Research Article

Vitamin D Status, Menstrual Pain Severity, and Functional Impairment Among Adolescent Girls in Pakistan: An Observational Study

Dr Aimen Tayyab[1], Anum Hamid[2], Alishba Sajjad[3], Amir Taj Khan[4], Rashad Mahmood Mirza[5], Asma Iqbal[6], Haroon Al Rasheed[7*]

[1]MBBS, FCPS, MRCOG 2 Gynae Obs, Consultant Gynae Obs, Haripur International Hospital, Dept Gynae Obs
[2]Lecturer (National Excellence Institute)
[3]Margalla Institute of Health Sciences, Rawalpindi
[4]Senior Register, Internal medicine Prime Medical and Dental College, Visting professor AHS KMU
[5]Affiliation / Institution: Department of Normal Physiology, Bukhara State Medical Institute named after Abu Ali ibn Sina, Bukhara, Uzbekistan. ORCID iD: 0009-0002-9781-0829
[6]Senior Registrar, Karachi Medical and Dental College
[7*]University of Balochistan.

aimentayyab.253@gmail.com[1] , anumhamid852@gmail.com[2] , alishbasajjad01@gmail.com[3] dramirtajkhan@yahoo.com[4] , rashadphysiology@gmail.com[5] , asmanadeemkhan@hotmail.com[6] rharoon47@gmail.com[7*]

Corresponding Author: Haroon Al Rasheed

Abstract

Background: Primary dysmenorrhea is common among Pakistani adolescent girls and disrupts school and daily life. Vitamin D modulates endometrial prostaglandin synthesis, but evidence linking vitamin D to dysmenorrhea, menstrual pain severity and functional impairment in Pakistani adolescents is lacking.

Objective: To assess dietary vitamin D intake, its determinants and its association with primary dysmenorrhea among adolescent girls in Pakistan, and to synthesise evidence on vitamin D status, menstrual pain severity and functional impairment.

Methods: Secondary analysis of open data from a population-based household survey of 405 post-menarcheal girls aged 10–19 years in rural Sindh. Energy-adjusted vitamin D intake was analysed with multivariable linear regression for its determinants, and with Firth logistic regression, restricted cubic splines and E-values for its association with dysmenorrhea. We also reviewed published evidence on vitamin D status, menstrual pain severity and functional impairment.

Results: Among 395 girls, 61.0% reported dysmenorrhea. Median vitamin D intake was 0.24 µg/day, and 99.7% were below the requirement of 10 µg/day. Intake was lower with low household dietary diversity (−55%), underweight (−31%) and no schooling. Girls with dysmenorrhea had lower intake (median 0.20 vs 0.31 µg/day; p = 0.025; crude OR per SD 0.78, 95% CI 0.63–0.96), but the association was attenuated after adjustment (OR 0.86, 95% CI 0.65–1.13), mainly by family history. An inverse association was observed among girls aged ≥15 years (OR 0.67, 95% CI 0.46–0.97; interaction p = 0.051). In the evidence synthesis, published serum data from rural Sindh showed that 81.1% of adolescent girls were vitamin D deficient (mean 25-hydroxyvitamin D 14.9 ng/mL), but no Pakistani study has linked vitamin D to pain severity or functional impairment, and no study anywhere has examined school absence.

Conclusion: Almost no rural Pakistani adolescent girl in this sample consumed enough vitamin D. Its association with dysmenorrhea is not independent of family history but may exist in older adolescents. Studies measuring serum 25-hydroxyvitamin D, pain severity and functional outcomes, and supplementation trials, are needed.

Keywords: Primary dysmenorrhea; Vitamin D status; Dietary vitamin D intake; Menstrual pain severity; Functional impairment; Adolescent girls; Pakistan; Secondary data analysis.

1. Introduction

Primary dysmenorrhea is cramping menstrual pain in the absence of pelvic pathology and is one of the most common health problems of adolescent girls [1]. A meta-analysis of more than 21,000 young women estimated a prevalence of about 71% and found that one in five missed school or university because of menstrual pain [2]. The burden is heavy in Pakistan as well. Among 484 female students in Sargodha, 20.9% reported severe and 48.9% moderate menstrual pain; 40.5% reported moderate problems with usual activities, 11.8% were unable to perform them, and 33.5% reported extreme pain or discomfort [3]. In a survey of 2,000 schoolgirls across four provinces, 19.3% missed two school days each month, pain was the most frequent reason for staying at home (54.5%), and 46% were unable to carry out daily activities during menstruation [4]. Menstruation-related absence has been described as a neglected threat to girls' education in Pakistan [5]. In rural Sindh, a population-based household survey found that 60.4% of post-menarcheal girls aged 10–19 years had primary dysmenorrhea [6].

