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ROLE OF VITAMIN D IN THE FORMATION OF ABNORMAL UTERINE BLEEDING IN THE PUBERTATE PERIOD

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Abstract. Role of vitamin D in the formation of abnormal uterine bleeding in the pubertate period. Dynnik V.O., Dynnik O.O., Druzhinina A.E. The problem of the participation of vitamin D in the pathogenesis of various diseases has been attracted the attention of scientists in recent years. Vitamin D deficiency is regarded as a global problem that increases the risk of many chronic diseases. More recently, the emphasis has shifted to the non-skeletal effects of vitamin D. A lot of literature suggests that vitamin D plays an important role in the regulation of processes in the ovaries that determine female fertility, female reproductive potential. Vitamin D receptor enzymes involved in its metabolism are expressed in both the central and peripheral reproductive organs (hypothalamic-pituitary system, ovaries, uterus, placenta). All of this suggests that vitamin D is involved in the regulation of menstrual function. The aim of the study is to identify the relationships between gonadotropic and steroid hormones with the content of vitamin D in menstrual disorders in adolescent girls by type of abnormal uterine bleeding (AUB). 167 adolescent girls with AUB were examined. The levels of Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), Prolactin (PRL), total estradiol (E₂), testosterone (T), cortisol (C), vitamin D were determined. It was revealed that in 92.1% of girls with AUB have a decrease in the level of vitamin D, and 53.9% of them have a sharp reduce of its. A factorial model of the relationship between pituitary-gonadal hormones and vitamin D, on the basis of which it may be assumed that vitamin D makes a significant contribution to the formation of AUB in adolescence. It participates in the mechanisms of stimulation of gonadotropins, affects the activity of sex hormones through the promoters of receptors located both in the hypothalamic-pituitary region and in the periphery in the ovaries. This is evidenced by factor weights, which serve as analogues of the correlation ratios and show the degree of interconnection between the variables in the factors selected. The changes in the content of vitamin D revealed indicate the need and expediency of its determination in the blood of patients with AUB during puberty for early diagnosis of its abnormalities and timely prevention of serious complications in the future.

Реферат. Роль вітаміну D у формуванні аномальних маткових кровотеч у пубертатному періоді. Диннік В.О., Диннік О.О., Дружиніна А.Є. Останні роки увагу вчених привертає проблема участі вітаміну D в патогенезі різних захворювань. Дефіцит вітаміну D розцінюється як глобальна проблема, яка збільшує ризик багатьох хронічних захворювань. Останнім часом акцент змістився на несkeletalні ефекти вітаміну D. Чимало літературних джерел свідчить, що вітамін D відіграє важливу роль у регуляції процесів у яєчниках, які визначають жіночу фертильність, жіночий репродуктивний потенціал. Ферменти рецептора вітаміну D, які беруть участь у його метаболізмі, експресуються як у центральних, так і в периферичних репродуктивних органах (гіпоталамо-гіпофізарній системі, у яєчниках, матці, плаценті). Усе це передбачає участь вітаміну D в регуляції менструальної функції. Мета дослідження - виявлення зв'язків гонадотропних, стероїдних гормонів із вмістом вітаміну D при порушеннях менструальної функції в дівчат-підлітків за типом аномальних маткових кровотеч (АМК). Обстежено 167 дівчат-підлітків з АМК. Визначалися рівні лютеїнізуючого гормону (ЛГ), фолікулоstimулюючого гормону (ФСГ), пролактину (ПРЛ), загального естрадіолу (Е₂), тестостерону (Т), кортизолу (К), вітаміну D. Виявлено, що в 92,1% дівчат з АМК реєструється зниження рівня вітаміну D, причому в 53,9% з них вміст вітаміну D – різко знижений. Побудована факторна модель взаємозв'язку гіпофізарно-гонадних гормонів із вмістом вітаміну D, на підставі якої можна припустити, що вітамін D чинить істотний внесок у формування АМК в підлітковому віці. Він бере участь у механізмах стимуляції гонадотропнів, впливає на активність статевих гормонів через промотори рецепторів, розташованих як у гіпоталамо-гіпофізарній ділянці,

