NSJC «WEST KAZAKHSTAN MARAT OSPANOV MEDICAL UNIVERSITY»

ANNOTATION
of the dissertation
aimed at obtaining the degree of doctor of philosophy (PHD)

Effect of vitamin D on cortisol levels in adolescent girls with primary dysmenorrhea

Educational program 8D10102 - "Medicine"

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RELEVANCE

Primary dysmenorrhoea (PD) is a significant medical and social problem that significantly reduces the quality of life of adolescent girls. Despite numerous studies, the pathogenesis of this disease is not fully understood. In recent years, there has been increasing interest in the role of vitamin D in various physiological processes, including the regulation of inflammation and pain sensitivity. There is also growing interest in studying its effects on the reproductive system.

Primary dysmenorrhoea is often accompanied by increased stress levels, which is reflected in increased cortisol concentrations. Cortisol, as a stress hormone, plays an important role in the pathogenesis of many diseases, including chronic pain. Studying the effect of vitamin D on the course of primary dysmenorrhoea and cortisol levels will allow us to evaluate its potential role in the prevention and treatment of this disease.

According to the literature data of domestic and foreign colleagues, the prevalence of primary dysmenorrhoea among adolescent girls ranges from 8% to 90%. And the severe course of primary dysmenorrhoea, which leads to reduced social activity and loss of work capacity, accounts for 15% of cases, making this pathology a serious medical and social problem that needs to be addressed.

Currently, a large number of published scientific studies related to various treatment strategies and tactics indicate the importance of vitamin D levels in the development of dysmenorrhoea.

Vitamin D is known for its multiple effects as its receptors are present in various tissues of the body, including the female reproductive organs.

Our domestic scientists considered PD from the position of treatment and correction, while scientists from foreign countries investigated possible causes of PD in adolescent girls for further prevention of reproductive health disorders.

Uncompensated deficiency of some substances causes not only functional but also metabolic disorders.Therefore, as a result of negative effects on the development of the growing organism, such as stress, malnutrition and deficiency of basic vitamins and minerals, have a great impact on the reproductive health of girls.

Most of the research results of scientists from far and near abroad have shown that 70-80% of female patients with PD have inadequate 25(OH) vitamin D levels. The use of vitamin D preparation in different dosages in patients with PD increases the content of 25(OH) vitamin D in the blood, reduces the intensity and duration of pain, thereby improving the course of primary dysmenorrhoea.

Since PD is accompanied by different degrees of pain sensations, it causes not only physical but also emotional stress in adolescent girls and young women. In many scientific works of foreign authors, in order to study pain syndrome and various kinds of stress, the level of cortisol in different biomaterials (blood, saliva, hair and urine) was determined as a biomarker of stress.

Saliva cortisol testing is becoming increasingly popular as a stress assessment method because it is noninvasive, readily available, and can be sampled multiple

times throughout the day, providing a more complete picture of the circadian rhythm of cortisol.

In a study, scientists from Indonesia found that the average cortisol levels in the group with chronic primary dysmenorrhoea were higher compared to the group without dysmenorrhoea, and the cortisol levels in the two groups were within the normal range.

Studies have shown that vitamin D deficiency may be associated with elevated cortisol levels. This may be because vitamin D is essential for the normal functioning of the hypothalamus and pituitary gland, which control the production of cortisol. An excess of which can lead to increased sensitivity to pain, which can exacerbate PD symptoms.

Vitamin D can affect the expression of genes associated with cortisol production. Studies have shown that vitamin D can reduce the expression of genes encoding enzymes involved in cortisol synthesis, and vitamin D can also reduce the activity of cortisol receptors, resulting in reduced sensitivity of cells to cortisol. The results of other studies have shown that vitamin D can affect the metabolism of cortisol, accelerating its excretion from the body, thus leading to a decrease in its levels.

Despite the large number of studies on the role of vitamin D in various physiological processes, data on its effect on the course of primary dysmenorrhoea and cortisol levels in adolescent girls remain limited, especially in our country.

The idea of the study is fundamentally different: firstly, there has been no treatment with vitamin D among adolescents with PD in Kazakhstan; secondly, studies around the world show contradictory findings on the effect of vitamin D on cortisol levels and the course of primary dysmenorrhoea.

