked endocrine tests*

Patients	Test							
	LH (IU/L)	FSH (IU/L)	T (nmol/L)	DHEA-S (μmol/L)	Δ_4A (nmol/L)	E_2 (pmol/L)	SHBG (nmol/L)	E_2/T
Tanner 1								
Normal values *	0.54 ± 0.2*	0.6 ± 0.4†	0.6 ± 0.6‡	2.3 ± 1.4	1.6 ± 0.6	18 ± 11	68 ± 41	30§
1	0.3	1.0	0.6	0.4	0.3	22	—	36.6
2	0.3	0.9	0.6	0.4	0.5	22	—	36.6
3	0.4	0.4	0.9	—	—	36	83	40
4	0.3	1.3	2.3	2.3	2.1	43	42.5	18.7
5	0.2	0.7	1.0	1.5	2.2	—	63	—
Tanner 3								
Normal values	0.8 ± 0.34	1.9 ± 1.2	2.4 ± 1.2				68 ± 41	7.5§
6	0.4	1.2	5.3	—	—	44	35	8.3
7	1.0	1.6	4.0	4.3	1.6	25.6	106	6.4
Tanner 4								
Normal values	1.34 ± 0.4	2.1 ± 1.6	10 ± 4.0					
8	0.4	0.8	11.3	3.5	3.7	70	—	6.2
9	0.6	1.3	35	4.35	3.5	125	46	3.6
Tanner 5								
Normal values	1.9 ± 0.8	2.6 ± 2.0	16.3 ± 5.2	6.2 ± 3.2	3.3 ± 1.6	80 ± 52	30 ± 16	4.9§
10	0.6	0.4	23.4	—	—	—	14	
11	1.3	1.3	20	13.3	7.0	168	70	8.4
12	1.5	1.7	20.3	6.5	11.4	55	—	2.7
13	0.9	2.2	16.5	—	—	66	—	4.0
14	4.3	1.7	18.3	3.4	3.0	92	39	5.0
15	1.1	1.4	33	4.0	7.6	66	—	2.0
16	0.8	1.2	16.5	—	—	80	—	4.8

* Mean ± 2 SD.
† Normal values for LH and FSH are from Dickerman et al, 1976.
‡ Normal values for T are from Topper et al, 1982.
§ Mean E_2/mean T.

2). Five midpubertal and adult patients (6, 7, 9, 10, and 15) had elevated baseline T levels, and two other adult patients (11 and 12) had elevated androgen levels (other than T). Thus, including the prepubertal patient, eight patients had elevated androgens and two of these (9 and 10) also had elevated E_2 levels.

Human chorionic gonadotropin stimulation was performed on six adult patients (Fig 3). Patients 13, 16, and 12 had baseline levels within 2 SD of the mean and normal responses to HCG (average increment, 15.6 nmol/L). The three other patients had basal T levels greater than 2 SD above the mean and responded to HCG with an average increment of only 3.5 nmol/L (Fig 3).

TABLE 4. *Baseline and peak LH and FSH levels of GnRH testing*

Patient	LH (IU/L)		FSH (IU/L)	
	Baseline	Peak	Baseline	Peak
7	0.5	2.4	1.4	2.2
12	1.5	10.2	2.8	4.9
13	0.9	3.5	2.2	2.8
14	1.5	5.8	1.3	2.6
15	1.1	4.5	1.4	2.1
16	1.1	4.2	1.1	1.2

As indicated above, four of the six patients who had stimulation tests had undergone unprovoked studies several years earlier. The results of these earlier studies are depicted in Table 3. At that time, two of those four patients (16 and 13) had low LH and only patient 7 had elevated T levels.

The level of SHBG was normal in eight of nine patients, including patients 4, 6, and 9 who had elevated T levels. Patient 11 had increased SHBG levels. His T level was at the upper normal range, and his DHEA-S and Δ_4A were also increased.

Discussion

The baseline studies and stimulation tests showed borderline to low basal LH levels in most of the patients, and blunted LH and FSH responses to GnRH stimulation. None of the patients had clinical evidence of hypogonadism, but overt hypogonadism apparently is rare in men with Wilson's disease. Elevated androgen levels were found in one half of the patients. Three patients who had elevated baseline T had only a modest response to hCG stimulation, while those with normal baseline levels had normal responses. The level of SHBG was normal in all but one patient tested, including the three patients with elevated T levels.

