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HORMONAL STATUS AND BONE HEALTH IN BULGARIAN ADOLESCENTS WITH SECONDARY AMENORRHOEA

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Abstract

A prospective study was conducted including 67 girls with secondary amenorrhoea (excluding cases with PCOS, medical disorders and steroid therapy) and 38 healthy controls. The following indicators were compared between the two groups: serum levels of LH, FSH, estradiol, Ca, P and vitamin D, z-scores for bone mineral density, osteocalcin and β-CrossLaps, and prevalence of vitamin D deficiency. The group with secondary amenorrhoea was additionally subdivided according to the main cause – alimentary deficiency, excessive physical exercise and stress-induced. LH, FSH and estradiol levels were compared between the three subgroups.

Ca, P and vitamin D levels in the studied and the control groups were not significantly different. The following indicators were significantly lower in the studied group compared to the control one: prolactin (291.74 mIU/ml and 336.24 mIU/ml), estradiol (322.43 pmol/ml and 475.45 pmol/ml) and LH (3.79 IU/L and 4.99 IU/L) levels, LH/FSH ratio (0.55 and 0.96) and z-score (−0.48 and 0.47). FSH was significantly higher in the studied group (6.90 IU/L) compared to the control one (5.44 IU/L). The serum levels of LH and estradiol were significantly lower in the subgroup with secondary stress-induced amenorrhoea (1.73 IU/L and 220.60 pmol/ml) compared to the subgroups with amenorrhoea due to alimentary deficiency (4.68 IU/L and 394.68 pmol/ml) or extreme physical exercise (3.74 IU/L and 323.91 pmol/ml).

In girls with secondary amenorrhoea the levels of osteocalcin were significantly lower compared to the ones in the control group (26.41 and 30.99 ng/ml)

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while β-CrossLaps levels were similar (0.34 and 0.98 ng/mL). High prevalence of vitamin D deficiency was observed in both the patients with secondary amenorrhoea (71.6%) and the control group (65.8%).

Significant changes in hormone levels and bone health were observed in Bulgarian adolescent girls with secondary amenorrhoea as well as high prevalence of vitamin D deficiency in both the studied and the control groups. Early diagnosis and timely interventions are needed to improve bone health in adolescents with secondary amenorrhoea.

Key words: secondary amenorrhoea, bone health, hormonal status, bone turnover markers, vitamin D deficiency

Introduction. Amenorrhoea (absence of menses) can be a transient, intermittent, or permanent condition resulting from dysfunction of the hypothalamus, the pituitary gland, the ovaries, the uterus, or the vagina. It is often classified as primary (absence of menarche by the age of 15 or thereafter), or secondary (cessation of menses for more than three months in girls or women who previously had regular menstrual cycles or for six months in girls or women who had irregular menses [1,2]. The prevalence of secondary amenorrhoea (excluding pregnancy as a cause) is around 3–5%. No evidence suggests that the incidence of secondary amenorrhoea is related to race. The most common causes for secondary amenorrhoea are conditions, that affect the normal function of hypothalamus-pituitary-ovarian axis (HPO axis) [3].

There are three types of functional hypothalamic amenorrhoea (FHA): resulting from weight loss, resulting from excessive physical exercise and stress-induced. All of them are characterized by hypogonadotropic hypogonadism and irregular/inhibited GnRH pulsatile secretion resulting in imbalance of LH and FSH secretion. Consequently, a myriad of hormonal irregularities occurs manifested with heavy hypoestrogenism [4]. *Anorexia nervosa* (AN) is a type of eating disorder that most commonly affects girls in adolescence. Adolescence is a critical period for bone accretion, and more than half of the peak bone mass is accumulated during teenage years [5,6]. Reduction of bone mineral density is detected even after a short period after the onset of AN. Moreover, girls with early onset of AN (early after menarche) have lower bone mass than the patients with late onset (after 20 years of age). Osteopenia is not only a result of the alimentary deficit, but of hormonal imbalance as well. Adolescent girls with AN have reduced areal bone mass density (BMD) [7], suppression of bone formation and resorption markers [9], and reduced bone accrual as measured by lumbar spine BMD [8].

Bulimia nervosa (BN) is the second most common eating disorder affecting adolescent girls, with prevalence 1–2%. The mechanism of secondary amenorrhoea in patients with BN is not clear enough. Patients usually have normal weight, but the low serum levels of LH and FSH indicate inhibition of the hypothalamic function. It is a well-known fact that 25–30% of the patients with BN have previous history of AN [10–12].

Systematic heavy physical exercise in young girls is most commonly associated with three types of inhibition of the reproductive function: late menarche, abnormal luteal phase of the menstrual cycle and amenorrhoea [13, 14]. Significant osteopenia of the trabecular bones occurs in female athletes with a history of amenorrhoea.

