Correlation of sex steroids with IGF-1 and IGFBP-3 during different pubertal stages

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Insulin-like growth factor 1 (IGF-1) is the major factor that affects linear bone growth. Also, androgens and estrogens are necessary for increasing longitudinal bone growth during sexual maturation. The aim of this study was to investigate the relationships among IGF-1 axis and sex steroids during pubertal development in healthy adolescents. In this cross-sectional study, IGF-1, IGF binding protein-3 (IGFBP-3) and sex steroid levels (estradiol in girls, testosterone in boys) of 205 healthy adolescents (101 female, 104 male) aged 9-17 years were measured. All subjects were apparently healthy, with no growth retardation and with skeletal ages appropriate for chronological ages, and none were taking medications known to influence calcium homeostasis. Greulich and Pyle's Radiographic Atlas of Skeletal Development of the Hand and Wrist was used for determination of skeletal ages. Tanner's classification was used to determine the pubertal developmental stage. Fasting blood samples were obtained from subjects between 09:00-10:00 h. Serum IGF-1 and IGFBP-3 levels differed significantly between pubertal developmental stages. Serum IGF-1 levels and IGF-1/IGFBP-3 ratios increased with proceeding stages and maximum mean values were found at stages III-IV in girls and at stage IV in boys. Estradiol levels of girls and testosterone levels of boys differed significantly between stages, and in both sexes, serum IGF-1 levels and IGF-1/IGFBP-3 ratios were significantly correlated with sex steroid levels. Increase in growth hormone secretion increases IGF-1 levels. Furthermore, increasing sex steroids with pubertal development increase the IGF-1 levels and IGF-1/IGFBP-3 ratios that affect bone growth.

Key words: sex steroids, IGF-1, IGFBP-3, pubertal stages.

The early phase of the pubertal growth spurt is related, at least in part, to sex steroid induced augmentation of growth hormone (GH) secretion[1,2]. Also, it is well established that androgens and estrogens are crucial for increased longitudinal bone growth during sexual maturation. Both estrogen and testosterone increase GH secretion and some of the effects of sex steroids on longitudinal bone growth occur through regulation of GH secretion[2-5]. However, increased sex steroids along with the completion of sexual maturation cause closure of epiphyseal plaques and skeletal maturation, thus consolidation of bone and cessation of longitudinal growth occur[3,6].

Insulin-like growth factor-1 (IGF-1) in the circulation shows its mitogenic activities by mediating most of the physiological actions of GH and is the major effector of bone growth[7-11]. It is reported that IGF-1 rapidly activates bone turnover[12,13] and IGFBP-3, the major binding protein (BP) of IGF-1 that has a direct role in the endocrine regulation of bone metabolism[14]. In puberty, both total IGF-1 and IGFBP-3 serum levels increase[15]. Furthermore, the molar ratio between IGF-1 and IGFBP-3 increases in puberty, suggesting that free IGF-1 increases in puberty when growth velocity is high[16,17].

In the literature, there are many studies investigating serum IGF-1 levels in relation with age and sexual developmental stages, but there are few studies investigating both IGF-1 axis and sex steroids and interrelationships between these parameters during pubertal development. As the results of these few studies conflict, this is still a new area for investigation.

The aim of this study was to investigate the relationships between pubertal development, IGF-1 axis and sex steroids (estradiol in girls, testosterone in boys) in healthy adolescents.

Material and Methods

Two hundred and five healthy children and adolescents (104 male, 101 female) aged between 9-17 years who were admitted to the adolescent outpatient clinic were included in this study. They were choosen according to the following criteria:

- Absence of growth retardation (none of the subject's height was below the 10th percentile according to the standards of Turkish children and adolescents).
- Skeletal age appropriate for chronological age.
- Absence of systemic disease.
- Absence of any signs of acute infection or inflammation at the time of blood sampling.
- No medication.