The idea of the study has a fundamental difference: firstly, in Kazakhstan, there was no vitamin D treatment among adolescents with PD; secondly, studies around the world have shown conflicting conclusions about the effect of vitamin D on cortisol levels and the course of primary dysmenorrhea. Previously obtained results of the university project on the topic "Assessment of the state of metabolism and bone mineral density in adolescent girls with primary dysmenorrhea in the Kazakh population" 2021-2023, indicate that among adolescent girls with primary dysmenorrhea, vitamin D deficiency and insufficiency amounted to 58% and 32%. These data served as a prerequisite for the development of the idea of this study.

Research purpose:

To evaluate the effect of prophylactic vitamin D supplementation on cortisol levels in adolescent girls with primary dysmenorrhoea before and after intervention.

Research objectives:

1. To compare vitamin D content in adolescent girls with primary dysmenorrhoea before and after intervention.
2.To investigate the daily rhythm of salivary cortisol in adolescent girls with primary dysmenorrhoea before and after intervention.

3.To study the relationship of prophylactic vitamin D supplementation with daily cortisol levels and pain intensity in adolescent girls with primary dysmenorrhoea.

Scientific novelty:
1. For the first time in Kazakhstan, a prophylactic dose of vitamin D was used to reduce pain intensity in primary dysmenorrhoea in adolescent girls.
2. The levels of 25(OH) vitamin D in blood serum and cortisol in saliva in adolescent girls with primary dysmenorrhoea in Aktobe region were estimated for the first time.
3. Correlations between vitamin D levels with pain intensity by VAS and daily cortisol rhythm among adolescent girls with primary dysmenorrhoea were found.

Theoretical and practical significance:
1. The obtained data on the effect of vitamin D on the course of primary dysmenorrhoea and pain intensity in adolescent girls will undoubtedly become additional useful information of timely preclinical examination for doctors of all spheres of health care as a method of selecting preventive measures to reduce pain intensity in PD.
2. The obtained results on the level of vitamin D will make it possible to make changes and additions to the protocol of treatment, early diagnosis and prevention of pain intensity in primary dysmenorrhoea. From the obtained data when taking prophylactic doses of vitamin D may become an important component of complex treatment, prevention and reduction of pain intensity in primary dysmenorrhoea in adolescent girls.

Provisions put forward for defence
1. Prophylactic vitamin D supplementation in adolescent girls with PD have a positive effect on the daily rhythm of cortisol in saliva.
2. The use of vitamin D in prophylactic doses (4000ME) helps to reduce the stress hormone (cortisol), also reducing the intensity of pain, which ultimately leads to an improvement in the course of PD in adolescent girls.

Approbation of work.
The main provisions of the dissertation work are stated at the extended meetings of the Academic Council and scientific-problem commission of the West Kazakhstan Marat Ospanov Medical University.

The results of the conducted study are presented at:
1. International Scientific and Practical Conference "MODERN MEDICINE: a NEW APPROACH and RELEVANT RESEARCH" among the medical educational organizations of Kazakhstan, FSU and beyond, confined to the World Osteoporosis Day (WOD) conducted within the framework of STP AR09563004 "Features of metabolism and the state of bone mineral density in adolescent girls with primary dysmenorrhea" Medicina (Kaunas) 2021;57(Supplement 2):17 (Kazakhstan, Aktobe, 20 October 20, 2021). Topic: «Vitamin D status in adolescent girls with primary dysmenorrhea», oral report;

2. LXII International Scientific Conference of Young Scientists "Science: Yesterday, Today, Tomorrow" dedicated to the 65th anniversary of the student scientific society of West Kazakhstan Marat Ospanov Medical University, Republic of Kazakhstan, Aktobe city, 27 April 2023 Aktobe, Kazakhstan. Topic: "The effectiveness of vitamin D in primary dysmenorrhoea in adolescent girls", oral report;
3. Conference “PHYSIOLOGY IN FOCUS 2023”, Organised by the Federation of European Physiological Societies (FEPS), 14-16 September 2023 г., Tallinn, Estonia. Topic: «Vitamin D and primary dysmenorrhea: RCTs», poster abstract;
4. IX Congress of Physiologists of Kazakhstan and Central Asia with international participation, dedicated to the 60th anniversary of NSJC "Medical University of Astana" 20-21 June 2024 on the basis of NSJC "Astana Medical University", Astana, Kazakhstan. Topic: «Evaluating the effectiveness of vitamin D in managing PMS symptoms in adolescent girls with primary dysmenorrhea», oral report.