так і на периферії в яєчниках. Про це свідчать факторні навантаження, які є аналогами коефіцієнтів кореляції і показують ступінь взаємозв'язку змінних у виділених факторах. Виявлені зміни вмісту вітаміну D свідчать про необхідність і доцільність його визначення в крові пацієнток з АМК періоду пубертату для ранньої діагностики його відхилень і своєчасної профілактики серйозних ускладнень у майбутньому.

In recent years, the attention of scientists has been drawn to the issue of the involvement of vitamin D in the pathogenesis of various diseases. Vitamin D deficiency is regarded as a global problem increasing the risk of many chronic diseases. More recently, the emphasis has shifted to the non-skeletal effects of vitamin D. A growing body of literature suggests that vitamin D plays an important role in the regulation of processes in the ovaries determining female fertility and reproductive capacity.

Vitamin D belongs to the steroid hormone family. In recent years, the spectrum of vitamin D-mediated effects has expanded and it is now recognized as a universal signaling molecule, not just a regulator of bone health and calcium homeostasis. The cellular effects of vitamin D are mediated primarily through the related intranuclear vitamin D receptor. Vitamin D receptor enzymes involved in its metabolism are expressed in both central and peripheral reproductive organs (hypothalamic-pituitary system, ovaries, uterus, placenta) [1, 2]. All this suggests that vitamin D is involved in the regulation of menstrual function. The data on this matter is ambiguous. The role of vitamin D was mostly studied in relation to dysmenorrhea, polycystic ovary syndrome [3, 4]. There are isolated works on the role of vitamin D in the formation of menstrual dysfunction [5]. However, virtually all these works indicate that vitamin D deficiency is harmful to reproductive health.

The effect of vitamin D deficiency on the health of children and adolescents has been studied for many years, yet information on the role of vitamin D in the formation of menstrual function in girls during puberty remains scant and ambiguous. Among the hypotheses of menstrual dysfunction with vitamin D deficiency, neurohumoral regulation of the hypothalamic-pituitary-ovarian system is considered the most important due to the fact that vitamin D receptors, in contrast to other vitamins, are located in the nuclei of various tissues and organs [6, 7].

For various reasons, the continuity between medical professionals who provide supervision and support from childhood to adulthood has been lost. Puberty is one of the most important stages of development in great need for harmonious and uninterrupted functioning of all systems. During this period, any adverse effects could lead to serious disorders on the part of puberty and the formation of menstrual function.

The issues of preserving the reproductive capacity of adolescents has recently become increasingly important. This can be explained not only by the crisis

demographic situation in the country, but also by the low reproductive capabilities of today's youth.

The high prevalence of menstrual irregularities - from 20 to 48% in the structure of gynecological diseases in adolescent girls is a common reason for referring to a pediatric gynecologist [8, 9]. The most severe disorders are abnormal uterine bleeding. Excessive blood loss is accompanied by serious abnormalities in various organs and systems.

Elucidation of new links in the pathogenesis of AUB during adolescence will make it possible to improve approaches to managing this disorder. There are descriptions of the interaction of the pituitary-gonadal complex and metabolic hormones, depending on the clinical variant of the course of the disease, the BMI on which basis it is formed [10]. We have not seen any works on the inclusion of vitamin D, a steroid hormone in abnormal uterine bleeding, into this process.

The aim of the study was to identify the relationship of gonadotropic, steroid hormones with the content of vitamin D in menstrual disorders in adolescent girls by type of abnormal uterine bleeding.

MATERIALS AND METHODS OF RESEARCH

The work was carried out based on the results of clinical and paraclinical examination of 110 adolescent girls aged 11-18 with AUB, who were treated in the Department of Pediatric Gynecology of the State Institution "Institute for Health Protection of Children and Adolescents of the National Academy of Medical Sciences". The level of physical development was determined based on the indicators of height, body weight and BMI index. The interpretation of the obtained results of anthropometry was carried out using age centile tables.