Pubertal development was staged according to Tanner's classification[18]. Pubic hair stages in the males and the breast stages in the females were used. Greulich and Pyle's Radiographic Atlas of Skeletal Development of the Hand and Wrist was used for determination of skeletal ages[19]. The number of cases in each stage and chronologic-bone ages in relation to pubertal stages are seen in Table I and Table II. Information about the aim of the study was given to the adolescents and their parents and informed consent was taken from the parents. Blood samples were obtained from subjects after fasting, between 09:00 and 10:00 h. In the cases having menstruation, blood samples were taken in the follicular phase of the cycle.

Serum estradiol and testosterone levels were measured using immunoassay kits Immulite 2000-Estradiol DPC and Immulite 2000-Total Testosterone DPC, respectively. Estradiol levels were expressed as pg/ml (picogram/millilitre) and testosterone levels were expressed as ng/ml (nanogram/millilitre). The estradiol levels below 20 pg/ml and testosterone levels below 20 ng/ml could not be measured quantitatively. Serum IGF-1 and IGFBP-3 levels were measured using IRMA (immunoradiometric assay) kits DSL-5600 Active Insulin-Like Growth Factor-I Coated Tube IRMA and IGFBP-3 IRMA C.T. (BC 1014), respectively, and both results were expressed as ng/ml.

One-way analysis of variance followed by a scheffe test were used to test the significance of the difference between IGF-1, IGFBP-3 and IGF-1/IGFBP-3 results at various pubertal stages. Kruskall-Wallis test was used to test the significance of the difference between estradiol levels in girls and testosterone levels in boys at various pubertal stage . Pearson correlation coefficient was used in the analysis of intercorrelations between the parameters investigated, but since some estrogen and testosterone levels were below detection limits, the lowest level of the assay detection limits were applied to these data. Forward stepwise multiple linear regression was also done in order to show the correlations between the presented data.

Table I. Chronologic and Bone Ages (mean±standard deviation, range) in Relation to Pubertal Stage in Girls

	Stage I (n=15)	Stage II (n=25)	Stage III (n=17)	Stage IV (n=22)	Stage V (n=22)
Chronologic Age	9.78±0.46 (9.25-10.75)	10.68±1.22 (9.0-13.75)	12.16±1.11 (10.5-14.0)	13.97±1.22 (12.0-16.0)	15.13±0.88 (13.25-16.5)
Bone Age	9.80±0.41 (9.0-10.0)	10.84±0.94 (9.0-13.0)	12.24±1.19 (11.0-14.0)	14.20±1.20 (12.0-16.0)	15.40±0.73 (14.0-17.0)

Table II. Chronologic and Bone Ages (mean±standard deviation, range) in Relation to Pubertal Stage in Boys

	Stage I (n=21)	Stage II (n=20)	Stage III (n=23)	Stage IV (n=26)	Stage V (n=14)
Chronologic Age	10.09±0.85 (9.0-12.0)	11.53±1.23 (9.0-13.5)	12.91±0.99 (11.0-15.75)	14.32±1.23 (12.0-16.5)	15.07±1.21 (13.25-17.0)
Bone Age	10.07±0.88 (9.0-11.5)	11.52±1.38 (8.0-14.0)	12.86±0.80 (11.0-15.0)	14.02±3.03 (13.0-17.0)	15.39±1.04 (14.0-17.0)

Results

Levels of IGF-1 and IGFBP-3 of 101 female and 104 male cases of our study population in relation to Tanner stages are seen in Tables III and IV, respectively. Estradiol levels in girls and testosterone levels in boys in relation to Tanner stages are seen in Tables V and VI, respectively.

As all estradiol and testosterone levels could not be measured quantitatively, the significance of the differences between estradiol levels at various pubertal stages in girls and testosterone levels at various pubertal stages in boys were evaluated with Kruskal-Wallis test. Estradiol levels (H=37.39 P=0.000) and testosterone levels (H=57.54 P=0.000) differed significantly between pubertal stages and increased with proceeding stages.