The pathophysiology of primary dysmenorrhea involves excess endometrial prostaglandin production after progesterone withdrawal, causing uterine hypercontractility, ischaemia and pain, accompanied by raised

inflammatory markers [1,7]. Vitamin D may modify this process, since its receptor is expressed in the female reproductive tract, where it regulates inflammatory cytokine and prostaglandin synthesis [8]. Observational studies have reported lower serum 25-hydroxyvitamin D [25(OH)D] in young women and adolescents with dysmenorrhea and inverse correlations with pain intensity [9–11], although one study of university students found no association with pain score [12]. Randomised trials and supplementation studies have reported reduced pain and analgesic use after high-dose cholecalciferol [13–17], including a prospective study of 85 deficient adolescents in Kazakhstan, in which weekly 50,000 IU for five months raised serum 25(OH)D from 13.5 to 58.4 ng/mL and reduced mean visual analogue pain scores from 8.7 to 4.8 [8]. Two meta-analyses of randomised trials confirmed a reduction in pain intensity with supplementation [18,19].

Vitamin D deficiency is highly prevalent in Pakistan, particularly among children and adolescents [20]. In rural Sindh, serum 25(OH)D was measured in 3,437 girls aged 15–18.9 years enrolled in the Matiari emPowerment and Preconception Supplementation (MaPPS) Trial: mean concentration was 14.9 ± 7.9 ng/mL and 81.1% were deficient (<20 ng/mL) [29]. Diets in the same population were monotonous, with only 13.5% of 24-hour recalls meeting minimum dietary diversity and consumption of animal-source foods strongly patterned by household wealth [30]. Yet the relationship between vitamin D and dysmenorrhea has not been examined in Pakistani adolescents. The original analysis of the Sindh survey examined nutritional, lifestyle and menstrual hygiene factors and reported associations with physical activity, sleep, BMI, vitamin B6 and carbohydrate intake [6], but did not focus on vitamin D. Because the anonymised survey data are openly available, we were able to examine this question in a population-based sample of Pakistani adolescents.

This study aimed (i) to describe dietary vitamin D intake among adolescent girls in rural Pakistan and identify sociodemographic and nutritional determinants of low intake; (ii) to assess whether dietary vitamin D intake is associated with primary dysmenorrhea after accounting for known risk factors, including the shape of any dose–response relationship; (iii) to explore whether this association differs by age group or calcium intake; and (iv) to synthesise the evidence linking vitamin D status, menstrual pain severity and functional impairment among adolescent girls, with a focus on Pakistan.

2. Methods

2.1 Design and data source

This is a secondary analysis of the open-access dataset (S1 Data) published with Rehman et al. (2026) in PLOS Global Public Health under a Creative Commons Attribution licence [6]. The original study was a cross-sectional household survey of post-menarcheal girls aged 10–19 years in Tando Mohammad Khan district, Sindh, Pakistan, selected by multistage cluster random sampling [6]. Data collection, consent procedures and ethical approval are described in the original publication. This report follows the STROBE guideline for cross-sectional studies [23].

2.2 Participants

The dataset contains 405 participants. Ten participants had missing dietary data and were excluded, leaving 395 for analysis.

2.3 Variables

Outcome. Primary dysmenorrhea (yes/no) as recorded in the dataset, following the original study's definition [6].

Exposure. Daily dietary vitamin D intake (µg/day), as derived by the original investigators from dietary interviews [6]. Because nutrient intakes correlate with total energy intake, vitamin D intake was energy-adjusted by the residual method [22]: log-transformed vitamin D intake was regressed on log-transformed energy intake, and the residuals plus the predicted value at mean energy were used. Energy intake (kcal/day) was calculated as 4 × carbohydrate + 4 × protein + 9 × fat (g/day). Energy-adjusted intake was analysed in tertiles and as a continuous standardised variable (per one SD). Adequacy was assessed against the Institute of Medicine Estimated Average Requirement (EAR) of 10 µg/day for vitamin D and 1,100 mg/day for calcium in girls aged 9–18 years [21].

Covariates. Age group (<15 or ≥15 years), BMI category (underweight, normal, overweight/obese), physical activity (active/inactive), sleep pattern (adequate/inadequate), family history of dysmenorrhea, age at menarche (≤12 or >12 years), socioeconomic status (low, middle, high) and energy intake, as coded in the dataset. Covariates were selected a priori based on the original study's findings [6].

Determinants of intake. Candidate determinants of vitamin D intake were age group, the girl's education (none vs any), maternal and paternal education (none vs some), socioeconomic status, household food insecurity (food

secure or mild, moderate, or missing [n = 57], as coded in the dataset), household dietary diversity (high, ≥7 food groups, vs low or medium, ≤6 food groups), stunting (height-for-age), BMI category and toilet facilities (improved vs unimproved) as a marker of household living conditions.