The girls were divided into two groups depending on their BMI index. Group I included 30 underweight girls - their average BMI was $16.35 \pm 1.11 \text{ kg/m}^2$. Group II included 80 patients with physiological BMI ($19.44 \pm 1.74 \text{ kg/m}^2$; $p < 0.00001$). The concentrations of Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), Prolactin (PRL), estradiol (E_2), testosterone (T), cortisol (C), vitamin D in blood serum were studied by enzyme immunoassay on a «Rayto RT 2100C» photometer using standard commercial kits from «Best Diagnostic» (Kyiv), according to the instructions for use of commercial reagent kits. Currently, the determination of the concentration of 25 (OH) D is considered the best method, since it reflects the intake of vitamin D from two sources - both through the skin and with food. A comparative analysis of the results of all parameters was carried

out. The comparison group consisted of 57 healthy peers with a normal menstrual cycle.

Statistical analysis was centered on the analysis of variance for basal hormone levels. The results are presented as tables of mean values, standard deviation (M±SD) and median (Me). System analysis was carried out using analysis of variance and factor analysis. The structure of factor models is presented in the form of a table containing information on the informative value of factors and factor weight ratios (FWRs). For statistical analysis, we used the SPSS-17 software package (license No. d47646601451106a68cd) and Microsoft Excel 2007 (license CMPJ6-YBX7P-CJPKF-62GPW-F).

All patients and their parents signed an informed assent form to participate in the clinical study. The study was conducted in compliance with the principles of the Declaration of Helsinki of the World Medical Association "Ethical principles of medical research with human participation as an object of research", the UN Committee on the Rights of the Child General Comment No. 5 (2003) on General measures of implementation of the Convention on the Rights of the Child, European Convention on the Adoption of Children (revised), European Convention on the Exercise of Children's Rights (CETS No. 202; 2008/2011)); Law of Ukraine dated April 26, 2001 No. 2402-III "On Child Protection" (as amended in 2002-2016), the Order of the Ministry of

Health of Ukraine "On Approval of the Procedure for Conducting Clinical Trials of Medicines and Expertise of Materials of Clinical Trials and Model Regulations on Ethics Commissions" No. 690 of 23.09.2009 (as amended in 2012-2015), about which there is an extract from the minutes of the meeting of the Committee on Bioethics and Deontology at the State Institution "Institute for the Protection of Children and Adolescents of the National Academy of Medical Sciences" (No. 8 from 15.09.2021)

RESULTS AND DISCUSSION

The study of the interaction of pituitary and steroid hormones with vitamin D in adolescent girls with AUB was carried out in two stages.

The first stage involved the study of hormonal parameters characteristic of modern patients with menstrual irregularities of the AUB type.

Based on the results of variance analysis, the levels of gonadotropic hormones in the blood serum had peculiarities depending on the BMI on the basis of which uterine bleeding was formed (Table 1). FSH concentration did not depend on BMI; however, LH values significantly differed between the selected groups and the comparison group. They were significantly higher in the adolescents with AUB than in the comparison group, and the average LH content was the highest in underweight girls. The level of PRL did not differ significantly between the study groups when compared.

Table 1

Statistical indicators of gonadotropic, steroid hormones and vitamin D in patients with AUB and different body weights

