NSJC «WEST KAZAKHSTAN MARAT OSPANOV MEDICAL UNIVERSITY»

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

The effect of prophylactic vitamin D supplementation on cortisol levels in adolescent girls with primary dysmenorrhea.

Educational program 8D10102 - "Medicine"

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Relevance

Primary dysmenorrhea (PD) is one of the most common gynecological problems in adolescence and, according to various epidemiological studies, occurs in 60–94% of girls and young women. PD is characterized not only by a pronounced pain syndrome but also by a significant reduction in quality of life, academic performance, and social activity, as well as by the development of anxiety and depressive disorders.

Current views on the pathogenesis of PD extend beyond the purely prostaglandin theory and increasingly consider it a complex neuroendocrine syndrome involving central pain mechanisms, chronic stress, and dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis. In this context, particular attention is drawn to cortisol - the key stress hormone, whose alterations in secretion and circadian rhythm are associated with increased pain sensitivity, neuroinflammation, and dysregulation of the menstrual cycle, especially during puberty.

In recent years, vitamin D has been considered an important modifiable factor in the pathogenesis of primary dysmenorrhea. A substantial body of evidence, including randomized controlled trials and meta-analyses, indicates that correction of vitamin D deficiency—typically using therapeutic or high doses—leads to a reduction in menstrual pain intensity, decreased need for nonsteroidal anti-inflammatory drugs, and improvement in patients' psychoemotional status. Most of these studies focus on the clinical effects of vitamin D on pain syndrome and the course of PD, and the dosages used generally exceed prophylactic levels.

At the same time, the influence of vitamin D on the neuroendocrine mechanisms of PD, particularly on cortisol levels and the functional state of the hypothalamic–pituitary–adrenal (HPA) axis, remains insufficiently investigated. Available data are fragmentary, often derived from heterogeneous samples of adult women or individuals with depressive disorders or metabolic disturbances, and only rarely from adolescent girls with primary dysmenorrhea. Studies specifically evaluating the effects of prophylactic doses of vitamin D, which are the safest and most applicable in adolescent practice are especially limited. There is a lack of systematic research assessing the relationship between correction of vitamin D deficiency and changes in cortisol secretion in adolescents with PD.

Thus, there is an evident gap between clinical data demonstrating the beneficial effects of vitamin D on the symptoms of primary dysmenorrhea and the limited understanding of its role in the regulation of cortisol and stress-associated neuroendocrine mechanisms in adolescents. In a context where the protection of adolescent reproductive health has been identified as a priority of the State Program for Healthcare Development of the Republic of Kazakhstan for 2020–2025, addressing challenges such as in-depth investigation of pathogenesis, early detection, and the development of new approaches to the correction of PD is of particular relevance.

Such research contributes to addressing key objectives of modern medicine, including a deeper understanding of the neuroendocrine mechanisms underlying the disease, substantiation of new preventive approaches, and the development of personalized, pathogenetically grounded strategies for patient management.

Research purpose:
To assess the effect of vitamin D on cortisol levels in adolescent girls with primary dysmenorrhea before and after administration of prophylactic doses of vitamin D and placebo.
Research objectives:
1. To investigate the circadian rhythm of salivary cortisol in adolescent girls with primary dysmenorrhea before and after administration of prophylactic doses of vitamin D and placebo.
2. To compare vitamin D levels in adolescent girls with primary dysmenorrhea before and after administration of prophylactic doses of vitamin D and placebo.
3. To examine the association between prophylactic vitamin D intake, daily cortisol levels, and pain intensity in adolescent girls with primary dysmenorrhea.
Scientific novelty:
1. For the first time in Kazakhstan, a double-blind randomized placebo-controlled trial using a prophylactic dose of vitamin D was conducted among adolescent girls aged 13–16 years with primary dysmenorrhea.
2. For the first time in adolescent girls with primary dysmenorrhea, the diurnal salivary cortisol rhythm (assessed four times per day) was evaluated before and after three months of prophylactic vitamin D and placebo administration.
3. For the first time, the effect of prophylactic vitamin D intake on the diurnal cortisol rhythm, as well as its relationship with pain intensity, was investigated in the examined girls.
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.
Key statements for defense
1. Prophylactic vitamin D supplementation in adolescent girls with primary dysmenorrhea does not disrupt the physiological diurnal pattern of cortisol secretion, but is associated with a favorable trend toward lower morning and evening cortisol levels, which corresponds to a reduction in pain intensity throughout the day.
2. A three-month course of prophylactic vitamin D at a dose of 4,000 IU results in a significant increase in serum vitamin D levels in adolescent girls with primary dysmenorrhea. The reduction in pain intensity observed with vitamin D supplementation is related to its modulatory effects on the hypothalamic-pituitary-adrenal axis, as evidenced by correlations between vitamin D status, cortisol levels, and pain intensity in primary dysmenorrhea.

