ONCOLOGY

Osteogenic Sarcoma and Androgens

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Endocrinologic examination of 320 male patients with primary osteogenic sarcoma reveals predominance of hyperandrogenemia characterized by decreased levels of sex hormone-binding globulin, elevated testosterone concentration, and increased free androgen index. The occurrence of androgen receptors was similar in the cytosolic fraction of osteogenic sarcoma (56%), benign tumors and tumor-like bone lesions (50%), while their content was higher in osteogenic sarcoma, implying an unfavorable prognosis of metastasizing.

Key Words: *osteogenic sarcoma; testosterone; androgen receptors; sex hormone-binding globulin*

Osteogenic sarcoma (OS) comprises 30-60% of primary malignant bone tumors, it is often developed at puberty, and is characterized by an extremely aggressive course, rapid hematogenic metastasizing, and high resistance to therapy [5,9]. Based on clinical experience and analysis of published data, we have defined two common characteristics of OS: it predominates in males and is often developed during the pubertal period [9,14]. The incidence of the disease is particularly high at puberty, when the secretion of gonadal hormones begins.

Our objective was to evaluate the androgenic status of patients with OS (total and free serum testosterone contents, albumin, and sex hormone-binding globulin concentrations) and to determine the sensitivity of primary tumors to androgens by the androgen receptor (AR) content.

MATERIALS AND METHODS

Three hundred and twenty patients with OS were enrolled in the study. Most of them (76%) were in their twenties. Generalized process (metastases in the lungs) was diagnosed in 7% of the patients.

Clinical, roentgenological, endocrinological, and morphological were employed to evaluate the status of the patients. Angiography, radioactive isotope scanning of the skeleton, liver, and kidneys, computer tomography, ultrasound examination, and biochemical evaluation of hepatic, renal, and pancreatic functions were performed.

Clinical and roentgenological diagnosis was confirmed by histological evidence. The following morphological variants of OS were identified according to classification [14]: osteoblastic (37%), anaplasia (18.6%), telangiectatic (14.8%), periosteal (7.6%), fibrohistiocytic (10%), parosteal (3.1%), highly differentiated (1.3%), and chondroblastic (7.6%) [14]. In most patients (74%) the tumor was localized in the epimetaphysis of the bones forming the knee joint.

The study also included 22 patients with benign neoplasms and tumor-like bone lesions: chondroblastoma (5), enchondroma (1), osteoblastoma (2), osteoid-osteoma (1), osteochondral exostosis (10), benign fibrous histiocytoma (1), aneurysmal bone cyst (1), and ossifying hematoma (1).

Serum contents of total testosterone (Ts), sex hormone-binding globulin (SHBG), and albumin

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TABLE 1. Blood Endocrine Parameters of Male Adolescents with Primary OS

Parameter	Medium height		Tall		Retarded physical development	
	controls	patients	controls	patients	controls	patients
SHBG, nM	28.8±2.1	20.6±0.9	28.7±1.9	20.6±2.5	56.0±8.5	39.4±3.9
Total Ts, nM	14.4±3.8	15.7±3.4	18.0±3.7	14.1±2.5	11.8±4.0	8.4±2.2
Free Ts, %	2.64±0.07	3.09±0.05	2.54±0.13	3.07±0.11	1.76±0.09	2.17±0.14
SHBG-bound Ts, %	44.3±1.6	34.5±1.1	44.6±2.7	35.3±2.4	56.6±3.2	54.5±3.1
Albumin-bound Ts, %	55.9±1.5	62.4±1.1	52.8±2.7	61.5±2.3	43.2±5.8	43.3±2.8
Free androgen index	47.7±5.8	81.9±17.3	59.6±14.5	69.9±5.6	21.2±6.8	24.7±10.6

were determined in 125 patients that received no specific therapy and 125 healthy adolescents (control). The patients and healthy subjects were assigned into pairs matching in age, body weight and height, and secondary sexual characteristics. The data on endocrinological status were compared within a case—control epidemiological study. Physical development of the adolescents was evaluated as described [7]. The concentration of SHBG and total Ts was measured by the radioimmunoassay method using Farmos Diagnostica kits. The albumin concentration was determined in a Grainer automatic analyzer using bromcresol green. Free, albumin- and SHBG-bound Ts levels were calculated as described elsewhere [12, 18]. The index of free serum androgens was calculated by the method [13].