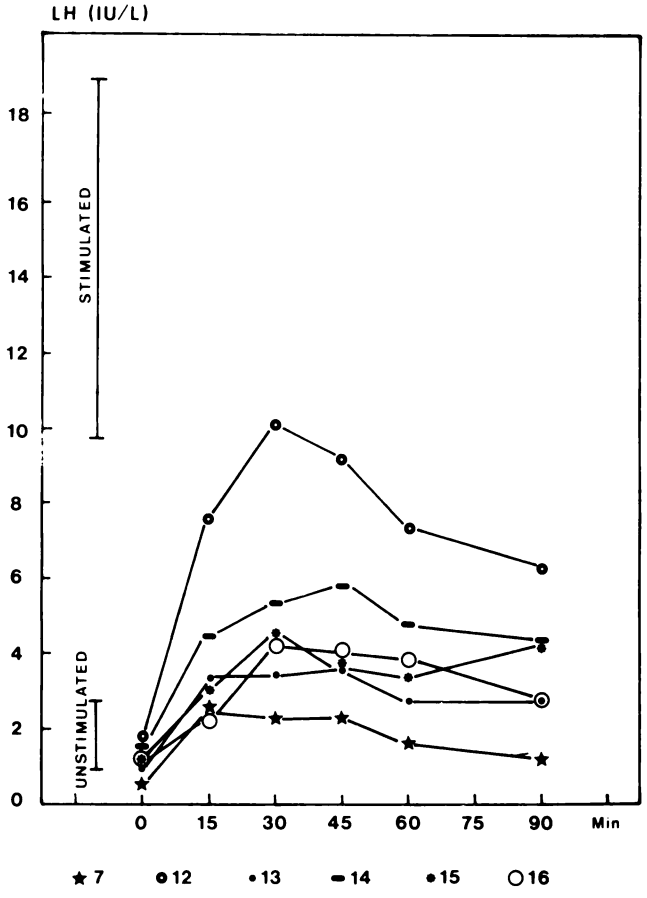

FIG. 1. GnRH stimulation test. The means ± 2 SD are indicated on the left. Bottom: baseline levels. Top: stimulated peak levels. Blunted LH response is evident in five patients.

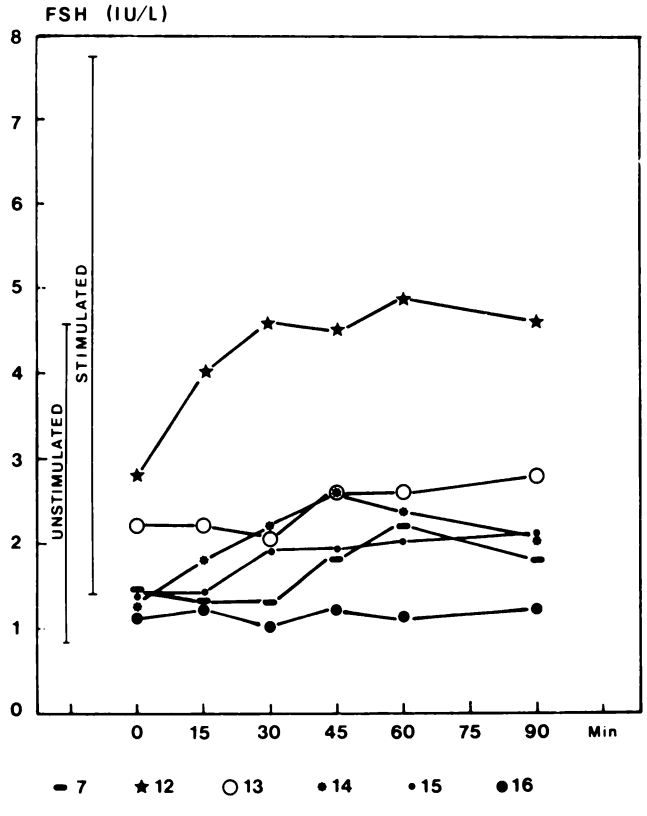

FIG. 2. GnRH stimulation test. The means ± 2 SD are indicated on the left. Bottom: baseline levels. Top: stimulated peak levels. Blunted FSH response is evident in five patients.