3. Regression analysis demonstrates that vitamin D status serves as an independent protective factor against pain in primary dysmenorrhea, whereas elevated morning and evening cortisol levels represent significant risk factors for greater pain severity.

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.

A total of 8 scientific publications have been produced on the topic of the dissertation, including 2 articles in international journals indexed in the Web of Science and Scopus databases – «Bangladesh Journal of Medical Science» (56th percentile in 2025) and «Endocrine and Metabolic Science» (33rd percentile in 2025); 3 articles in scientific journals recommended by the Committee for Quality Assurance in Science and Higher Education of the Republic of Kazakhstan— Reproductive Medicine and Astana Medical Journal; and 3 conference abstracts published in the proceedings of international conferences.

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:

This research study was conducted at the Department of Normal Physiology of Marat Ospanov West Kazakhstan Medical University, as well as at the JSC "Consultative and Diagnostic Center of the Regional Perinatal Center" and the "OLIMP" Clinical Diagnostic Laboratory. The study constitutes a component of the research and technical project entitled "The Effect of Vitamin D on the Neuroendocrine Regulation of the Menstrual Cycle in Adolescent Girls with Primary Dysmenorrhea," carried out within the framework of the university's intramural research funding program in 2022–2023.

The study protocol was approved by the Local Ethics Committee of Marat Ospanov West Kazakhstan Medical University (Protocol No. 9 dated November 19, 2021).

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

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

The onset and greatest severity of primary dysmenorrhea symptoms are most commonly observed between the ages of 13 and 16 years. This is associated with the physiological changes occurring in the female body during puberty. The mean age at menarche ranges from 11 to 14 years.

Inclusion criteria:

- adolescent girls aged 13–16 years;
- regular menstrual cycle (24–38 days) with menarche established for at least 1 year;
- newly diagnosed primary dysmenorrhea;
- participants reporting menstrual pain rated ≥3 and <9 points on the Visual Analog Scale (VAS).

Exclusion criteria:

- girls with pelvic diseases or congenital anomalies of the pelvic organs;
- history of pelvic surgery;
- documented neurological or psychiatric disorders in the medical history;
- adolescent girls using hormonal medications.

Sample size estimation was performed using the online calculators Epi Info™ and Raosoft. The following parameters were applied: a 95% confidence level, 80% statistical power, and a 5% margin of error. Based on formula-based calculations, the recommended sample size was 66 participants per group. Allowing for a 20% attrition rate, the final target sample size was set at n=132+20%=158 for the study.

Educational sessions and informational meetings were conducted in 18 schools in Aktobe, and lists of adolescent girls with primary dysmenorrhea were compiled.

To ensure objectivity, participants were allocated to study groups using a randomization procedure. An independent expert involved at the early stage of the study generated a sequence of unique random numbers using specialized software corresponding to the total number of participants. Using a random allocation algorithm, the adolescents were assigned either to the vitamin D group (n = 96),

receiving vitamin D_3 (4000 IU tablets, manufactured in Poland), or to the placebo group (n = 95), receiving a placebo tablet identical in color, appearance, taste, and smell and without physiological activity, administered daily for three months. The study medications were packaged and labeled by the independent expert to ensure that neither participants nor investigators were aware of group assignments. The allocation code was disclosed only after study completion. This approach minimized systematic bias, enhanced objectivity, and ensured baseline comparability between groups.

At present, there is no universal global consensus regarding the optimal dose of vitamin D supplementation. The selected dosage was based on current international recommendations, according to which preventive doses of up to 10,000 IU are considered acceptable. A daily dose of 4,000 IU is recognized as a safe upper intake level for the general population, including children.

A placebo is a preparation that does not contain an active pharmacological substance and is indistinguishable from the investigational product in appearance and organoleptic properties. The placebo tablets used in this study were manufactured by TK Pharm Aktobe LLP (Kazakhstan), a company licensed for pharmaceutical activities (License No. 64566579DD dated March 26, 2019). Production was carried out in compliance with the Good Manufacturing Practice standards of the Republic of Kazakhstan (GMP RK). Product quality compliance was confirmed through appropriate testing (Test Report No. 25 dated June 11, 2022).