Endocrinological parameters were analyzed in three groups of patients. Group 1 consisted of 60 patients of medium height aging 16.2±0.2 years, height 171.2±0.8 cm, and body weight 61.2±1.7 kg. The degree of sexual maturity was estimated as 2nd-3rd in 12 of them, 3rd-4th in 23, and 4th-5th in 25. The control group included 60 healthy subjects of medium height, aging 15.7±0.2 years, height 170.6± 0.96 cm, and body weight 65.8±1.8 kg; 23 of them were of the 2nd-3rd degree of sexual maturity, 23 were of the 3rd-4th, and 14 were of the 4th-5th.

Group 2 included 55 tall patients aging 16.6±0.4 years, height 181.6±0.6 cm, and body weight 70.0± 3.1 kg; 10 patients were of the 3rd degree of sexual maturity, 23 patients were of the 3rd-4th, 22 patients were of the 4th-5th. The controls were 55 healthy subjects aging 16.5±0.4, years, height 182.7±0.4 cm, weight 68.5±2.6 kg; 10 of them were of the 2nd-3rd degree of sexual maturity, 23 were of the 3rd-4th, 22 were of the 4th-5th.

Group 3 consisted of 10 patients with delayed physical (predominantly sexual) development; age 16.7±0.6 years, height 164.5±1.9 cm, body weight 55.7±3.6 kg; 7 patients were of the 0-1st degree of sexual maturity and 3 patients were of the 1st-2nd degree. Clinical and endocrinological examination revealed constitutional-somatogenic delay of sexual development in 4 patients, microgenitalism in 2 patients, and the incorrect puberty syndrome in 4 patients. The control group consisted of 10 individuals with delayed sexual development aging 16.5± 0.4 years, height 160.4±3.0 cm, weight 54.0±5.2 kg. Six of them were of the 0-1st degree of sexual maturity and 4 were of the 1st-2nd.

The AR content was determined in the cytosolic fractions of 80 OS and 28 benign tumors and tumor-like osteogenic lesions as described elsewhere [17]. None of the patient received any therapy prior to the determination. 5-α-Dihydro[1,2,4,5,6,7-^{3}H]-Ts (Amersham) was used as a ligand. The ligand was at least 98% pure after purification by a previously described method [1]. Specific binding was determined at 10 nM radioligand (saturating concentration) and 100-fold excess of 5-α-dihydrotestosterone (Koch-Light Laboratories) to suppress nonspecific binding and expressed as fmol bound steroid/mg total protein in the sample. Binding with progesterone receptors was suppressed by a 500-fold excess of triamcinolone acetonide (Sigma).

The results were analyzed using software for statistical processing of medical data.

RESULTS

There were no statistically significant differences in the total serum Ts content between group 1 patients (adolescents of medium height) and controls. Compared with the control, in OS patients the free Ts content was higher: 3.09±0.05% *vs.* 2.64±0.7%, $p< 0.05$ (Table 1); the SHBG content was lower that in the controls: 20.6±0.9 *vs.* 28.8±2.1 nM ($p<0.05$), and the index of free androgen was higher: 81.9±17.3 *vs.* 47.7±5.8, respectively. Although blood albumin levels were similar in patients and controls, the content of albumin-bound Ts was higher in patients, while the content of SHBG-bound Ts was higher in the controls.