2.4 Statistical analysis

Continuous variables are presented as median (IQR) and compared using the Mann–Whitney U or Kruskal–Wallis test; categorical variables are presented as n (%) and compared using the chi-square test. Characteristics were compared across vitamin D tertiles to identify potential confounders.

Crude associations were estimated with standard logistic regression. Because family history of dysmenorrhea separated the outcome strongly (89.3% vs 27.6% prevalence), adjusted models used Firth's penalised likelihood logistic regression, which reduces small-sample bias and gives finite estimates under near-separation, with profile penalised likelihood confidence intervals [24,25]. We estimated odds ratios (OR) with 95% confidence intervals for dysmenorrhea by vitamin D tertile (highest tertile as reference), for the lowest tertile versus the two higher tertiles combined, and per one-SD increase in energy-adjusted intake. Linear trend across tertiles was tested by entering tertile rank as a continuous term.

To identify covariates responsible for changes in the estimates, models were fitted adjusting for age and energy only, and for all covariates except family history. Effect modification by age group (<15 vs ≥15 years) and by calcium intake (below vs at or above the median, and continuous) was tested with likelihood ratio tests for product terms, and stratum-specific estimates are reported. Sensitivity analyses (i) additionally adjusted for calcium intake, (ii) used tertiles of intake without energy adjustment, and (iii) were restricted to girls aged 15 years and older. Two-sided $p < 0.05$ was considered significant, and interaction tests were interpreted as exploratory. Analyses were performed in Python (pandas, NumPy, SciPy, Matplotlib); the code is available on request. The public dataset does not contain sampling weights or cluster identifiers, so analyses are unweighted.

For the determinants analysis, log energy-adjusted vitamin D intake was regressed on all candidate determinants simultaneously by ordinary least squares. Coefficients were back-transformed and are reported as percentage differences in intake with 95% CIs. A constant of 0.01 µg was added before log transformation because some girls reported no intake. The shape of the association between energy-adjusted intake and dysmenorrhea was modelled with restricted cubic splines with three knots at the 10th, 50th and 90th percentiles [28], adjusted for the covariates above, overall and separately by age group, with median intake as the reference; the overall association and non-linearity were tested with likelihood ratio tests. Robustness to unmeasured confounding was assessed with E-values [27], after converting odds ratios to approximate risk ratios by the square-root transformation because dysmenorrhea was common (>15%).

2.5 Evidence synthesis

To place the survey findings in context, we conducted a targeted (non-systematic) review of evidence on each link in the proposed pathway between vitamin D status, menstrual pain severity and functional impairment in adolescent girls and young women. In November 2026, we searched PubMed, Google Scholar and the wider web using combinations of terms for dysmenorrhea or menstrual pain with vitamin D, 25-hydroxyvitamin D or cholecalciferol, and terms for dysmenorrhea with quality of life, school absenteeism or daily activities in Pakistan. We also checked the reference lists of retrieved articles and of two recent meta-analyses [18,19].

We prioritised (i) studies from Pakistan; (ii) studies in adolescents; (iii) studies that measured serum 25(OH)D or pain severity on a numerical or visual analogue scale; and (iv) randomised trials and meta-analyses. For each study we extracted the country, population, sample size, measures and main findings. Findings are summarised narratively for each link in the pathway (vitamin D status, menstrual pain severity and functional impairment) and in an evidence map (Table 6) that shows the evidence from Pakistan, the evidence from other countries and the contribution of the present analysis. Because the review was not systematic, its purpose is to identify the available evidence and gaps rather than to estimate pooled effects, and relevant studies may have been missed.

2.6 Ethics

This analysis used only anonymised, publicly available data and did not involve contact with participants; additional ethical approval was therefore not required. Ethical approval for the original survey is reported in the original publication [6].

3. Results

3.1 Participant characteristics

Of 395 girls analysed, 271 (68.6%) were aged 15 years or older, 240 (60.8%) were underweight and 214 (54.2%) reported a family history of dysmenorrhea. Primary dysmenorrhea was reported by 241 girls (61.0%). Dysmenorrhea was more common among girls with a family history of dysmenorrhea, those who were physically inactive, those with inadequate sleep and those with menarche after age 12 (Table 1).