Of the 191 participants initially enrolled, 168 adolescent girls completed follow-up. The main reasons for attrition were intercurrent illnesses (n = 9), relocation (n = 7), and irregular intake of the study medication (n = 7). Ultimately, 87 participants remained in the intervention group and 81 in the control group. Despite the reduction in sample size, the post hoc analysis indicated that the statistical power remained sufficient to detect significant between-group differences.

Subsequently, the study was conducted according to a standard protocol at the consultative and diagnostic center during appointments with a pediatric and adolescent gynecologist at the Regional Perinatal Center in Aktobe. The clinical evaluation included collection of complaints, detailed medical history, anthropometric measurements (body weight, height, and BMI), and assessment of pain intensity using the Visual Analog Scale (VAS).

The VAS is one of the most widely used tools for pain assessment. It is a numeric rating scale on which patients select a value from 0 to 10 corresponding to pain intensity. Pain severity was categorized as follows: 0 — no pain; 1–2 — mild pain; 3–4 — moderate pain; 5–6 — moderately severe pain; 7–8 — severe pain; 9–10 — unbearable pain. Adolescents reporting no pain or extreme pain requiring urgent medical care were not included in the study.

All adolescents with primary dysmenorrhea underwent pelvic ultrasonography to exclude organic pelvic pathology. Transabdominal ultrasound is a safe and noninvasive method for evaluating pelvic organs in adolescents. The procedure has no contraindications and was performed through the anterior abdominal wall.

At the beginning of the study, all participants and their parents received detailed information about the study objectives and procedures. Written informed consent was obtained from each participant and their legal guardians, emphasizing voluntary participation and the right to withdraw at any stage of the study.

Laboratory analyses were performed at the OLYMP Clinical Diagnostic Laboratory to determine salivary diurnal cortisol and serum 25-hydroxyvitamin D [25(OH)D] concentrations. Sampling was conducted twice: at baseline and after three months following the intervention.

Noninvasive sample collection (saliva sampling feasible in outpatient settings) is well suited for adolescents, as it minimizes stress associated with hospital visits and venipuncture.

Salivary cortisol (free cortisol) was measured using an electrochemiluminescence immunoassay on the automated immunoassay analyzer Cobas e411 (Roche Diagnostics, Switzerland). Mixed oral fluid samples were collected using the Salivette® Cortisol system (Cat. No. 51.1534.500) with a blue cap. To assess the circadian rhythm of cortisol, saliva was collected four times over 24 hours: in the morning (08:00–10:00), afternoon (12:00–14:00), evening (18:00–20:00), and night (22:00–00:00). Cortisol concentrations were determined in each of the four samples corresponding to these time intervals.

Serum 25(OH)D levels were measured by chemiluminescent immunoassay using the Cobas e411 analyzer (Roche Diagnostics, Switzerland). Venous blood samples (up to 3 mL) were collected into red-top vacutainer tubes. Vitamin D status was classified as follows: ≥30 ng/mL — sufficient; 20–29 ng/mL — insufficient; <20 ng/mL — deficient.

Statistical analysis

Data collection, primary data systematization, and database construction were performed using MS Excel 2021. Statistical analyses and graphical presentation of results were conducted using SPSS 26 (IBM SPSS Statistics, USA) and GraphPad Software Prism 9 (Version 9.5.1, 2023).

The first stage of statistical processing involved testing for normality using the Kolmogorov–Smirnov test and the Shapiro–Wilk W test, depending on sample size, as well as graphical assessment through histograms. Descriptive statistics were then applied. For normally distributed data, the mean (M), standard error of the mean (SEM), and standard deviation (SD) were calculated. For non-normally distributed data, the median (Me) and interquartile range (IQR, 25th–75th percentiles) were reported.

For hypothesis testing, the independent samples Student's t-test was used for normally distributed variables, whereas the Mann–Whitney U test served as the nonparametric alternative. Statistical significance for the t-test was determined by comparison with critical values, with $p \leq 0.05$ considered significant. For the Mann–Whitney test, significance was established when the calculated U value was equal to or less than the critical value. The Wilcoxon signed-rank test was applied for comparisons of paired non-normally distributed data.