Hormonal indicators	Statistical indicators	Groups			
		I	II	All	Comparison
E ₂ , nmol/l	n	20	48	68	57
	M±SD	0.41±0.17	0.38±0.22	0.39±0.21	0.34±0.21
	Me	0.39	0.34	0.35	0.3
T, nmol/l	n	18	45	63	57
	M±SD	1.98±1.28	1.91±1.09	1.9±1.14	2.85±1.21
	Me	1.47	1.62	1.57	2.78
C, nmol/l	n	11	48	59	57
	M±SD	579.6±250.1	424.6±258.0	453.49±261.6	491.76±217.63
	Me	500.7 ¹⁾²⁾	394.7 ¹⁾	395.6	424.0
FSH, mIU/ml	n	23	70	93	57
	M±SD	6.88±2.43	6.79±2.53	6.81±2.49	6.12±2.35
	Me	6.16	6.35	6.34	6.09
LH, mIU/ml	n	23	70	93	57
	M±SD	9.21±4.89	7.53±5.33	8.11±5.39	4.34±3.22
	Me	8.85 ¹⁾²⁾	6.56 ¹⁾²⁾	6.81	3.82
Prolactin, mIU/l	n	23	70	93	57
	M±SD	395.3±191.88	449.34±280.81	435.97±261.72	342.87±155.76
	Me	342.1	382.7	376.5	298.32
Vitamin D, ng/ml	n	12	40	52	20
	M±SD	16.87±7.62	20.09±7.21	19.34±7.37	20.35±3.05
	Me	15.42	17.95	17.75	20.35

Notes: ¹⁾p<0.05 between the I and II groups; ²⁾p<0.001 in relation between I or II group to the comparison group.

The average content of steroid hormones in the blood serum of girls with AUB had its own characteristics. Comparison of T and estradiol levels did not reveal significant differences when comparing the groups with each other or with the control group. The concentration of cortisol varied significantly among girls of different groups. The average C values in underweight adolescents exceeded the same both in the group with standard BMI and in the comparison group. The content of C in adolescents with physiological BMI did not exceed the control values. It can be assumed that abnormal uterine bleeding was a more stressful situation for underweight girls than for adolescents with physiological BMI parameters.

Average levels of vitamin D did not differ significantly when compared between the groups and the

comparison group and corresponded to the reduced indicators of the content of vitamin D in blood serum. In isolated cases, the level of vitamin D was within the range of physiological values, was reduced in 92,1% of cases, and more than half of patients had it sharply reduced (53.9%).

The second stage of the study involved a systematic analysis of the relationship between vitamin D and the level of gonadotropins and steroid hormones. The method of factor analysis was used to construct a model of the association of vitamin D with pituitary-gonadal hormones (Table 2). According to factor analysis, 3 factors were identified, the total influence of which explains 76.8% of the total variance.

Table 2

Structure of the factor model of the relationship between gonadotropic, steroid hormones and vitamin D

Factors	Factor variance, %	Variable names	Factor weights
F1	40.7	LH	0.88
		FSH	0.67
		PRL	0.51
		Testosterone	0.89
		Vitamin D	0.67
F2	20.1	Cortisol	0.96
		PRL	0.54
F3	15.9	Estradiol	0.92
		FSH	-0.31
		Vitamin D	0.54

The most significant F1 describes 40.7% of the variance of the system of characteristics considered. It indicates the inclusion of vitamin D into the regulation of the menstrual cycle and reflects the mechanism of influence of the content of vitamin D on the formation of the reproductive system during puberty primarily at the level of the central core and testosterone. Literature data indicate multiple receptors in the reproductive organs, including the hypothalamus and pituitary gland. The literature

contains conflicting information on the relationship between vitamin D and fertile hormones. There is evidence of a change in the sensitivity of ovarian tissues to FSH under the influence of vitamin D [11]. A study conducted in Germany, which included 351 women of the European race of reproductive age, revealed a negative correlation between vitamin D, total testosterone, free androgen index, LH [12]. However, there is information on the direct dependence of the level of vitamin D and T in the literature.

Evidence obtained from experimental studies in primates suggests that T stimulates follicular maturation and accumulation of androgens in the ovaries. This is important both for the development of follicles and the proliferation of granulosa cells, and this effect is potentiated by the activity of FSH receptors, and a sufficient level of vitamin D may be able to enhance these processes [13]. These data have been confirmed by the studies on healthy women, not suffering from obesity [14].