Eleven of our 16 patients had clinical or biochemical evidence of liver disease. Since patients with neurologic disease are considered survivors of the hepatic phase (Danks, 1989), a degree of liver damage may be expected in patients who present with neuropsychiatric manifestations. Chronic liver disease is usually associated with mildly elevated plasma estrogens, significant reduction in testosterone, and elevated SHBG (Mellinger, 1985). Hormonal imbalance (Mellinger, 1985) or a preferential estrogen bioavailability (Terasaki et al, 1988) may lead to feminization, which is well recognized in alcoholic cirrhosis but was also reported by Sherlock (1981) in men with Wilson's disease. The clinical findings and the endocrine studies of our patients fail to support Sherlock's note, but her observation was probably made in the presence of advanced liver disease in patients who, at that time, did not have the benefit of treatment. Indeed, several of our patients had somewhat elevated E_2 in addition to increased androgen levels. We assume that the elevated E_2 level in these patients resulted from hepatic damage and decreased estrogen degradation (Mellinger, 1985).

Rare cases of gynecomastia associated with D-penicillamine (D-PA) administration have been reported, but no endocrine dysfunction has been found (Reid et al, 1982). In our study, 11 patients received D-PA and five of the patients were never treated (including two in whom stimulation tests were performed). Similar results were obtained in both groups, suggesting that the gonadotropin and androgen anomalies are not D-PA associated.

Almost all women with untreated Wilson's disease have amenorrhea or oligomenorrhea resulting in impaired fertility. Recently, we reported low E_2, high T, and mildly elevated Δ_4A levels in affected women. It was suggested that copper interference with ovarian follicular aromatase activity (which converts T to E_2 and Δ_4A to estrone) may explain the ovulatory disturbances (Kauschansky et al, 1987). While such interference will severely affect a woman, the intratesticular conversion of T to E_2 in the man does not appear to be important to spermatogenesis or the regulation of feedback mechanisms. Thus, copper intoxication would affect the female reproductive system more severely and may explain the elevated androgen levels observed in our patients.

Most of our patients had low or borderline levels of LH. Dynamic GnRH testing performed on six of the patients

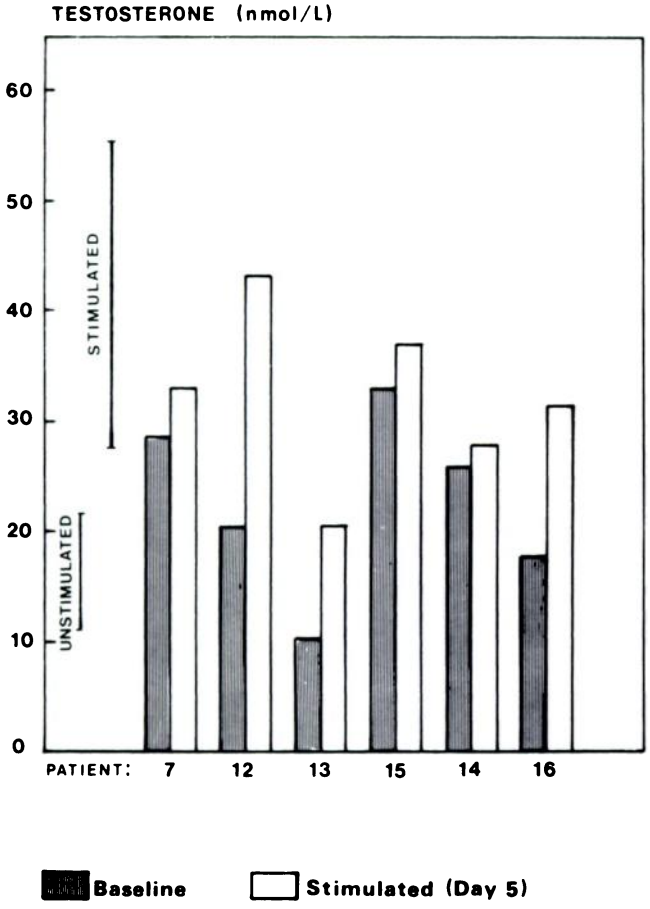

FIG. 3. HCG test. The means ± 2 SD are indicated on the left.

demonstrated blunted LH and FSH responses in all but one patient. This finding probably reflects a deficiency in the pituitary functional reserve of the gonadotroph. Hypothalamic-pituitary failure was previously considered by Monfort et al (1978) and by Chihara and Fujita (1984) to explain the findings in their patients. Copper accumulation in the hypophysis and, possibly, increased androgen levels could be responsible for the blunted GnRH response.

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