Comparisons of proportions in 2×2 contingency tables were performed using Pearson's chi-square (χ^2) test when expected frequencies exceeded 10. McNemar's

test was applied to paired categorical data obtained from the intervention and control groups before and after treatment. Nominal variables were presented as absolute values and percentages (n (%)). Associations between categorical variables were evaluated using contingency tables and Pearson's χ^2 test.

Correlation analysis was performed using Spearman's rank correlation coefficient (r) with corresponding p values. The strength of correlations was interpreted according to the Chaddock scale: <0.1 — negligible; 0.1–0.3 — weak; 0.3–0.5 — moderate; 0.5–0.7 — noticeable; 0.7–0.9 — high; >0.9 — very high. Statistical significance was set at $p \leq 0.05$; $p < 0.01$ indicated high significance, and $p \leq 0.001$ indicated very high significance.

To assess the effect of prophylactic vitamin D supplementation on the diurnal cortisol rhythm in adolescents with primary dysmenorrhea, linear regression analysis was performed. Serum 25(OH)D concentration after supplementation was used as the independent variable, while salivary cortisol levels (morning, afternoon, evening, and night) were analyzed as dependent variables. Linear regression enabled estimation of the direction and strength of associations, and regression coefficients with significance levels were calculated for each model. Analyses were conducted both for individual time points and for overall circadian patterns of cortisol secretion in relation to 25(OH)D levels.

Binary logistic regression was used to identify independent predictors of pain persistence and to explore potential cause–effect relationships. This approach supported the development of a prognostic model for the risk of persistent pain after vitamin D supplementation. Model construction proceeded in stages. Initially, univariate analysis evaluated each variable separately to identify predictors significantly associated with pain. Variables included vitamin D level, morning, afternoon, evening, and night cortisol levels, as well as age, BMI, menstrual duration, and physical activity. Subsequently, variables demonstrating significant associations were entered into a multivariable model, allowing estimation of each predictor's contribution while adjusting for confounders. Variables without statistical significance (daytime and nighttime cortisol, age, BMI, menstrual duration, and physical activity) were excluded from the final model.

Odds ratios (ORs) with 95% confidence intervals (95% CI) were calculated for each retained predictor. The diagnostic performance of the model was evaluated using receiver operating characteristic (ROC) curve analysis. The optimal cutoff point was determined using the Youden index, enabling calculation of sensitivity and specificity.

Data were presented as percentages with confidence intervals and as medians with interquartile ranges [Q1; Q3]. A p value < 0.05 was considered statistically significant.

RESEARCH RESULTS

A total of 168 adolescent girls with primary dysmenorrhea were included in the analysis. To exclude the influence of baseline differences on study outcomes, a comparative analysis of the groups was performed with respect to age, anthropometric parameters, pain intensity on the VAS, and laboratory measures in blood and saliva prior to intervention.

In the intervention and control groups, the median age was 14 [13; 15] years; height, 163 [158; 165] cm and 160 [157; 165] cm; weight, 50 [46; 55] kg and 51 [47; 55] kg; body mass index (BMI), 19.3 [17.3; 20.9] kg/m² and 19.7 [17.8; 20.8] kg/m²; and pain intensity on the VAS, 6 [4; 8] points, respectively.

Comparison of baseline diurnal cortisol rhythm, assessed from four saliva samples throughout the day, revealed no significant differences between the groups prior to administration of prophylactic vitamin D or placebo. Morning cortisol levels were 14.8 [8.8; 20.4] nmol/L versus 14.5 [9.8; 20.9] nmol/L ($p = 0.818$); afternoon cortisol, 5.6 [4.1; 8.5] nmol/L versus 5.5 [3.8; 8.2] nmol/L ($p = 0.712$); evening cortisol, 3.8 [2.1; 5.3] nmol/L versus 3.4 [2.0; 5.5] nmol/L ($p = 0.639$); and night cortisol, 1.5 [1.5; 3.5] nmol/L versus 1.5 [1.5; 2.5] nmol/L ($p = 0.437$). No statistically significant differences were observed in serum 25(OH)D levels: 12.5 [9.2; 15.9] ng/mL in the intervention group and 13.9 [10.1; 19.9] ng/mL in the control group ($p = 0.163$).

These results indicate that, prior to administration of prophylactic vitamin D or placebo, baseline measures in both groups were comparable and showed no statistically significant differences.

