ARTICLE

GENOTYPING OF VITAMIN D ASSOCIATED SNPs IN FEMALE STUDENTS

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ABSTRACT

The reproductive health of students deserves attention due to the high social expectations of this group of young people. It has now been established that vitamin D deficiency is an etiopathogenetic factor in the development of a wide range of diseases. Vitamin D receptors are found in reproductive tissues, including the ovaries, uterus, placenta, testes, and in the pituitary gland. Vitamin D deficiency in women is associated with an increased risk of developing reproductive dysfunction, which led to our study. The availability of vitamin D was studied in female students with menstrual irregularities and a physiological menstrual cycle. Vitamin D metabolism is genetically predetermined; it was decided to study the genetic component that could cause vitamin D deficiency in female students with menstrual irregularities and genotyping genes (GC gene, rs2282679; CYP2R1 gene, rs2060793; VDR gene, rs2228570. VDR gene, rs222857070) involved in the metabolism of vitamin D. The study of the level of vitamin D was carried out using BIOMEDICAGRUPPE enzyme-linked immunosorbent assay kits (Germany). Genotyping was carried out by real-time PCR using TaqMan probes according to the protocol of the manufacturer (SibDNA LLC, Novosibirsk). Vitamin D deficiency was found in all female students with menstrual irregularities, in girls - students with a physiological menstrual cycle, vitamin D levels were within the limits of normal exogenous supply. Genotyping revealed in female students with menstrual irregularities variations in GC, rs2282679; CYP2R1, rs2060793; VDR, rs2228570 causing vitamin D deficiency, and variations of all three genes contributed to the development of vitamin D deficiency. In female students of the control group, for the most part, variations in the genes providing physiological metabolism of vitamin D were revealed. Prescribing a vitamin D drug will probably have a beneficial effect on the reproductive function of female students with menstrual irregularities and with a genetic predisposition to reduce the transport of vitamin D in the body and decrease the function of converting vitamin D into an active ligand for the vitamin D receptor.

KEY WORDS
reproductive health, genotyping, female students, menstrual irregularities, vitamin D deficiency

INTRODUCTION

The population is mainly reproduced by the population of fertile age 20-29 years, which accounts for 49% of the total number of abortions and 64% of the total number of births [1, 2]. In this regard, the reproductive health of students deserves special attention. The need for self-fulfillment as a spouse and parent refers to the basic needs of a person at the age to which students belong [3, 4].

Vitamin D is an important pre-hormone involved in many metabolic processes. It has now been established that vitamin D deficiency affects a wide range of acute and chronic diseases [5, 6]. WHO experts (2010) emphasized that vitamin D status is very important in preventing a large number of abnormalities in the functioning of the human body. The association of vitamin D deficiency with many reproductive health problems can be assumed with a high degree of probability since VDR and 1a-hydroxylases are found in reproductive tissues, including the ovaries, uterus, placenta, testes, and in the pituitary gland [7, 8, 9].

Experimental studies have shown that the level of 2D3 equal to 1.25 (OH) determines the modulation of ovarian activity and with the development of vitamin D deficiency in female rats' hyper gonadotropic hypogonadism, uterine hypoplasia, impaired folliculo genesis, abnormal follicular development, and infertility were observed [10].

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Vitamin D deficiency in women is associated with an increased risk of developing menstrual dysfunction, which led to our study.

Objective. To study the availability of vitamin D in female students with menstrual irregularities and conduct genotyping of genes (*GC* gene, rs2282679; gene *CYP2R1*, rs2060793; gene *VDR*, rs2228570. Gene *VDR*, rs2228570) involved in the metabolism of vitamin D.

MATERIALS AND METHODS

The blood level of vitamin D was determined in girls with menstrual irregularities - 28 subjects (study group); 30 subjects with a physiological menstrual cycle made up the control group. Written consent from the subjects was taken and the study was approved by the Kazan University ethical committee. Blood sampling was carried out from the cubital vein into a test tube in a volume of 5.0 ml. The study of vitamin