Publications on the topic of dissertation.
On the subject of the thesis 14 scientific printed works have been published, including 1 article - in the edition indexed in the information base Web of Science and Scopus - "European Review for Medical and Pharmacological Sciences" (78th percentile in 2023); 2 articles - in scientific publications recommended by the Committee for Quality Assurance in Science of the Republic of Kazakhstan; 3 theses - in collections of international conferences, 1 - poster abstract, 1 - patent for utility model of RK, 1 - author's certificate, 5 - acts of introduction of scientific research results.

Personal contribution
The author recruited adolescent girls into the study, obtained written informed consent from parents (or guardians) and adolescent girls for participation and laboratory tests, referred them for laboratory tests, monitored the participants before and after the intervention, created an electronic database, and performed statistical processing and analysis of the results of the study. The author wrote the chapters of the dissertation, prepared publications and reports.

OBJECT AND METHODS OF RESEARCH:

The main research work was carried out by the Department of Normal Physiology as a fragment of the scientific and technical project "Effect of vitamin D on neuroendocrine regulation of menstrual cycle in adolescent girls with primary dysmenorrhea" within the grant programme 2022-2023. This research work was carried out at the level of West Kazakhstan Marat Ospanov Medical University, the Department of Normal Physiology and JSC "KDC of the Regional Perinatal Centre", as well as KDL "OLYMP".

The research work is approved by the protocol № 9 of the local ethical committee of West Kazakhstan Marat Ospanov Medical University from 19.11.2021.

The clinical part of this scientific study was conducted at the Regional Perinatal Center of JSC “Consultative and Diagnostic Center”, and the laboratory analysis was carried out on the basis of KDL "OLYMP", located at Gaziza Zhubanova Street 3m and Aliya Moldagulova Avenue 57v of Aktobe city.

Study design: double-blind randomised placebo-controlled trial.

Object of study: adolescent girls with primary dysmenorrhoea aged 13-16 years.

At the age of 13-16 years that the onset and the greatest severity of symptoms of primary dysmenorrhoea are most often noted. This is due to the physiological changes occurring in the girl's body during puberty. The average age of menarche is 11-14 years.

Inclusion criteria:

- Adolescent girls 13 to 16 years of age;
- Regular menstrual cycle (within 21-35 days) and menarche within 1 year;
- First identified sign of primary dysmenorrhoea;
- Girls who described pain on a visual analogue scale (VAS) 3≤ и 9> score;

Exclusion criteria:

- Girls with diseases and anomalies of the pelvic organs;
- After surgical treatment of the pelvic cavity;
- A history of neurological and psychiatric abnormalities;
- Adolescent girls on hormonal medication;

The online calculators "Epi InfoTM" and on the Raosoft.com website were used to calculate the sample size. The following indicators were used to calculate the sample size: confidence interval - 95%, power of the study - 80%; margin of error was - 5%; confidence level - 95%, calculations using the formula determined the recommended sample size - 66 participants in each group, total number n=132+20% =158 for this study.

Lectures and awareness-raising activities were conducted in 18 schools of Aktobe city, and lists of adolescent girls with PD were obtained.

To ensure the objectivity of the study, adolescent girls were randomly assigned to groups. An independent expert, recruited at an early stage of the study, used specialised software to generate a sequence of unique random numbers corresponding to the total number of participants. Then, using a random allocation algorithm, the participants were divided into a study group (n=96) receiving vitamin D (4000 IU, tablets, manufacturer Poland) and a control group (n=95) receiving a placebo (pacifier tablet - a type of drug that does not differ from the study drug in colour, appearance, taste and smell and has no effect on the body) daily for three months. The drugs were packaged and labelled by an independent expert so that neither the study participants nor the researchers knew which drug each group was receiving. Only at the end of the study did the researchers have access to a list of participant numbers and their corresponding dosage forms. This approach minimised systematic error, maximised the objectivity of the study results and ensured that the groups were comparable in terms of key characteristics.

Currently, there is no worldwide agreement on the optimal dose of vitamin D supplements. This dosage has been chosen taking into account the current

recommendations in various countries, where it is accepted that the acceptable preventive dose is up to 10,000 IU; a dose of 4,000 IU is a safe dose with an upper limit of daily intake for all and also for children from birth.