Comparative analysis of the diurnal cortisol rhythm in the intervention and control groups before and after administration of prophylactic vitamin D and placebo

In the intervention group, comparative analysis of the diurnal cortisol rhythm before and after vitamin D supplementation showed a trend toward decreased cortisol levels throughout the day. Specifically, morning cortisol decreased from 14.8 [8.8; 20.4] to 12.8 [7.5; 18.1] nmol/L ($p = 0.134$), and evening cortisol decreased from 3.8 [2.1; 5.3] to 3.1 [1.9; 4.6] nmol/L ($p = 0.178$). Afternoon cortisol showed a slight increase from 5.6 [4.1; 8.5] to 6.0 [3.9; 9.6] nmol/L ($p = 0.365$), and night cortisol slightly increased from 1.5 [1.5; 3.5] to 2.0 [1.5; 3.6] nmol/L ($p = 0.437$).

In the control group, analysis of diurnal cortisol in adolescents with primary dysmenorrhea revealed a significant decrease in morning cortisol after three months of placebo administration, from 14.5 [9.8; 20.9] to 10.1 [6.7; 16.1] nmol/L ($p = 0.001$). Afternoon, evening, and night cortisol levels did not change significantly: afternoon cortisol remained 5.5 [3.8; 8.2] vs. 5.5 [3.1; 8.1] nmol/L ($p = 0.874$), evening cortisol 3.4 [2.0; 5.5] vs. 3.3 [2.0; 5.6] nmol/L ($p = 0.914$), and night cortisol 1.5 [1.5; 2.5] vs. 1.7 [1.5; 3.0] nmol/L ($p = 0.227$).

Comparative analysis of the diurnal cortisol rhythm after administration of prophylactic vitamin D and placebo showed statistically significant differences between groups only for morning cortisol. Morning cortisol in the vitamin D group was 12.8 [7.5; 18.1] nmol/L, compared to 10.1 [6.7; 16.1] nmol/L in the placebo

group, with the difference reaching statistical significance (p = 0.003). No significant between-group differences were observed for afternoon (6.0 [3.9; 9.6] vs. 5.5 [3.1; 8.1] nmol/L; p = 0.570), evening (3.1 [1.9; 4.6] vs. 3.3 [2.0; 5.6] nmol/L; p = 0.649), or night cortisol (2.0 [1.5; 3.6] vs. 1.7 [1.5; 3.0] nmol/L; p = 0.679). Overall, there was a trend toward a reduction in cortisol levels during the day.

Comparative analysis of serum vitamin D levels in the intervention and control groups before and after administration of prophylactic vitamin D and placebo

Comparative analysis of serum 25-hydroxyvitamin D [25(OH)D] levels in adolescent girls with primary dysmenorrhea before and after administration of prophylactic vitamin D or placebo revealed significant between-group differences. In the intervention group, 25(OH)D increased from 12.5 [9.2; 15.9] ng/mL to 28.6 [23.5; 36.9] ng/mL (p = 0.0001), whereas in the control group, levels decreased from 13.9 [10.1; 19.9] ng/mL to 11.7 [8.8; 17.1] ng/mL (p = 0.001). Thus, after three months of intervention, 25(OH)D levels in the intervention group were 2.5 times higher than in the control group, confirming the efficacy of prophylactic vitamin D supplementation in correcting deficiency.

Comparative analysis of pain intensity in the intervention and control groups before and after administration of prophylactic vitamin D and placebo

After three months of prophylactic vitamin D or placebo administration, both groups demonstrated a trend toward reduced pain intensity as measured by the VAS. The most pronounced decrease was observed in the vitamin D group, with median pain scores declining from 6 [4; 8] to 3 [2; 3] points, indicating a statistically significant reduction in pain intensity among adolescents (p = 0.0001). In the placebo group, a slight decrease in VAS scores was observed, from 6 [4; 8] to 5 [4; 6] points; this 1-point reduction was not statistically significant ($p \geq 0.05$).

Correlation analysis between diurnal cortisol rhythm, pain intensity, and vitamin D levels in adolescents with primary dysmenorrhea

In the intervention group, a moderate positive correlation was observed between morning cortisol levels and pain intensity (r = 0.34, p = 0.002). Additionally, a noticeable positive correlation was found for evening cortisol (r = 0.51, p = 0.0001), and night cortisol showed a moderate positive association with pain intensity (r = 0.38, p = 0.0001). These findings indicate that changes in the diurnal rhythm of cortisol throughout the day correspond to the intensity of pain experienced by adolescents.