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D levels was carried out using BIOMEDICAGRUPPE enzyme-linked immunosorbent assay kits (Germany). To determine 25-OH Vitamin D, an enzyme immunoassay was used to quantify 25-hydroxyvitamin D and other hydroxylated metabolites in serum (IDS OCTEIA 25-Hydroxy Vitamin D Kit). Principle of the IDS OCTEIA 25-Hydroxy Vitamin D test is based on an enzyme-linked immunosorbent assay, which quantifies 25-OH Vit D and other hydroxylated metabolites in serum and plasma. Calibrators, controls, and samples are diluted with biotinylated 25-OH Vit D. During the reaction of 25-OH Vit D, calibrators, controls and samples compete with biotinylated 25-OH Vit D for binding sites on highly specific sheep anti-25-OH Vit D antibodies, sorbed in the wells of the tablet for 2 hours at room temperature. After aspiration of the reagent and washing, horseradish peroxidase conjugated with avidin is added to the plate. A complex is formed, which is quantitatively determined further during incubation with the TMB substrate. The intensity of the developed color is inversely proportional to the content of 25-OH Vit D in the standard / sample / control. Based on the measurement results of the controls, a calibration curve is constructed; the concentration of 25-OH Vit D in the samples is determined using this curve. The recognized criterion for assessing the exogenous availability of vitamin D is the level of 25-OH D in the blood: normal blood levels - 20-35 ng/ml, deficiency - 10-20 ng/ml, deficiency - hypovitaminosis - 10 ng/ml and lower, vitamin deficiency - below 5 ng/ml. Hypervitaminosis D - above 70 ng/ml.

Genotyping of the following genes was carried out on the *GC* gene, rs2282679; *CYP2R1* gene, rs2060793; *VDR* gene, rs2228570. *VDR* gene, rs2228570. Genotyping was carried out by real-time PCR using TaqMan probes according to the protocol of the manufacturer (SibDNA LLC, Novosibirsk). DNA was isolated from peripheral blood leukocytes by phenol-chloroform extraction followed by precipitation with 96% ethanol. After drying, the DNA was diluted in distilled water and used as a template for PCR.

Statistical processing of the research results was carried out using Statistica for Windows software packages (version 6.1) using parametric and nonparametric statistics methods (Student t-test, Mann-Whitney test).

RESULTS

We analyzed the anamnesis of the menstrual function of female students with menstrual irregularities and found that the first menstruation occurred at 14 years old and at 15 and 16 years old, then periods of absence of menstruation from 5-6 months to 1-2 years, until they started to seek medical help.

It was revealed that in students with menstrual irregularities, a low level of vitamin D was found - 11.7 ± 1.8 ng/ml ($p \geq 0.01$).

The study of the content of vitamin D in the blood showed in female students with a physiological menstrual cycle (control group) the level of vitamin D within the normal exogenous supply equal to 22.5 ± 1.3 ng/ml ($p \geq 0.01$).

Vitamin D metabolism is genetically predetermined; it was decided to study the genetic component that could cause vitamin D deficiency in female students with menstrual irregularities and severe vitamin D deficiency. Genotyping of the following genes was carried out: gene *GC*, rs2282679; *CYP2R1* gene, rs2060793; *VDR* gene, rs2228570. the *VDR* gene, rs2228570, because variations of these genes are involved in the metabolism of vitamin D in the body.

The *GC* gene, rs2282679 is a multifunctional protein; it binds to vitamin D and its plasma metabolites and transports them to tissues. Subjects with a C/C or A/C genotype with a genetic marker may be more prone to low levels of vitamin D due to reduced ability to transport vitamin D in the body, and the variation of A/A of this gene provides the physiological metabolism of vitamin D, corrected by the diet.

The *CYP2R1*, rs2060793 gene encodes members of the cytochrome P450 enzyme superfamily. Proteins of monooxygenases of Cytochrome P450, which catalyze many reactions, are involved in the metabolism of drugs and the synthesis of cholesterol, steroids and other lipids. This enzyme is a microsomal vitamin D hydroxylase that converts vitamin D into an active ligand for the vitamin D receptor. There is a C/T gene variation: T/T - the microsomal vitamin D hydroxylase enzyme that converts vitamin D into an active ligand for the vitamin D receptor; C/C or C/T genotypes with a genetic marker predisposed to low levels of vitamin C, as in these cases a decrease in the function of converting vitamin D into an active ligand to the vitamin D receptor is observed.

The function of the *VDR*, rs2228570 gene is the most studied: the T/T genotype provides a physiological level of vitamin D3; C/T gene variation causes a more than 1.5-fold increased risk of lowering the level of circulating active form of vitamin D; C/C gene variation causes a more than 3.7-fold increased risk of lowering the level of circulating active form of vitamin D.