Patients with FHA induced by *psychosomatic stress* display increased cortisol serum levels and inhibited/irregular LH pulsatile secretion [15, 16]. The mechanism of either somatic or psychical stress induced hypothalamic dysfunction is yet to be determined.

Hyperprolactinemia is a heterogenous syndrome that can be classified as either primary (in prolactinoma patients) or secondary – as a result of macroprolactinemia, medications or stress. This condition occurs in less than 1% of the general population but is more common in women with secondary amenorrhoea – 10–40%. The hyperprolactinemic hypogonadism is associated with different abnormalities in the cyclic ovarian function – e.g. anovulatory cycles, shortening of the luteal phase, oligomenorrhoea, secondary amenorrhoea [17].

The aim of the study was to compare the serum levels of LH, FSH, estradiol, prolactin and bone turnover markers (osteocalcin and β-CrossLaps) in patients with different types of secondary amenorrhoea and healthy controls, as well as the z-scores for BMD and the prevalence of vitamin D deficiency in patients with secondary amenorrhoea and healthy controls.

Materials and methods. A prospective study was conducted between 2015 and 2019 at the University Obstetrics and Gynecology Hospital "Maichin Dom" in Sofia. The participants in the study were between 12 and 18 years old. Of them 67 had secondary amenorrhoea and 38 were healthy controls. The patients with secondary amenorrhoea were additionally subdivided according to the main cause for the menstrual disturbance – stress, alimentary deficiency or extreme physical exercise. All subjects with severe chronic medical conditions, congenital musculoskeletal disorders and girls on corticosteroid therapy for more than 3 months were excluded from the study, as well as the patients with polycystic ovarian syndrome. For each patient an individual profile was completed documenting: menarche, eating habits, chronic conditions, physical activity, medical history, family history. The following parameters were evaluated for each patient: puberty development according to Tanner staging, BMI, general and gynecological status, ultrasound evaluation of the small pelvis, spinal osteodensitometry by the DXA method (currently, gold standard for measuring bone mineral density). The data from the osteodensitometries was presented as a z-score (standard deviations in the expected values for individuals of the same sex, age and body measurements). The LH, FSH, prolactin, estradiol, Ca, P and vitamin D serum levels as well as the serum markers for bone turnover – osteocalcin (marker for bone formation) and β-CrossLaps (maker for bone resorption) were measured during the early follicular phase (3rd–5th day) of a spontaneous menstrual cycle or

progestin-induced uterine bleeding. All samples were collected in the morning between 8 AM and 10 AM following standard conditions. Data were processed using statistical software IBM SPSS Statistics 25.0 and MedCalc Version 14.8.1. P values less than 0.05 were defined as statistically significant.

Results. The serum levels of Ca, P and vitamin D were not statistically different between the patients with secondary amenorrhoea and the healthy controls (2.29 and 2.32 mmol/L, add the remaining) – Table 1. The serum levels of prolactin were significantly lower in patients with secondary amenorrhoea (219.74 mIU/L) compared to the corresponding in the control group (336.24 mIU/L). The mean z-score value was significantly lower in the secondary amenorrhoea group (−0.48) compared to the controls (0.47). A statistically significant difference was found for the serum levels of estradiol and the gonadotropin hormones (LH and FSH), all of them having lower values in the patients with secondary amenorrhoea (estradiol – 322.43 IU/L, LH – 3.79 UI/L, FSH – 6.90 UI/L) compared to the control group (estradiol – 475.45 IU/L, LH – 4.99 UI/L, FSH – 5.44 UI/L). The LH/FSH ratio was significantly lower in patients with secondary amenorrhoea compared to the control group (Table 1).

When comparing the LH, FSH and estradiol levels in the three subgroups with secondary amenorrhoea (alimentary, extreme physical exercise, stress) significantly lower levels of LH (1.73 UI/L – below the reference range) and estradiol (220.60 pmol/ml) were found in the group with stress-induced amenorrhoea (Table 2).

T a b l e 1

Comparative analysis of Ca, P, vitamin D, prolactin, LH, FSH, estradiol levels, LH/FSH and z-scores in healthy controls and girls with secondary amenorrhoea ($^*p < 0.05$)

Indicators		Healthy controls			Girls with secondary amenorrhoea		
		N	$\overline{X}$	SD	N	$\overline{X}$	SD
Ca	mmol/L	38	2.32	0.14	67	2.29	0.14
P	mmol/L	38	1.32	0.17	67	1.27	0.20
Vitamin D	ng/ml	38	18.16	7.00	67	18.50	8.30
Prolactin	mUI/L	38	336.24	134.28	67	219.74*	133.91
LH/FSH		38	0.96	0.35	67	0.55*	0.54
z-score		12	0.47	0.86	26	−0.48*	1.21
Estradiol	pmol/ml	38	475.45	276.35	67	322.43*	206.68
LH	IU/L	38	4.99	1.88	67	3.79*	4.42
FSH	IU/L	38	5.44	1.88	67	6.90*	4.12