Assessment of the effect of vitamin D on cortisol levels after prophylactic supplementation revealed a moderate negative correlation between salivary cortisol and serum 25(OH)D concentrations in the intervention group. Higher vitamin D levels were associated with lower cortisol levels: morning cortisol (r = -0.40, p = 0.001) and afternoon cortisol (r = -0.25, p = 0.041). No significant correlations were observed between the diurnal cortisol rhythm and 25(OH)D levels in the control group.

Regression analysis of the diurnal cortisol rhythm depending on vitamin D levels in adolescent girls with primary dysmenorrhea after prophylactic supplementation

Linear regression analysis was conducted to evaluate the effect of prophylactic vitamin D on the diurnal cortisol rhythm, with salivary cortisol as the dependent variable and serum 25(OH)D as the independent variable. The analysis demonstrated a statistically significant moderate direct correlation ($r = 0.4$; $p < 0.001$). Specifically, an increase in serum 25(OH)D by 1 ng/mL was associated with a reduction in morning salivary cortisol by 0.37 nmol/L.

Prediction of pain probability in primary dysmenorrhea based on diurnal cortisol rhythm and vitamin D levels after prophylactic vitamin D supplementation

A predictive model was developed to estimate the probability of pain in adolescents with PD)based on morning, afternoon, evening, and night cortisol levels, serum vitamin D concentration, and additional factors including age, BMI, menstrual duration, and physical activity, using binary logistic regression after prophylactic vitamin D supplementation.

The resulting regression model was statistically significantly different from the null model (without predictors), confirming its adequacy in describing the data ($p < 0.001$). The pseudo-determination coefficient (Nagelkerke R^2) was 63.2%, indicating high predictive power.

After prophylactic vitamin D supplementation, each 1 ng/mL increase in serum vitamin D was associated with a 1.045-fold decrease in the odds of experiencing pain. Conversely, a 1 nmol/L increase in morning cortisol increased the odds of pain by 1.093-fold, and a 1 nmol/L increase in evening cortisol increased the odds by 1.225-fold.

Regression coefficients indicated that post-intervention morning and evening cortisol levels were positively associated with pain probability, whereas serum vitamin D concentration was inversely associated with pain risk. Other factors, including afternoon and night cortisol levels, age, BMI, and physical activity, did not have statistically significant effects on outcomes.

ROC analysis confirmed good predictive performance of the model, with an area under the curve (AUC) of 0.756 (95% CI: 0.684–0.828; $p < 0.001$). The optimal probability threshold, determined using the Youden index, was 66.5%. Logistic function values of $P \geq 66.5\%$ indicated high risk of pain, while $P < 66.5\%$ indicated low risk. At this threshold, the model demonstrated 62.0% sensitivity and 76.3% specificity, reflecting a balanced ability to identify true positive cases while minimizing false positives.

Thus, the developed model can be effectively applied for risk stratification and prognostic evaluation of pain severity in adolescent girls with primary dysmenorrhea.

CONCLUSIONS

1. In adolescent girls with primary dysmenorrhea, the diurnal rhythm of cortisol remained stable before and after prophylactic vitamin D supplementation, with no statistically significant differences between the intervention and control groups. However, in the intervention group, there was a trend toward decreased morning cortisol (from 14.8 [8.8; 20.4] to 12.8 [7.5; 18.1] nmol/L) and evening cortisol (from 3.8 [2.1; 5.3] to 3.1 [1.9; 4.6] nmol/L) compared to baseline, which was associated with reduced pain intensity throughout the day.

2. After administration of prophylactic vitamin D and placebo, a significant between-group difference in serum vitamin D levels was observed: 28.6 [23.5; 36.9] ng/mL in the intervention group versus 11.7 [8.8; 17.1] ng/mL in the control group ($p = 0.0001$).

3. Following prophylactic vitamin D supplementation, a significant positive correlation was found between evening cortisol and pain intensity ($r = 0.51$, $p = 0.0001$), along with a moderate negative correlation between morning cortisol and vitamin D levels ($r = -0.40$, $p = 0.001$). According to the regression model, each 1 ng/mL increase in serum vitamin D reduced the probability of pain in primary dysmenorrhea (AOR = 0.957; 95% CI: 0.929–0.986; $p = 0.004$), whereas increases in morning (AOR = 1.093; 95% CI: 1.033–1.157; $p = 0.002$) and evening cortisol (AOR = 1.225; 95% CI: 1.048–1.430; $p = 0.011$) were significant predictors of increased risk of pain.