Placebo is a preparation that does not contain an active pharmacological substance and is externally and organoleptically identical to the medicinal product involved in the clinical trial. The placebo tablets used in the study were manufactured by TK Pharm Aktobe LLP, Kazakhstan, which has a licence for pharmaceutical activity No. 64566579DD dated 26.03.2019. The production of products is carried out in accordance with the requirements of the Good Manufacturing Practice Standard of the Republic of Kazakhstan (GMP RK). To confirm the compliance of placebo tablets with the quality requirements, appropriate tests were conducted (test report No. 25 dated 11 June 2022).

Of the 191 participants, 167 adolescent girls completed the study. Attrition was mainly due to illness (n=9), change of residence (n=7) and irregular use of the drug (n=8). Despite the attrition, the analyses showed that the statistical power of the study remained sufficient to detect significant differences between the groups.

Then the study was conducted according to the standard protocol, in the consultative-diagnostic centre, at the appointment of child and adolescent gynaecologist of the Regional Perinatal Centre of Aktobe city. At the appointment, complaints were collected, complete anamnesis, measurement of anthropometric indicators (weight, height, BMI), assessment of pain intensity using visual analogue scale (VAS).

To assess pain, one of the most common tools is the visual analogue scale (VAS), Visual Analog Scale (VAS). This common method is a numerical rating scale where the patient selects a number between 0 and 10 to reflect the intensity of pain. When analysing the severity of pain, the following gradation of pain severity was adopted: 0 points to consider no pain, 1-2 points weak pain, 3-4 points moderate pain, 5-6 points moderately severe pain, 7-8 severe pain, 9-10 points intolerable pain. Adolescent girls with no pain and with the highest degree of pain that required urgent medical attention were not included in the study.

In addition, all adolescent girls with PD underwent pelvic ultrasound (USG) to rule out organic changes in the pelvic organs. Transabdominal ultrasound is a safe and painless way to examine the pelvic organs of adolescent girls. The procedure has no contraindications and was performed through the anterior abdominal wall of the abdomen.

At the beginning of the study, all adolescent girls and their parents were thoroughly informed about the aims of the study and the procedures during the study. Written informed consent was obtained from each participant, emphasising voluntary consent to participate or the right to refuse participation at any stage of the study.

Laboratory tests were performed in the KDL "OLYMP" for the determination of 25(OH) vitamin D in serum and daily cortisol in saliva. The analyses were taken at baseline and three months after the intervention. Vitamin D levels $\geq$30 ng/ml were considered normal, between 20-30 ng/ml insuficiency and <20 ng/ml deficiency, respectively.

Determination of the content of 25(OH) vitamin D in the blood serum was carried out by chemiluminescent immunoassay, which is performed on the automatic immunological analyzer "Cobas E411" manufactured by "Roshe Diagnostics", Switzerland. Vitamin 25(OH) D - sampling is done from venous blood in a red vacutainer with a red cap up to 3 ml of blood.

Non-invasive collection of the test material (saliva collection is possible in outpatient settings) is easy to perform among adolescents, as it allows avoiding stressful situations associated with visiting a hospital and taking blood from a vein.

Determination of the level of cortisol in saliva (free salivary cortisol) was carried out by electrochemiluminescent immunoassay, which is performed on the automatic immunological analyzer "Cobas E411" manufactured by "Roshe Diagnostics", Switzerland. Mixed oral saliva samples were collected using a dedicated Salivette Cortisol system (Cat. No. 51.1534.500) with a blue cap. To analyze the circadian rhythm of cortisol, study participants collected saliva four times a day at the following time intervals: morning (8:00-10:00), afternoon (12:00-14:00), evening (18:00-20:00), and night (22:00-00:00).

Methods of statistical analysis

The collection, systematization of primary information and the formation of a database were carried out in MS Excel 2021. Statistical processing and graphical presentation of the research results were carried out using SPSS 26 (IBM SPSS Statistics, USA) and GraphPad SoftWare, LLC Prism 9 Version 9.5.1 (733) 2023 programs.

The first stage of statistical processing was to check the obtained data for normal distribution using the Kolmogorov-Smirnov criterion, the Shapiro-Wilk's W test depending on the size of the sample under study, and graphically by constructing distribution histograms.

Next, descriptive statistics methods were applied. In the case of normal data distribution, the arithmetic mean (M), standard error (m) and standard deviation (SD) were calculated; in the case of data deviation from the normal distribution, parameters such as the median (Me), interquartile range (25th – 75th quartiles, IQR) were also calculated.