Table 1. Characteristics of participants by primary dysmenorrhea status (n = 395)

Characteristic	Category	n	Dysmenorrhea, n (%)	p
Age group	<15 years	124	68 (54.8)	0.112
	≥15 years	271	173 (63.8)	
BMI	Underweight	240	141 (58.8)	0.058
	Normal	138	93 (67.4)	
	Overweight/obese	17	7 (41.2)	
Physical activity	Active	190	100 (52.6)	0.001
	Inactive	205	141 (68.8)	
Sleep pattern	Adequate	292	168 (57.5)	0.023
	Inadequate	103	73 (70.9)	
Family history of dysmenorrhea	No	181	50 (27.6)	<0.001
	Yes	214	191 (89.3)	
Age at menarche	≤12 years	115	59 (51.3)	0.015
	>12 years	280	182 (65.0)	
Socioeconomic status	Low	134	73 (54.5)	0.129
	Middle	130	81 (62.3)	
	High	131	87 (66.4)	

3.2 Dietary vitamin D and calcium intake

Vitamin D intake was extremely low: median 0.24 µg/day (IQR 0.09–0.58; mean 0.56 ± 1.16). Only one girl (0.3%) reached the EAR of 10 µg/day, and 87.1% consumed less than 1 µg/day. Calcium intake was also low (median 276 mg/day, IQR 203–389), with 99.5% below the EAR. Girls with dysmenorrhea had lower vitamin D intake than those without, whereas calcium and energy intake did not differ (Table 2).

Table 2. Daily dietary intake by primary dysmenorrhea status, median (IQR)

Nutrient	Dysmenorrhea (n = 241)	No dysmenorrhea (n = 154)	p*
Vitamin D (µg/day)	0.20 (0.09–0.51)	0.31 (0.11–0.70)	0.025
Calcium (mg/day)	281 (197–404)	275 (212–381)	0.948
Energy (kcal/day)	1,404 (1,146–1,712)	1,332 (1,083–1,645)	0.177

*Mann–Whitney U test.

3.3 Characteristics by vitamin D intake tertile

Compared with the highest tertile, girls in the lowest tertile of energy-adjusted vitamin D intake more often had a family history of dysmenorrhea (62.9% vs 50.0%) and were more often physically inactive (55.3% vs 40.2%). Calcium and energy intake did not differ across tertiles (Table 3).

Table 3. Characteristics by tertile of energy-adjusted dietary vitamin D intake, n (%) or median (IQR)

Characteristic	Lowest (n = 132)	Middle (n = 131)	Highest (n = 132)	p
Vitamin D intake (µg/day)	0.06 (0.00–0.10)	0.24 (0.17–0.32)	0.88 (0.56–1.29)	<0.001
Calcium intake (mg/day)	289 (183–470)	288 (212–374)	266 (204–358)	0.142
Energy intake (kcal/day)	1,395 (1,146–1,725)	1,344 (1,126–1,633)	1,400 (1,122–1,744)	0.602
Age ≥15 years	95 (72.0)	83 (63.4)	93 (70.5)	0.276
Underweight	85 (64.4)	84 (64.1)	71 (53.8)	0.396†

Characteristic	Lowest (n = 132)	Middle (n = 131)	Highest (n = 132)	p
Physically inactive	73 (55.3)	79 (60.3)	53 (40.2)	0.003
Inadequate sleep	33 (25.0)	36 (27.5)	34 (25.8)	0.896
Family history of dysmenorrhea	83 (62.9)	65 (49.6)	66 (50.0)	0.049
Menarche >12 years	99 (75.0)	94 (71.8)	87 (65.9)	0.257
Low socioeconomic status	46 (34.8)	38 (29.0)	50 (37.9)	0.376†
Primary dysmenorrhea	91 (68.9)	74 (56.5)	76 (57.6)	0.072

†p for the full three-category variable.

3.4 Vitamin D intake and dysmenorrhea

The prevalence of dysmenorrhea was 68.9% in the lowest tertile, 56.5% in the middle and 57.6% in the highest tertile (Figure 1).