Genotyping revealed in female students with menstrual irregularities variations in *GC*, rs2282679; *CYP2R1*, rs2060793; VDR, rs2228570 causing vitamin D deficiency, and variations of all three genes contributed to the development of vitamin D deficiency. In female students of the control group, for the most part, variations in the genes providing physiological metabolism of vitamin D were revealed - a variation of the T/T of the VDR gene, a variation of the A/A of the *GC*, rs2282679 gene, and T/T variation of the *CYP2R1*, rs2060793 gene.

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Table 1: Genotyping results of female students with menstrual irregularities

Indicators	Vitamin D (ng/ml)	*VDR*, rs2228570			*CYP2R1*, rs2060793			*GC*, rs2282679		
		C/C	C/T	T/T	C/C	C/T	T/T	C/C	A/C	A/A
Girls with menstrual irregularities (28)	11.7 ±1.8	11	11	6	12	7	9	8	16	4
Girls with a physiological menstrual cycle (30)	22.5 ±1.3	3	7	21	4	6	20	1	6	23

According to the results of genotyping, vitamin D was prescribed to girls with menstrual dysfunction. The dose of the vitamin D depended on the genotype. Girls with the genotypes C/C, C/T of *VDR*, rs2228570, C/C, C/T of *CYP2R1*, rs2060793, and C/C, A/C of *GC*, rs2282679, which do not provide physiological metabolism of vitamin D, were prescribed a dose of 4000 U/day, and those with genotypes that ensure normal metabolism of vitamin D, - T/T (VDR, rs2228570); T/T (*CYP2R1*, rs2060793) and A/A (*GC*, rs2282679) in the absence of the C/C, C/T, A/C genotypes of other genes, were prescribed a dose of 2000 U/day. Of the 55 girls with menstrual dysfunction, 26 took vitamin D at the recommended dose, and 29 girls did not.

Vitamin D levels are re-examined 3-4 months after the start of taking the recommended doses. The study of vitamin D availability in girls with menstrual dysfunction who took vitamin D revealed a significant increase in vitamin D levels - 19.4 ± 0.6, and positive changes in the functioning of the reproductive system were observed (the number of painful menstruations, manifestations of premenstrual syndrome, and heavy menstruation). And the girls with menstrual dysfunction who did not take vitamin D had no increase in vitamin D levels observed and there were also no positive changes in the functioning of the reproductive system. According to our recommendation, girls with menstrual dysfunction continued taking vitamin D in recommended doses for another 5-6 months. And 10 months after the start of the daily intake of vitamin D, its level was measured. In girls with menstrual dysfunction, the level of vitamin D increased during the intake period and corresponded to the physiological norm. At the same time, 27.3% of girls with menstrual dysfunction noted a lack of manifestations of premenstrual syndrome and algomenorrhea, as well as significant positive changes. And the girls with menstrual dysfunction who did not take vitamin D had no increase in vitamin D levels and positive changes in the functioning of the reproductive system, that is, there were manifestations of premenstrual syndrome, algomenorrhea, and hyper polymenorrhea.

SUMMARY

Thus, according to the results of the study, it can be reliably stated that vitamin D deficiency is a risk factor for the development of disorders of reproductive and menstrual function and the physiological level of vitamin D contributes to the restoration of physiological menstrual function. As WHO experts already emphasized (2010), the status of vitamin D is very important in the prevention of various deviations in the functioning of organ systems, which indicates the advisability of prescribing vitamin D preparations differentially, taking into account the genotype. The effectiveness of the proposed measures is confirmed by a decrease of 27.3% among female students with menstrual irregularities.

CONCLUSION

Studies have shown the presence of vitamin D deficiency in female students with menstrual irregularities. Moreover, vitamin D deficiency is associated with a genetic predisposition to reduce the transport of vitamin D in the body and decrease the function of converting vitamin D into an active ligand to the vitamin D receptor. According to the results of the study, it was concluded that prescribing a vitamin D drug will probably have a beneficial effect on the reproductive function of female students with menstrual irregularities and with a genetic predisposition to reduce the transport of vitamin D in the body and decrease the function of converting vitamin D into an active ligand for the vitamin D receptor.

CONFLICT OF INTEREST

There is no conflict of interest.

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