To test the hypothesis about differences in means for data subject to normal distribution, Student's t-test was used for unpaired samples; in case of deviation from normal distribution, its nonparametric analogue, Mann–Whitney U-test, was calculated. The obtained values of Student's t-test were assessed by comparison with critical values. Differences in indicators were considered statistically significant at a significance level of $p \leq 0.05$. The calculated values of Mann–Whitney U-test were compared with critical values at a given significance level: if the calculated U-value was equal to or less than critical, the statistical significance of the differences was recognized. When comparing quantitative indicators whose distribution differed from normal in two related groups, the Wilcoxon test was used.

Comparison of percentages in the analysis of four-field contingency tables was performed using the Pearson chi-square test (for values of the expected

phenomenon greater than 10). McNemar's test was used to analyze paired data obtained from the main and control groups before and after the intervention.

Nominal data were described with absolute values and percentages (n (%)). Comparison of nominal data was carried out by constructing contingency tables and calculating the Pearson chi-square ($\chi 2$) criterion, which allows assessing the significance of differences in the studied groups. The calculated value of the Pearson $\chi 2$ criterion was compared with the critical value, and if it was greater than the critical value, a conclusion was formulated about the presence of a statistical relationship between the studied phenomena at the appropriate level of significance.

To identify correlation relationships between variables and their statistical significance, the correlation analysis method was used to calculate the Spearman rank correlation coefficient (r) and its significance level (p). The values of the correlation coefficient r were interpreted in accordance with the Chaddock scale: less than 0.1 - no relationship, 0.1-0.3 - weak, 0.3-0.5 - moderate, 0.5-0.7 - noticeable, 0.7-0.9 - high and more than 0.9 - very high. Differences between indicators at an error probability level of $p \leq 0.05$ were considered statistically significant. At a value of $p < 0.01$, the significance of differences was assessed as very high, and at a value of $p \leq 0.001$ - equal to 99.9%.

To construct a mathematical prognostic model, the decision tree method was used based on the CHAID (Chi-square automatic interaction detection) principle to assess the effect of vitamin D intake on other variables.

Binary logistic regression was performed to identify the dependence of the probability of developing primary dysmenorrhea on the content of vitamin D and the daily rhythm of cortisol. The sensitivity and specificity of the prognostic models were also determined. All data are expressed as frequency percentages with CI and median [Q1; Q3], where $p<0.05$ was considered statistically significant.

RESEARCH RESULTS

A total of 167 adolescent girls with PD were analyzed. In the study and control groups, the median age of adolescent girls was 14 [13; 15] and 14 [13; 15] years, height 163 [158; 165] and 160 [157; 165] cm, weight 50 [46; 55] and 51 [47; 55] kg, BMI 19.3 [17.3; 20.9] and 19.7 [17.8; 20.8] kg/m2, and pain intensity according to VAS 6 [4; 8] and 6 [4; 8] points.

Comparative analysis of baseline data on the content of 25(OH) vitamin D in the blood serum in the main and control groups before the intervention was 11.5 [9.2; 15.9] ng/ml and 13.9 [10.1; 19.9] ng/ml ($p= 0.163$). According to the level of cortisol in saliva in the study and control groups before the intervention, no differences were found between the groups. The level of morning cortisol was 14.8 [8.8; 20.4] nmol/l and 14.5 [9.8; 20.9] nmol/l ($p=0.818$), daytime cortisol 5.6 [4.1; 8.5] nmol/l and 5.5 [3.8; 8.2] nmol/l ($p=0.712$), evening cortisol 3.8 [2.1; 5.3] nmol/l and 3.4 [2.0; 5.5] nmol/l ($p=0.639$), night cortisol 1.5 [1.5; 3.5] nmol/l and 1.5 [1.5; 2.5] nmol/l ($p=0.437$).

The obtained data indicate that before the intervention, the indicators of the two groups were comparable and had no significant differences.

Frequency analysis of vitamin D levels among adolescent girls with PD before and after the intervention.

Before the start of the study, according to the results of vitamin D content, insufficiency was detected in 17.4% (95% CI: 11.1-22.4%) of adolescent girls, while deficiency was shown in 82.6% (95% CI: 76.9-88.4%).