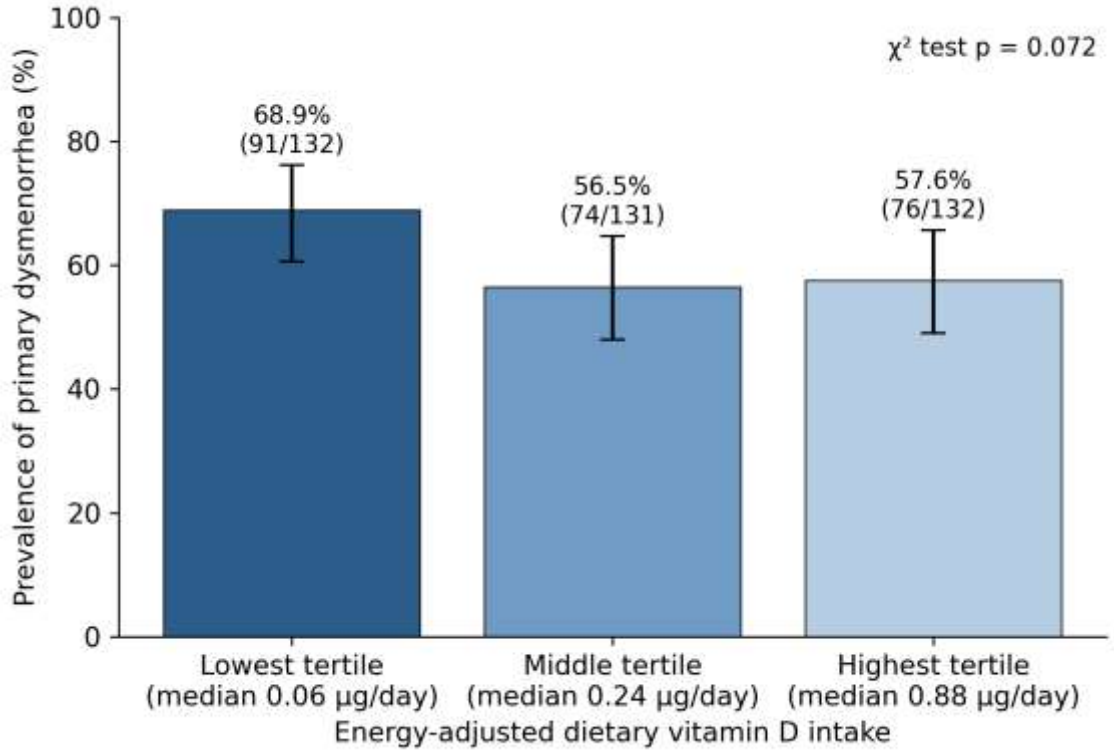


Figure 1. Prevalence of primary dysmenorrhea by tertile of energy-adjusted dietary vitamin D intake (n = 395). Error bars show 95% Wilson confidence intervals; tertile labels give median intake.

In crude analysis, higher energy-adjusted intake was associated with lower odds of dysmenorrhea (OR per SD 0.78, 95% CI 0.63–0.96; p = 0.022), and girls in the lowest tertile had higher odds than those in the two higher tertiles (OR 1.67, 95% CI 1.08–2.60; p = 0.023). After full adjustment, these associations were attenuated and no longer statistically significant (Table 4).

Table 4. Association between energy-adjusted dietary vitamin D intake and primary dysmenorrhea

Exposure	Crude OR (95% CI)	p	Adjusted OR (95% CI)*	p
Lowest tertile vs highest	1.64 (0.99–2.71)	0.056	1.32 (0.68–2.57)	0.411
Middle tertile vs highest	0.96 (0.59–1.56)	0.859	0.99 (0.52–1.91)	0.987
Trend per tertile	—	—	0.87 (0.62–1.22)	0.417
Lowest tertile vs higher two	1.67 (1.08–2.60)	0.023	1.33 (0.74–2.37)	0.341
Per 1-SD increase	0.78 (0.63–0.96)	0.022	0.86 (0.65–1.13)	0.283

*Firth penalised logistic regression with profile likelihood confidence intervals, adjusted for age group, BMI category, physical activity, sleep pattern, family history of dysmenorrhea, age at menarche, socioeconomic status and energy intake.

In the fully adjusted model (Supplementary Figure S1), family history of dysmenorrhea (OR 22.72, 95% CI 12.84–42.30) and inadequate sleep (OR 2.23, 95% CI 1.19–4.28) were associated with higher odds of dysmenorrhea, underweight with lower odds than normal BMI (OR 0.54, 95% CI 0.29–0.96), and energy intake with slightly higher odds (OR 1.06 per 100 kcal, 95% CI 1.00–1.13). Estimates from Firth regression were close to those from standard logistic regression (family history OR 26.4 with standard estimation), so the large estimate for family history is unlikely to be an artefact of sparse data.

3.5 Explaining the attenuation

Adjusting for age and energy alone left the lowest-tertile estimate almost unchanged (OR 1.63, 95% CI 0.98–2.70). Adjusting for all covariates except family history gave OR 1.53 (95% CI 0.90–2.60). Most of the attenuation was therefore due to family history, and a smaller part to physical activity.

3.6 Effect modification

The association between vitamin D intake and dysmenorrhea differed by age group with borderline significance (Supplementary Table S1). Among girls aged 15 years and older, each one-SD increase in energy-adjusted intake was associated with 33% lower odds of dysmenorrhea (OR 0.67, 95% CI 0.46–0.97), whereas no association was seen among younger girls (OR 1.18, 95% CI 0.71–1.96; interaction p = 0.051). Calcium intake did not modify the association (interaction p = 0.63 for continuous calcium and 0.77 for calcium above vs below the median).

Spline analysis supported these findings (Figure 2). Across all girls, the odds of dysmenorrhea declined gradually with increasing intake, but the overall association was not significant (p = 0.53). Among girls aged 15 years and older, the odds declined steadily across the intake range (overall p = 0.088), whereas no consistent pattern was seen among younger girls (p = 0.73). There was no evidence of non-linearity in any group (all p ≥ 0.62).