Serum IGF-1 levels differed significantly between the pubertal developmental stages (in girls, F=26.562 P=0.000; in boys,

Table III. Levels of IGF-1 and IGFBP-3 in Relation to Pubertal Stage in Girls

	Stage	N	Mean±SE	Range
IGF-1 (ng/ml)	1	15	224.26[a]±62.40	106.00-304.00
	2	25	320.04[b]±62.80	179.00-410.00
	3	17	414.58[c]±60.98	245.00-495.00
	4	22	418.22[c]±75.48	240.00-520.00
	5	22	390.18[c]±64.89	263.00-519.00
IGFBP-3 (ng/ml)	1	15	2910.13[a]±438.82	2069.00-3532.00
	2	25	3237.68[ab]±393.01	2700.00-4096.00
	3	17	3789.29[b]±682.44	2638.00-5510.00
	4	22	3751.77[b]±680.54	2585.00-6076.00
	5	22	3560.50[b]±596.03	2633.00-4790.00

Statistical differences between the stages without a common letter are significant for each parameter.
IGF-I: insulin-like growth factor-I, BP-3: binding protein-3.

Table IV. Levels of IGF-1 and IGFBP-3 in Relation to Pubertal Stage in Boys

	Stage	N	Mean±SE	Range
IGF-1 (ng/ml)	1	21	181.37[a]±72.88	77.10-407.00
	2	20	259.52[b]±109.09	80.50-490.00
	3	23	363.08[c]±81.55	206.00-492.00
	4	26	429.65[c]±47.76	315.00-535.00
	5	14	416.78[c]±59.73	292.00-499.00
IGFBP-3 (ng/ml)	1	21	2785.00[a]±347.89	2177.00-3723.00
	2	20	3158.65[ab]±734.98	2124.00-5517.00
	3	23	3605.91[bc]±506.86	2564.00-4638.00
	4	26	3742.76[c]±374.57	3130.00-4457.00
	5	14	3813.35[c]±489.65	2972.00-4517.00

Statistical differences between the stages without a common letter are significant for each parameter.
IGF-I: insulin-like growth factor-I, BP-3: binding protein-3.

Table V. Estradiol Levels in Relation to Pubertal Stage in Girls

Stage	N	n (%)	Median	Minimum	Maxmimum
1	15	2 (13)	39.65	22.6	56.7
2	25	13 (52)	39.10	20.2	66.3
3	17	15 (88)	49.80	23.3	119.0
4	22	20 (90)	53.15	24.0	178.0
5	22	19 (86)	82.40	23.1	183.0

N= number of girls in that stage.
n= number of girls whose estradiol levels could be measured quantitatively.

Table VI. Testosterone Levels in Relation to Pubertal Stage in Boys

Stage	N	n (%)	Median	Minimum	Maxmimum
1	21	4 (19)	25.65	21.2	132.0
2	20	12 (60)	100.75	32.6	555.0
3	23	21 (91)	261.00	55.0	1268.0
4	26	26 (100)	290.50	48.5	743.0
5	14	14 (100)	372.50	218.0	532.0

N=number of boys in that stage.
n=number of boys whose testosterone levels could be measured quantitatively.

F=40.478 P=0.000). Serum IGF-1 levels increased with proceeding stages and maximum mean values were found at stages III-IV in girls and at stage IV in boys (Table III and VI).

Serum IGFBP-3 levels differed significantly between the pubertal developmental stages in girls, F=7.506 P=0.000; in boys, F=15.350 P=0.000). IGF-1/IGFBP-3 ratios differed significantly between the pubertal developmental stages (in girls, F=10.865 P=0.000; in boys, F=26.293 P=0.000). IGF-1/IGFBP-3 ratios increased with proceeding stages and maximum mean values were found at stages III-IV in girls and stage IV in boys (Fig. 1).

Both serum IGF-1 levels and IGF-1/IGFBP-3 ratios significantly correlated with sex steroid levels in both sexes (Table VII). Furthermore, in stepwise regression analysis the variances in IGF-1, IGFBP-3 and IGF-1/IGFBP-3 levels were best explained by the variances in pubertal stages, by 34, 13 and 21% in girls and by 56, 35 and 45% in boys, respectively (multiple R-square, p=0.000). Addition of estradiol and testosterone levels to the analysis improved the explanation rates of IGF-1/IGFBP-3 by 4 and 7% in girls and boys, respectively (p=0.000). Testosterone improved the explanation rates of IGF-1 by 5% in boys (p=0.000).