Variance analysis of F1 eigenvalues did not reveal statistically significant differences depending on the body weight of adolescent girls.

The structure of the second factor (F2), describing 20.1% of the variability of the initial data is represented by only two highly interrelated hormones and indicates the existence of a positive association between PRL levels and cortisol. In our opinion, this factor indicates the interaction of stress-supporting and stress-limiting systems in the presence of AUB and reflects the balance of their interaction. The variance of the proper values of this factor relative to the observation group (with different BMIs) showed that underweight adolescents present a statistically significant higher average level of F2 ($p < 0.02$) in relation to patients with physiological body weight (Fig. 1).

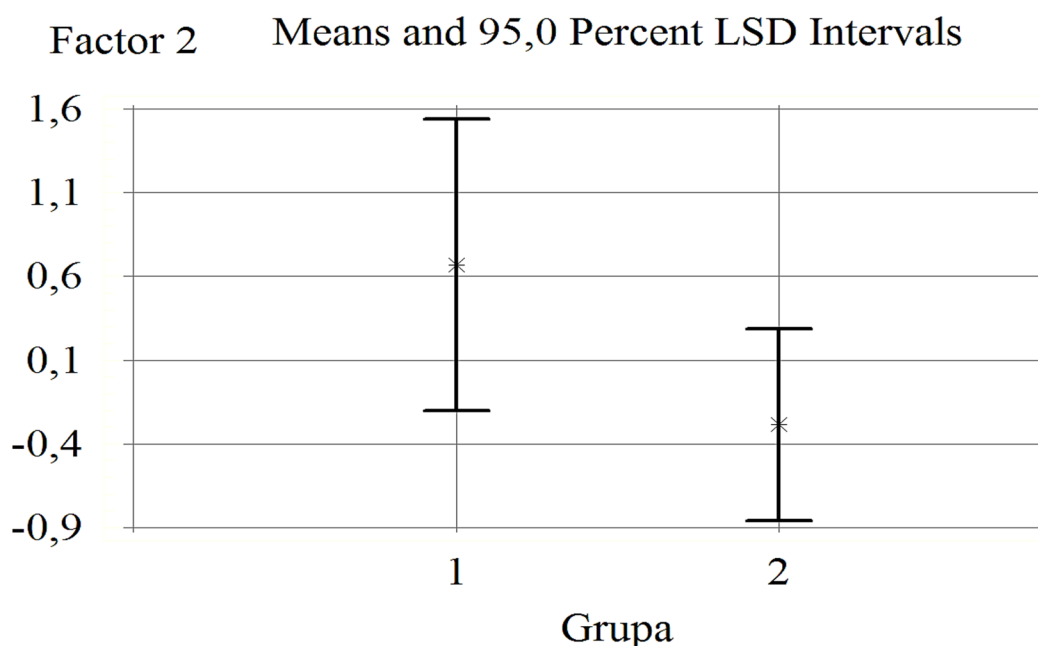


Fig. 1. Distribution of mean eigenvalues of Factor 2 in patients with AUB and different body weights

It can be concluded that this mechanism in AUB depends on the body weight of adolescent girls, on the basis of which uterine bleeding formed.

The third factor (F3) describes 15.9% of the variability of the variables and characterizes the relationship between FSH, estradiol and vitamin D. Moreover, relationship of FSH is negative. It illustrates the fact of a negative relationship of the pituitary hormone FSH (FWRs = -0.31) and the female sex hormone (estradiol – FWRs = 0.92) and vitamin D (FWRs = 0.54). At the same time, estradiol has a predominant influence. There are publications indicating that vitamin D regulates steroidogenesis in human ovarian cells. Taking estrogen-containing drugs increases the level of vitamin D in women’s bodies [15]. There is information that specific receptors for vitamin D are

present in the cells of various organs, including the ovaries, which makes it possible to consider it as a hormone capable of generating and modulating reactions in target tissues by regulating gene transcription. Thus, vitamin D is able to modulate ovarian activity [16], meaning that vitamin D is an important factor in estrogen biosynthesis. This correlation may possibly be explained by the association with the ability of vitamin D to act as a regulator of a number of enzymes involved in the regulation of the production of steroid hormones, including the sex hormones of the ovaries.