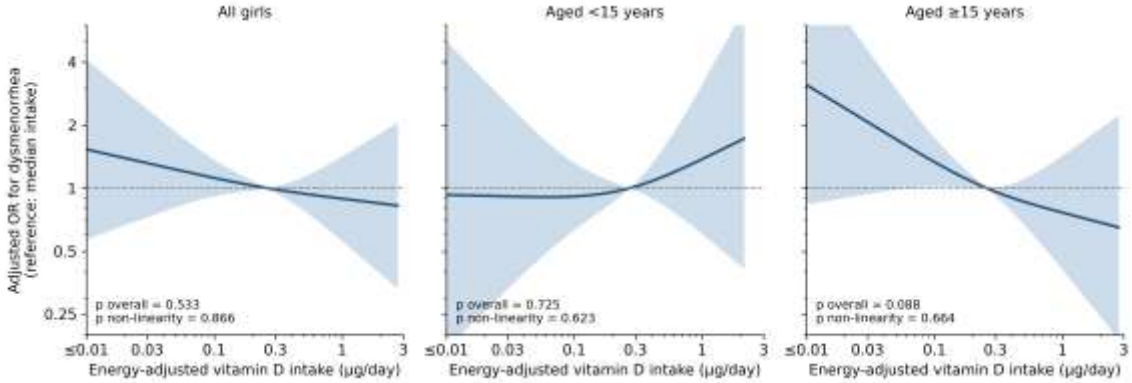


Figure 2. Adjusted odds ratios for primary dysmenorrhea across energy-adjusted dietary vitamin D intake, modelled with restricted cubic splines (three knots), overall and by age group. Shaded areas show 95% confidence intervals; the reference is median intake. Models are adjusted for the covariates in Table 4 (age group omitted in age-stratified models). Intake is shown on a log scale between the 2.5th and 97.5th percentiles.

3.7 Sensitivity analyses

Results were similar when calcium intake was added to the model (lowest vs highest tertile OR 1.44, 95% CI 0.72–2.87) and when tertiles of intake without energy adjustment were used (OR 1.42, 95% CI 0.71–2.84). Restricting the tertile model to girls aged 15 years and older (n = 271) gave a stronger estimate of borderline significance (OR 2.36, 95% CI 1.00–5.61; p = 0.051).

Unmeasured confounding. The E-value for the crude association per one-SD increase in intake was 1.52 (1.17 for the confidence limit), and for the lowest tertile versus the higher two tertiles 1.91 (1.24). For the association among girls aged 15 years and older, the E-value was 1.74 (1.14). An unmeasured confounder associated with both vitamin D intake and dysmenorrhea by a risk ratio of about 1.5–1.9 could therefore explain these associations. Family history of dysmenorrhea (approximate risk ratio 4.8) exceeded this threshold, consistent with the attenuation observed after adjusting for it.

3.8 Determinants of low vitamin D intake

Intake was low in every subgroup we examined (Table 5). In bivariate analysis, intake was lower among girls with no schooling (median 0.22 vs 0.31 µg/day; p = 0.006), girls whose mothers had no education (0.23 vs 0.30 µg/day; p = 0.040) and girls from households with low or medium dietary diversity (0.14 vs 0.25 µg/day; p = 0.046). In multivariable analysis, low or medium household dietary diversity (−55.0%, 95% CI −73.9 to −22.3) and underweight (−30.5%, 95% CI −48.7 to −5.8) were independently associated with lower energy-adjusted intake, and any schooling with higher intake (+44.2%, 95% CI +2.9 to +102.2). Socioeconomic status, food insecurity, parental education, stunting and sanitation were not independently associated. Together, these factors explained 7.3% of the variation in intake.

Table 5. Determinants of dietary vitamin D intake (n = 395)