Table 2

Comparative analysis of LH, FSH and estradiol levels in the subgroups with different causes for secondary amenorrhoea. LH – luteinizing hormone, FSH – follicle stimulating hormone ($^*p < 0.05$)

Indicators		Cause for secondary amenorrhoea								
		Alimentary deficiency			Extreme physical exercises			Stress		
		N	$\overline{X}$	SD	N	$\overline{X}$	SD	N	$\overline{X}$	SD
LH	IU/L	34	4.68	5.65	17	3.74	1.36	16	1.73*	1.99
FSH	IU/L	34	7.58	2.87	17	6.45	2.37	16	7.76	8.18
Estradiol	pmol/ml	34	394.68	246.68	11	323.91	70.91	10	220.60*	137.75

When comparing the markers for bone turnover, significantly lower value of osteocalcin was found in patients with secondary amenorrhoea (26.41 ng/mL) compared to healthy controls (30.99 ng/mL). However, the difference in β-Cross-Laps levels between the studied and the control groups was not statistically significant (0.34 ng/mL and 0.98 ng/mL) – Table 3.

Table 3

Comparison of osteocalcin and β-CrossLaps in healthy controls and girls with secondary amenorrhoea ($^*p < 0.05$)

Indicators		Healthy controls			Girls with secondary amenorrhoea		
		N	$\overline{X}$	SD	N	$\overline{X}$	SD
Osteocalcin	ng/mL	38	30.99	14.27	67	26.41*	10.99
β-CrossLaps	ng/mL	38	0.98	0.45	67	0.95	0.34

The prevalence of vitamin D deficiency in the studied group (71.6%) was slightly higher but not significantly different from the one in the control group (65.8%). In general, the prevalence of vitamin D deficiency for all the subjects included in the study was 69.4%, which is a relatively high figure (Table 4).

Discussion. The most common cause for secondary amenorrhoea in the studied populace was alimentary deficiency (34 patients 51%), followed by stress (16 patients 23.8%) and extreme physical exercise (17 patients 25.2 %). In all three groups of patients with secondary amenorrhoea, it was confirmed that the lower values of osteocalcin were significant compared to the values in healthy controls, which indicates suppressed bone turnover. In β-CrossLaps indications, there was no connection between the examined causes and the β-CrossLaps levels.

Table 4

Comparative analysis of vitamin D deficiency prevalence in the studied and the control groups and for the subjects enrolled in the study

Group	Patients with vitamin D deficiency	
	N	%
Healthy controls	38	65.8
Patients with secondary amenorrhoea	67	71.6
Whole sample	105	69.4

The comparative analysis of the studied groups for the indicators Ca, P, vitamin D, prolactin, LH/FSH and z-score confirmed no statistically significant difference for Ca and P between the four groups. Statistically significant difference was not observed between the two groups with respect to vitamin D deficiency which was considerable in both. The vitamin D deficiency in all subjects was 69.4%. In z-score, the average value was considerably higher in healthy controls than in the girls with secondary amenorrhoea, although there were pathological values of z-score (z-score ≤ -2 SD) in three patients with secondary amenorrhoea (4.5%). In all three cases, the main cause of secondary amenorrhoea was alimentary deficiency. The LH/FSH ratio was significantly lower in patients with secondary amenorrhoea compared to the control group, as a sign suppressed function of the hypothalamus and impaired coordination between the hypothalamus and the pituitary gland. Statistically significant lower values were found of the serum levels of estradiol and the gonadotropic hormones in the patients with secondary amenorrhoea compared to the control group, indicating status of hypogonadothropic hypogonadism, and as a consequence – hypoestrogenism.

The results of the comparative analysis of LH, FSH and estradiol due to alimentary deficiency, stress and excessive physical exercise in girls with secondary amenorrhoea determined that in the stress-affected patients, the average value of the indicators of LH and estradiol were significantly lower compared to the girls in the other subgroups (which were not statistically different from each other). The levels of FSH were not affected by the studied causes in a statistically significant way.

There was a considerably lower value of prolactin in girls with secondary amenorrhoea compared to the healthy controls, but both groups were in the reference range for prolactin.

The dependencies that were concluded in this study were:

- LH was inversely proportional and moderately correlated to z-score;

- FSH was proportional and lightly correlated to osteocalcin;
- Estradiol was proportional and lightly correlated to β-CrossLaps.

Conclusion. Early diagnosis and nutritional intervention for optimal weight management, prevention of stress and normal physical activities, consistent monitoring of bone health, and conservative treatment with pharmacologic options presently available are important to circumvent future impaired bone mineral density. More rigorous, multi-centre research, including intervention and prevention trials with long-term follow-up, are needed to determine the most effective means of bone loss prevention and catch-up bone accrual.

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