Variance analysis of the proper values of F3 revealed statistically significant differences depending on the body weight of adolescent girls ($p < 0.03$). It was significantly higher in patients with normal body weight (Fig. 2).

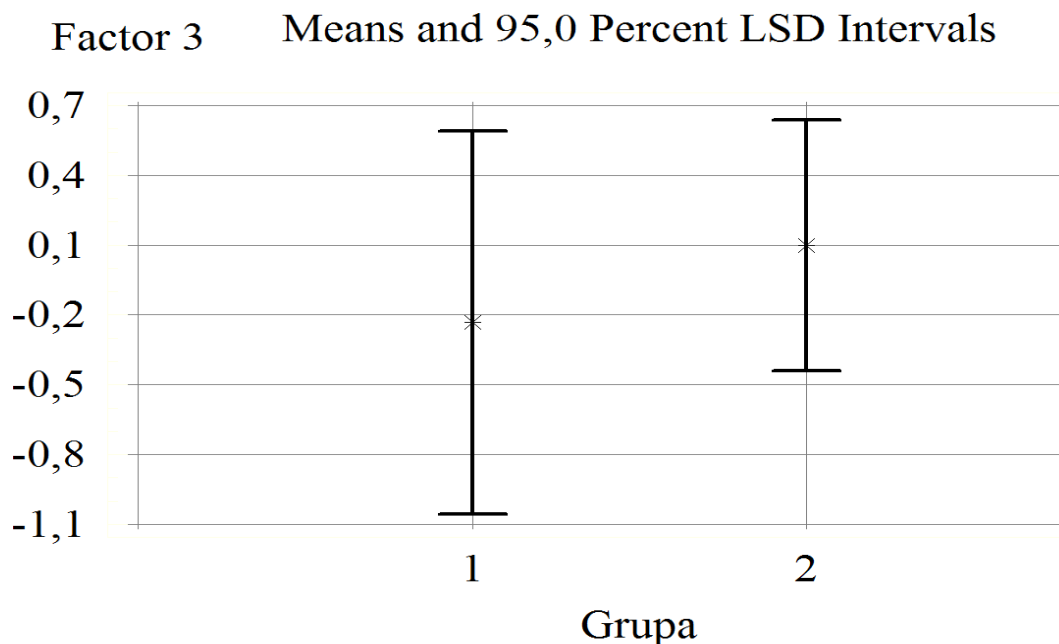


Fig. 2. Distribution of mean eigenvalues of Factor 3 in patients with AUB and different body weights

CONCLUSIONS

1. It was established that the course of abnormal uterine bleeding in modern girls is accompanied by decreasing of vitamin D in blood serum.

2. Systemic analysis has convincingly shown that vitamin D makes a significant contribution to the formation of abnormal uterine bleeding in adolescence. It is involved in the mechanisms of gonadotropin stimulation, affects the activity of sex hormones through the promoters of receptors located both in the hypothalamic-pituitary region and on the periphery in the ovaries. This is evidenced by factor loads, which are analogs of correlation coefficients and show the degree of interrelation of variables in distinguished factors.

3. The debut of the abnormal uterine bleeding during puberty occurs against the background of

disorders of both the hormonal component and changes in the content of vitamin D.

4. Further study of the problem of the inclusion of vitamin D in the formation of menstrual disorders, namely abnormal uterine bleeding can help develop new strategies for the prevention and treatment of this serious disease during puberty.

Contributors:

Dynnik V.O. – conceptualization, methodology, supervision;

Dynnik O.O. – formal analysis, validation;

Druzhinina A.E. – investigation, data curation.

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