Characteristic	Category	n	Median intake, µg/day (IQR)	In lowest tertile, %	Bivariate p	Adjusted % difference (95% CI)*	p
Age group	<15 years	124	0.24 (0.11–0.51)	29.8	0.725	Reference	
	≥15 years	271	0.23 (0.09–0.61)	35.1		−10.6 (−34.6 to 22.1)	0.481
Girl's education	None	262	0.22 (0.09–0.49)	35.9	0.006	Reference	
	Any	133	0.31 (0.13–0.79)	28.6		44.2 (2.9 to 102.2)	0.034
Mother's education	None	339	0.23 (0.09–0.53)	35.1	0.040	Reference	
	Some	56	0.30 (0.15–0.99)	23.2		29.1 (−18.7 to 105.1)	0.280
Father's education	None	221	0.23 (0.09–0.60)	33.9	0.759	Reference	
	Some	174	0.24 (0.10–0.56)	32.8		−12.0 (−35.9 to 20.8)	0.429
Socioeconomic status	Low	134	0.26 (0.09–0.65)	34.3	0.149	Reference	
	Middle	130	0.21 (0.09–0.47)	36.9		−24.8 (−47.7 to 8.1)	0.125
	High	131	0.28 (0.12–0.65)	29.0		−7.6 (−43.5 to 51.2)	0.753
Household food insecurity	Secure or mild	80	0.20 (0.09–0.49)	40.0	0.107	Reference	
	Moderate	258	0.24 (0.09–0.56)	33.7		39.8 (−7.2 to 110.5)	0.109
	Missing	57	0.33 (0.16–0.79)	22.8		44.3 (−11.7 to 135.8)	0.144
Household dietary diversity	High (≥7 groups)	367	0.25 (0.11–0.58)	31.9	0.046	Reference	
	Low/mediu	28	0.14 (0.00–0.63)	53.6		−55.0 (−73.9 to	0.004

Characteristic	Category	n	Median intake, µg/day (IQR)	In lowest tertile, %	Bivariate p	Adjusted % difference (95% CI)*	p
	m (≤6 groups)					−22.3)	
Stunting	No	302	0.23 (0.09–0.61)	34.8	0.780	Reference	
	Yes	93	0.28 (0.10–0.53)	29.0		8.9 (−22.0 to 52.1)	0.617
BMI	Normal	138	0.30 (0.11–0.66)	30.4	0.118	Reference	
	Underweight	240	0.22 (0.09–0.50)	35.4		−30.5 (−48.7 to −5.8)	0.019
	Overweight/obese	17	0.23 (0.12–0.63)	29.4		−8.8 (−55.7 to 87.6)	0.802
Toilet facilities	Improved	144	0.27 (0.11–0.62)	31.9	0.313	Reference	
	Unimproved	251	0.23 (0.09–0.56)	34.3		−7.3 (−35.4 to 32.9)	0.680

*Percentage difference in energy-adjusted intake from multivariable linear regression of log-transformed intake including all characteristics in the table (R^2 = 0.073). Bivariate p from Mann–Whitney U or Kruskal–Wallis tests of unadjusted intake.

4. Discussion

4.1 Principal findings

In this population-based sample of adolescent girls in rural Sindh, dietary vitamin D intake was extremely low, averaging about one-fortieth of the requirement, and almost no girl met the EAR. Intake was lowest among girls from households with low dietary diversity, underweight girls and girls who had never attended school. Girls with primary dysmenorrhea had lower vitamin D intake, but after adjustment, mainly for family history of dysmenorrhea, the overall association was weak and not significant. In exploratory analysis, an inverse, approximately linear association was seen among girls aged 15 years and older.

4.2 Comparison with previous studies

The direction of our crude findings agrees with observational studies that measured serum 25(OH)D and found lower vitamin D in girls and young women with dysmenorrhea and inverse correlations with pain severity [9–11], and with trials showing that vitamin D supplementation reduces menstrual pain [13–19]. In Turkish young women, those with dysmenorrhea had lower serum vitamin D and higher parathyroid hormone than controls, and pain scores correlated inversely with vitamin D (r = −0.713) [9]. In Kazakh adolescents, correlations between serum 25(OH)D and pain scores were very strong [8,11]. Our weaker, confounded association is consistent with the null finding for pain score among Iranian university students [12], and with the fact that we measured dietary intake rather than serum status.

Our finding that family history was the strongest correlate of dysmenorrhea matches findings among Pakistani university students, 58.1% of whom reported a family history [3], and the original analysis of this dataset [6]. The independent association of inadequate sleep also agrees with the original analysis [6].

4.3 Interpretation

We see three possible reasons for the attenuation. First, the crude association may be confounded: family history was more common in the lowest vitamin D tertile, which may reflect shared household diets, genetic factors or shared perceptions of menstrual pain within families. Physical inactivity, more common in the lowest tertile, may also reflect less time outdoors. Second, the very narrow, low range of intake limits the ability to detect an effect: even girls in the highest tertile consumed a median of under 1 µg/day, so the comparison is between very low and extremely low intake rather than between inadequate and adequate status. Third, dietary intake is a weak proxy for vitamin D status, which depends mainly on cutaneous synthesis from sunlight [26]. Measurement error of this kind usually biases associations towards the null.

The stronger association among older adolescents is biologically plausible. Primary dysmenorrhea usually develops once ovulatory cycles are established after menarche [1], so prostaglandin-mediated pain, the pathway that

vitamin D may influence [7,8], is more likely to be fully expressed in older girls. However, this finding came from an exploratory test with borderline significance and needs confirmation in a larger sample before it is acted on.