Before the start of the study, according to the results of vitamin D content in the study and control groups, the number of adolescent girls with vitamin D insufficiency was 11.5% (95% CI: 4.8 - 18.2%) and 22.5% (95% CI: 13.3 - 31.7%), deficiency 88.5% (95% CI: 81.8 - 95.2%) and 77.5% (95% CI: 68.3 - 86.7%), respectively.

After three months of taking vitamin D in the study group, the number of adolescent girls with normal levels of 25(OH) vitamin D was 46% (95% CI: 35.5 - 56.4%), insufficiency 43.7% (95% CI: 33.3 - 54.1%) and deficiency 10.3% (95% CI: 3.9 - 16.7%), in the control group 13.8% (95% CI: 6.2 - 21.3%) and 86.2% (95% CI: 78.7 - 93.8%), respectively.

Comparative analysis of vitamin D content in two groups before and after the intervention.

Comparative analysis of vitamin D content by median before and after the intervention in each group showed significant differences and significantly increased in the main group from 11.5 [9.2; 15.9] to 28.6 [23.5; 36.9] ng/ml (p=0.000), while in the control group it decreased from 13.7 [10.1; 19.8] to 11.6 [8.8; 16.5] ng/ml (p=0.000). The dynamics of 25(OH) vitamin D content demonstrates significant differences between the groups. Thus, the level of 25(OH)D in the main group increased by 2.4 times compared to the control group.

Comparative analysis of the daily cortisol rhythm in the study and control groups before and after the intervention.

In a comparative analysis of the daily cortisol rhythm before and after taking vitamin D, a decrease in cortisol levels during the day was observed in the study group. In the study group, a decrease in morning cortisol from 14.8 [8.8; 20.4] to 12.8 [7.5; 18.1] nmol / l (p = 0.134), evening cortisol from 3.8 [2.1; 5.3] to 3.1 [1.9; 4.6] nmol / l (p = 0.178) and a slight increase in daytime cortisol from 5.6 [4.1; 8.5] to 6.0 [3.9; 9.6] nmol / l (p = 0.365) and nighttime cortisol from 1.5 [1.5; 3.5] to 2.0 [1.5; 3.6] nmol/l (p=0.437).

Comparative analysis of daily cortisol in adolescent girls with primary dysmenorrhea in the control group showed a significant decrease in morning cortisol after three months of taking placebo: from 14.5 [9.8; 20.9] nmol/l to 10.1 [6.7; 16.1] nmol/l (p=0.001). At the same time, daytime, evening and nighttime cortisol values did not change significantly, maintaining their initial levels. Daytime cortisol before the intervention was 5.5 [3.8; 8.2] nmol/l after 5.5 [3.1; 8.1] nmol/l (p=0.874), evening cortisol up to 3.4 [2.0; 5.5] nmol/l after 3.3 [2.0; 5.6] nmol/l (p=0.914) and night cortisol up to 1.5 [1.5; 2.5] nmol/l after 1.7 [1.5] (p=0.227).

Comparative analysis of the daily cortisol rhythm did not reveal statistically significant differences between the groups. Morning cortisol after the intervention

in the main group was 12.8 [7.5; 18.1] nmol/l and in the control group 10.1 [6.7; 16.1] nmol/l (p=0.907), daytime cortisol 6.0 [3.9; 9.6] nmol/l and 5.5 [3.1; 8.1] nmol/l (p=0.570), evening cortisol 3.1 [1.9; 4.6] nmol/l and 3.3 [2.0; 5.6] nmol/l (p=0.649), night cortisol 2.0 [1.5; 3.6] nmol/l and 1.7 [1.5; 3.0] nmol/l (p=0.679), respectively. The data obtained indicate that in both the main and control groups, the daily rhythm of cortisol corresponded to the daily rhythm, which indicates the preservation of the hypothalamic-pituitary-adrenal system

Comparative analysis of pain intensity according to VAS in two groups before and after the intervention.

After three months of taking vitamin D and placebo, a tendency towards a decrease in pain intensity according to VAS was observed in both groups. However, the most pronounced decrease in pain intensity was found in the main group taking vitamin D, it decreased from 6 [4; 8] to 3 [2; 3] points, which showed a significant improvement in the course of primary dysmenorrhea in adolescent girls (p=0.000). Whereas in the control group, a slight decrease in pain intensity was observed from 6 [4; 8] to 5 [4; 6] points ($p \geq 0.05$).

Correlation analysis was conducted between vitamin D levels, pain intensity, and the daily rhythm of cortisol among adolescent girls.