TABLE 2. Levels of Free and SHBG-Bound Testosterone (Ts) in Adolescents of Medium Height with OS at Different Periods of Puberty

Periods of puberty, years	Number of observations	SHBG, nM	Total Ts, nM	Free Ts, %	SHBG-bound Ts, %	Albumin-bound Ts, %
Control						
14	12	41.0±1.8	13.5±0.9	2.52±0.16	47.01±3.30	50.31±3.20
15	11	35.8±2.2	18.4±5.5	2.46±0.24	48.02±2.41	49.54±2.30
16	12	29.4±2.1	13.9±4.7	2.61±0.14	45.02±3.12	52.39±2.90
17	13	24.2±2.2	13.5±4.5	2.84±0.10	37.10±3.82	60.06±3.70
18	12	21.4±1.2	12.8±3.7	2.89±0.03	35.01±2.10	62.11±3.40
Patients with OS						
14	12	23.0±2.5	15.9±6.5	2.96±0.20	37.64±4.24	69.40±3.60
15	11	20.7±2.1	14.3±2.3	3.07±0.01	35.27±2.20	61.66±2.57
16	12	20.9±2.3	15.7±2.4	3.10±0.11	34.70±2.40	62.20±3.0
17	13	19.7±1.6	17.2±2.9	3.23±0.12	32.07±2.50	64.70±3.80
18	12	20.7±3.1	15.7±3.1	3.08±0.02	37.00±4.01	59.91±3.51

It was interesting to compare the SHBG, total Ts, free Ts, and albumin-bound Ts blood at different periods of puberty in group 1 patients and healthy subjects (Table 2). Both in patients and controls, the mean Ts concentration remained practically unchanged during 5 years of puberty. However, in healthy subjects the Ts content was higher at the early stage of puberty and varied in a wide range during other stages. The SHBG content in patients did not change during puberty, being lower than in the controls. A negative correlation between the mean SHBG content and age at different stages of puberty was established in the control group (r=-0.72, p<0.01). In controls, the free Ts content gradually increased, while in patients it remained high at all stages of puberty. In addition, SHBG-bound Ts level was higher in the control, while in patients the content of albumin-bound Ts was higher at all stages of puberty. The index of free androgens was always higher in patients than in controls (Table 2).

In group 2 (tall adolescents), the blood content of free Ts was significantly (p<0.05) higher in patients than in controls (3.07±0.11 *vs.* 2.54±0.13%), the SHBG content lower (20.6±2.5 *vs.* 28.7±1.9 nM), and the index of free androgens tended to increase (Table 1). The total Ts concentration was similar in patients and controls: 14.1±2.5 and 18.0± 3.7 nM, respectively. The albumin-bound Ts content was higher in patients, while the level of SHBG-bound Ts was higher in controls (Table 3). It should be noted that at all stages of puberty the content of SHBG-bound Ts was lower and the free Ts level was higher in patients than in controls (Table 3).

In group 3 patients (delayed sexual development), the free Ts content was significantly higher and the SHBG content was lower than in controls: 2.17±0.14% *vs.* 1.76±0.09% for Ts and 39.4±3.9 *vs.* 56.0±8.5 nM for SHBG-bound Ts. There were no significant differences between patients and controls in other parameters (Table 1).

Blood concentration of SHBG markedly decreased, while that of free Ts increased in patients with generalized OS (metastases in the lungs). These changes did not depend on the time when the metastases were detected: during or after adjuvant therapy.

Cytosolic AR were identified in 56% of tumors, their content varying in a wide range (10-198 fmol/mg protein) and their occurrence depending on the histological variant of OS. Almost 50% poorly differentiated OS (osteoblastic, anaplasia, and telangiectatic) contained these receptors. However, the content of AR did not depend on the degree of OS differentiation. In addition, both the AR occurrence and content were not associated with the size of the primary tumor focus and its length over the marrow cavity. The occurrence of AR in benign tumor and tumor-like osteogenic lesions was similar to that in OS (50%), the AR content, however, being significantly lower than in OS: 28.5±5.2 *vs.* 52.3±6.2 fmol/mg protein.