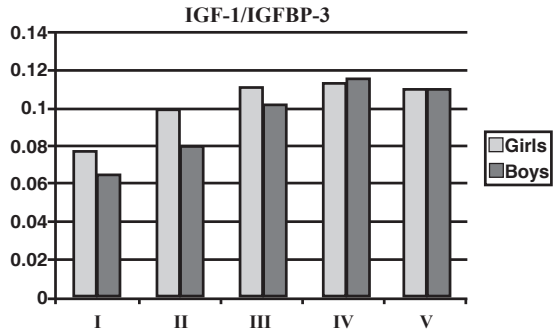

Fig. 1. IGF-1/IGFBP-3 ratios in relation to pubertal developmental stages.
IGF: insulin-like growth factor-1; BP-3: binding protein-3.

Table VII. Relationships of Estradiol in Girls and Testosterone in Boys with IGF-1, IGFBP-3 and IGF-1/IGFBP-3

		Estradiol	Testosterone
IGF-1	PC	.362**	.628**
	P	.000	.000
IGFBP-3	PC	.109	.428**
	P	.280	.000
IGF-1/IGFBP-3	PC	.401**	.606**
	P	.000	.000

PC: Pearson correlation coefficient.
** p<0.01.
IGF-1: insulin-like growth factor-1; BP-3 binding protein-3.

Discussion

In puberty, both total IGF-1 and IGFBP-3 levels increase. Furthermore, the molar ratio between IGF-1 and IGFBP-3 increases, suggesting that free IGF-1 increases in puberty when growth velocity is high. This is also shown by measuring serum free IGF-1 levels[10,16,17].

Juul et al.[15] reported that mean serum IGF-1 concentrations increased slowly in prepubertal children, with a further steep increase during puberty; after puberty a subsequent continuous fall in circulating IGF-1 levels was apparent throughout adulthood. The large variation in serum IGF-1 levels during puberty was diminished when data were separated according to sex and Tanner stage of puberty. They observed that serum IGF-1 levels increased with increasing Tanner stages of puberty, with maximal mean IGF-1 concentrations in Tanner stage IV for boys and in Tanner stages III-IV for girls. Luna et al.[20] also investigated serum IGF-1 levels in healthy adolescents, and reported that the rise in IGF-1 levels corresponds better with Tanner stage of the adolescents than with their chronological age. Like in the previous study, they observed the maximum IGF-1 levels in stage III-IV girls and in stage IV in boys.

In our study, serum IGF-1 levels also differed significantly between the pubertal developmental stages. The increase in serum IGF-1 levels from stage I to stage II and from stage II to stage III was found to be statistically significant in both sexes, but IGF-1 levels did not differ significantly between stages III-IV-V. Maximum mean IGF-1 levels were observed at breast stage III-IV in girls and at pubic hair stage IV in boys, as reported in the recent literature.

We also measured IGFBP-3 levels, the major binding protein of IGF-1 and found that serum IGFBP-3 levels differed significantly between the pubertal developmental stages. Maximum mean IGFBP-3 levels were found at stage III-IV in girls and at stage IV-V in boys. These findings support the study of Wilson et al.[21].

We also observed that IGF-1/IGFBP-3 ratios differed significantly between the pubertal developmental stages. IGF-1/IGFBP-3 ratios

increased with proceeding stages, and maximum mean values were found at stages III-IV in girls and at stage IV in boys. However, we could not find any other study in the literature investigating this ratio or serum free IGF-1 levels according to pubertal developmental stages.

As IGF-1 is the major factor that effects linear bone growth[7-11] and because both androgens and estrogens are necessary during sexual maturation for increasing longitudinal bone growth[2-5], we investigated the relationship between IGF-1 axis and sex steroids in puberty. We found that estradiol levels of girls and testosterone levels of boys differed significantly between pubertal stages and increased with proceeding stages. In both sexes, serum IGF-1 levels and IGF-1/IGFBP-3 ratios were significantly correlated with sex steroid levels. We thus conclude that increasing sex steroids with pubertal development in healthy adolescents increase IGF-1 levels. In the literature, there are different reports and conclusions about the effects of sex steroids on IGF-1 levels in puberty.