The correlation analysis revealed a negative moderate relationship between vitamin D levels and pain intensity according to VAS r=-0.3 (p=0.041) in the study group after the intervention, which indicates that a decrease in vitamin D levels is accompanied by an increase in the severity of pain during PD. And in the control group, no correlation was found between the above parameters.

The correlation analysis in the study group revealed a moderate positive relationship between the pain intensity according to VAS and morning cortisol r=0.34 (p=0.002). Similarly, the level of night cortisol showed moderate positive significant relationship with the pain intensity r=0.38 with p=0.000, which confirms the dynamics of changes in the cortisol level throughout the day depending on the pain intensity according to VAS. Noticeable positive correlation was also found between the pain intensity and evening cortisol r=0.51 (p=0.0001). The results of the study demonstrate that an increase in the intensity of pain sensations assessed by VAS is accompanied by an increase in the cortisol level, which indicates an increase in the stress response.

When assessing the effect of vitamin D on the cortisol level before and after the intervention in the study group, a significant moderate negative correlation was found between the vitamin D content with morning (r=-0.4, p=0.001) and daytime (r=-0.3, p=0.04) cortisol in adolescent girls with PD. The higher the vitamin D level, the lower the cortisol level in the morning and afternoon. Correlation analysis did not reveal a statistically significant relationship between the vitamin D content in the blood serum and evening and nighttime cortisol levels in the saliva in adolescent girls with PD. In the control group, compared with the main group, the data obtained did not confirm the presence of significant correlations between the daily cortisol level and the vitamin D content.

A regression analysis was performed to study the relationship between taking prophylactic doses of vitamin D and pain intensity and the daily rhythm of cortisol in adolescent girls with PD.

Based on the values of the regression coefficients, the factors vitamin D, morning and evening cortisol have a direct relationship with the likelihood of improving the course of PD. Regression analysis showed that taking vitamin D reduces the odds of PD and the effect on pain intensity by 2 times (95% CI: 1.29-2.98) p = 0.002, a decrease in morning cortisol reduces the odds of PD and pain intensity by 3.4 times (95% CI: 1.15-9.79) p = 0.027, a decrease in evening cortisol reduces the odds of PD and pain by 4.1 times (95% CI: 1.12-14.63) p = 0.033.

The resulting regression model is statistically significant (p=0.0001). Based on the value of the Nagelkerk determination coefficient, model (1) determines 17.7% of the variance in the probability of improving the course of PD, which is manifested by a decrease in pain intensity.

The obtained results indicate the presence of various mechanisms of interaction between vitamin D, cortisol and pain intensity in primary dysmenorrhea. This in turn may be due to the fact that normalization of vitamin D levels in adolescent girls with primary dysmenorrhea has a positive effect on metabolic processes in the body and the production of dopamine, which affect the decrease in cortisol, leading to a decrease in pain intensity.

Based on the results obtained, the following conclusions were made.

CONCLUSIONS

1. Among all examined adolescent girls with primary dysmenorrhoea before the intervention, vitamin D deficiency was 82.7% and insufficiency was 16.7%. After the intervention, a significant difference(p= 0.0001) was found between the study and control groups in terms of vitamin D content: after prophylactic vitamin D supplementation was 28.6 [23.5; 36.9] ng/ml and after placebo supplementation was 11.7 [8.8; 17.1] ng/ml, respectively.

2. In the examined adolescent girls with primary dysmenorrhea before and after the intervention the daily cortisol rhythm was maintained and there were no significant differences between the groups. However, the study group tended to have lower morning and evening cortisol compared to baseline, which in turn was manifested by a reduction in pain intensity during the day.

3. A moderate negative correlation was found between vitamin D content with morning cortisol r=-0.4 (p=0.001) and with pain intensity r=-0.3 (p=0.041). Also, a significant positive noticeable correlation was found between pain intensity (VAS) with evening cortisol r=0.51 (p=0.0001). Vitamin D supplementation reduced the odds of PD and effect on pain intensity by 2-fold (95% CI: 1.29-2.98) p=0.002, while reduction in morning and evening cortisol respectively reduced the odds by 3.4-fold (95% CI: 1.15-9.79) p=0.027 and 4.1-fold (95% CI: 1.12-14.63) p=0.033.

Further in-depth studies are needed to differentiate the vitamin D-cortisol relationship in more detail.