The presence of AR in a typical histological variant of OS is an unfavorable prognostic factor concerning metastasizing. Sixty-seven percent of tumors with an AR content of 10 fmol/mg protein and higher metastasized within one year from the start of therapy, while only 28% of tumors without these receptors produced metastases during the same observation period, i.e., the occurrence of metastasizing was 2-fold lower. It should be noted that the duration of the metastases-free period as a function of the presence of AR receptors was recorded in patients with typical OS (n=61) after surgical re-

TABLE 3. Blood Levels of Free and Bound Ts in Tall Adolescents with OS at Different Periods of Puberty

Periods of puberty, years	Number of observations	SHBG, nM	Total Ts, nM	Free Ts, %	SHBG-bound Ts, %	Albumin-bound Ts, %
Control						
14	10	44.0±0.4	13.2±1.3	2.06±0.12	56.60±2.71	41.34±2.56
15	12	33.3±0.4	15.9±5.9	2.47±0.13	47.91±2.76	49.62±2.63
16	11	25.6±3.8	23.4±4.5	2.38±0.16	42.12±2.75	55.50±2.62
17	10	20.6±4.5	21.9±6.4	2.82±0.13	40.57±3.50	56.61±3.72
18	12	20.3±0.7	15.6±0.8	2.97±0.12	36.04±2.10	60.99±2.0
Patients with OS						
14	10	22.0±3.1	11.4±2.0	2.90±0.12	38.90±2.45	58.19±2.58
15	12	22.9±3.3	11.9±1.0	2.93±0.16	38.24±3.44	58.82±2.37
16	11	22.9±2.3	13.7±2.4	2.95±0.11	37.95±2.25	59.09±2.25
17	10	16.4±2.0	15.2±3.4	3.34±0.07	29.70±1.54	66.95±1.47
18	12	19.0±2.1	18.3±3.1	3.23±0.08	32.00±2.42	64.74±2.30

moval of the primary tumor and two courses of adjuvant chemotherapy: adriamycin and CAP regimen [9].

It has been generally recognized that growth, development, and proliferation of human OS are hormone-dependent [6,15,16]. Sex steroids are an important factor strongly associated with the incidence of OS at puberty [2,3]. Previously, we showed that Ts but not estradiol-17β stimulate the growth of human OS cells inoculated into thymus-free male rats [4]. Proceeding from these observations, we decided to compare blood contents of Ts and SHBG in adolescents with OS and healthy subjects.

Our results indicate that hyperandrogenemia prevails among patients with OS (65%), which manifests itself as a decrease in serum concentration of SHBG at normal total Ts level and increased free Ts concentration and free androgen index compared with the control and does not depend on the degree of physical development.

In patients with generalized tumor, the content of biologically active free Ts markedly increased, while the SHBG concentration decreased. It was reported that serum SHBG concentration is inversely related to the free Ts content [19]. Consequently, blood concentration of SHBG determines the level of free (biologically active) Ts. Free sex steroids are capable of binding to the target tissues and induce cellular responses to the hormonal signal [11,19]. Previously, it was shown that the total serum Ts concentration is increased even in clinically manifested hyperandrogyny, although not in all cases [10].

This is confirmed by the observation that in patients with OS the concentration of free Ts is increased, while the total Ts content remains unchanged. Free steroid hormones play an important role in latent hyperandrogyny coinciding with idiopathic primary cutaneous virilism [9].

The etiology of hyperandrogenemia and its potential sources in individuals suffering from OS are unknown. However, previously we reported that there is no difference between basal levels of major cortical androgens (androstenedione and dehydroepiandrosterone sulfate) in patients with OS and healthy subjects. In addition, it remains unclear whether hyperandrogenemia precedes the development of OS or occurs concomitantly with it from the beginning.

We also showed specific binding of 5-α-dihydrotestosterone to specific proteins with protein receptors in the cytosolic fraction of OS. This suggests that the tumor is sensitive to androgens. Bearing in mind the relationship of hyperandrogenemia with the development of OS, generalization of the process, the presence of AR in primary tumor, and stimulation of cultured human OS cells by Ts, the feasibility of antiandrogene therapy is worth discussing.

On the basis of published evidence and our long-term experience in the investigation of hormone metabolism of patients with hormone-dependent neoplasms, we think it reasonable to apply antiandrogen therapy in males with OS in combination with drugs suppressing hormone binding to tumor cell receptors and Ts biosynthesis in the gonads. Presumably, at this stage of investigation these preparations should be used in conventional regimes of adjuvant therapy.

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