Garnier et al.[22] studied 78 prepubertal and 57 early pubertal patients referred for short stature and reported that in the early stages of puberty, testosterone in boys increased the serum levels of IGF-1 provided that GH secretion was normal. In girls, serum estradiol correlated negatively with the levels of IGF-1, so they concluded that this correlation needs to be confirmed in a larger series of female adolescents.

Rosenfield et al.[23] evaluated 20 male and 21 female healthy adolescent volunteers for at least two years and, in contrast to the previous study, reported that in girls, both estradiol and testosterone were found to correlate independently with IGF-1. However, in boys, neither estradiol nor testosterone was significantly correlated with IGF-1.

As was found in our study, Cook et al.[24] showed that there was significant correlation between sex steroids and IGF-1 levels in both sexes. Fifty-eight healthy adolescents (34 male, 24 female) were investigated, and estradiol levels in girls and testosterone levels in boys were found to be significantly correlated with IGF-1 levels.

There are some studies in the literature reporting that in adolescent girls, serum IGF-1 levels were correlated with estradiol levels, and that the administration of estradiol in physiologic doses that stimulate normal pubertal development increases the serum IGF-1 concentration gradually in hypogonadotropic females[2,23,25]. It is also reported that in hypogonadotropic males or in boys with constitutional delayed puberty, induction of puberty by administrating testosterone in physiologic doses increases serum IGF-1 levels[5,25,26]. The data obtained from these studies suggest that in healthy adolescents, physiologic increases in sex steroid levels increase serum IGF-1 levels. The significant correlation between sex steroids and IGF-1 levels in our study supports this hypothesis as well.

İnsulin-like growth factor-1 levels increase during puberty so that at mid-puberty the mean value is 2.5 to 3 times higher than adults values[7,8,15]. Although pubertal IGF-1 secretion is stimulated by sex steroids, IGF-1 levels decrease in adults, while estradiol and testosterone levels are still high, suggesting that the effect of sex steroids on IGF-1 secretion is biphasic. This is also confirmed by animal studies in recent years. Crawford and Hadelsman[27] conducted a longitudinal study of puberty in male hamadryas baboons over three years to examine the role of androgens in initiating the pubertal rise in circulating IGF-1 levels. Prepubertally castrated baboons had no significant rise in IGF-1 levels; however, administration of testosterone resulted in a close approximation of the normal pubertal rise in IGF-1. In another study, castration of sexually mature male baboons had no effect on serum IGF-1 levels. This indicates that androgens are the predominant determinant of circulating IGF-1 in the male baboon, and that this is an uniquely pubertal phenomenon.

Wilson[28] ovariectomized juvenile female rhesus monkeys and treated them with estradiol, which significantly increased serum IGF-1 and IGFBP-3 levels. In another study, he reported that estradiol replacement in ovariectomized monkeys significantly elevated serum concentrations of IGF-1 during early adolescence, but significantly decreased serum IGF-1 in young adult females. In contrast to the age-specific effect on IGF-1, estradiol replacement elevated serum IGFBP-3 at all ages. So this differential effect resulted in a decrease in the molar ratio of IGF-1 to IGFBP-3 from early adolescence through adulthood[29].

The effects of sex steroids on longitudinal bone growth and thus bone formation during puberty are biphasic. Low concentrations of sex steroids

in the early stages of puberty stimulate, and higher concentrations inhibit, bone formation[3,30]. The in vitro study of Bitbol and Garabedian[6] testing the hypothesis that bone sensitivity to estrogens differs with the pubertal status supports this conclusion. They cultured human osteoblasts from 14 girls (3-18 years) and examined the effects of repeated weekly doses of 17 β-estradiol (10 pM-10 Nm) on bone formation markers, and reported that prepubertal and early pubertal human osteoblasts appear to be specifically sensitive to picomolar doses of estradiol, suggesting that this hormone is a crucial regulator of bone metabolism even before puberty[6].

In conclusion, in both sexes, serum IGF-1 levels and IGF-1/IGFBP-3 ratios were significantly correlated with sex steroid levels. This correlation indicates that increasing sex steroids with pubertal development increase the IGF-1 levels and IGF-1/IGFBP-3 ratios that affect bone growth.

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