The determinants of low intake are consistent with the main dietary sources of vitamin D, which are animal-source and fortified foods [21], and with findings from the MaPPS Trial in rural Sindh, where household wealth was the strongest predictor of dietary diversity and consumption of flesh foods ranged from 10.9% in the poorest to 41.1% in the least poor wealth quintile [30]. Households with less diverse diets are less likely to include these foods, and underweight may reflect generally poor diet quality. In our data the socioeconomic index was not independently associated with intake, possibly because dietary diversity, which lies on the pathway between wealth and intake, was in the same model. The association with schooling may reflect nutrition knowledge or household resources. However, these factors explained only 7% of the variation in intake: intake was low almost everywhere, which argues for population-wide measures rather than narrow targeting.

4.4 Vitamin D status, menstrual pain severity and functional impairment among adolescent girls in Pakistan

Low vitamin D status may increase endometrial prostaglandin production [7,8] and so worsen menstrual pain, which in turn keeps girls out of school and away from their daily activities [2–4]. Our analysis tests only one step in this chain. Table 6 summarises the evidence for each step and where our study fits.

Vitamin D status. Serum data from rural Sindh show that most adolescent girls there are vitamin D deficient. In the MaPPS Trial in Matiari district, another rural district of Sindh, mean serum 25(OH)D among 3,437 girls aged 15–18.9 years was 14.9 ± 7.9 ng/mL, 81.1% were deficient (<20 ng/mL), and 91.0% had at least one of iron deficiency anaemia, vitamin A deficiency or vitamin D deficiency [29]. In a dietary subgroup of the same trial (n = 390), deficiency was again 81.1% (mean 15.1 ± 8.5 ng/mL), and flesh foods appeared in only 26.1% and eggs in 10.5% of 24-hour recalls [30]. These serum findings are consistent with our observation that dietary vitamin D intake was negligible: with almost no dietary supply, vitamin D status in these girls depends on skin synthesis from sunlight [26], which covering clothing and time indoors limit. Girls doing unskilled manual labour, commonly outdoors, had lower odds of deficiency than girls in all other occupations, and girls with secondary or higher education had higher odds of deficiency than those with no education (OR 1.67, 95% CI 1.17–2.38) [29], probably reflecting less time outdoors. In our data, by contrast, schooling went with higher dietary intake. For girls who attend school, a slightly better diet may therefore be offset by less time in the sun. The serum concentrations in Pakistani girls are similar to those of adolescents with dysmenorrhea in Kazakhstan (13.5–16.2 ng/mL) [8,11], in whom supplementation reduced menstrual pain.

Menstrual pain severity. Among Pakistani female students, 48.9% reported moderate and 20.9% severe menstrual pain on a numerical rating scale [3]. Studies that measured both serum 25(OH)D and pain severity found strong inverse correlations, for example r = −0.71 in Turkish young women [9] and r = −0.89 in Kazakh adolescents, in whom supplementation reduced mean pain scores from 8.7 to 4.8 [8]. In our data, dysmenorrhea was recorded only as present or absent, so we could not test whether vitamin D intake relates to pain severity. This is the main gap in our study.

Functional impairment. Dysmenorrhea interferes with the daily lives of many Pakistani girls. Among students in Sargodha, 40.5% reported moderate problems with usual activities and 11.8% were unable to perform them, while 26.2% reported extreme anxiety or depression [3]. Among 2,000 schoolgirls across four provinces, 19.3% missed two school days a month and 46% could not carry out daily activities during menstruation [4]. No study, in Pakistan or elsewhere, has examined whether vitamin D status or supplementation reduces school absence or functional impairment; trials have reported only analgesic use [13,16,17].

Table 6. Evidence on the pathway from vitamin D to functional impairment in adolescent girls

Link	Evidence from Pakistan	Evidence from other countries	Contribution of this study
Low vitamin D status or intake	Serum 25(OH)D 14.9 ng/mL and 81.1% deficient among 3,437 rural Sindh girls aged 15–18.9 years [29]; poor dietary diversity, patterned by wealth [30]; deficiency most prevalent in children and	Deficient serum levels in adolescents with dysmenorrhea [8,11]	Intake below requirement in 99.7%; lowest with low dietary diversity, underweight and no schooling

Link	Evidence from Pakistan	Evidence from other countries	Contribution of this study
	adolescents nationally [20]		
Vitamin D and presence of dysmenorrhea	None before this study	Lower serum 25(OH)D in cases than controls [9–11]	Lower intake in girls with dysmenorrhea; not independent of family history; possible association at age ≥15
Vitamin D and menstrual pain severity	None	Inverse correlations [8,9]; reduced pain with supplementation [13–19]	Not measurable (binary outcome)
Pain and functional impairment	Impaired usual activities, quality of life and school attendance [3–5]	Absence from school or university in one in five young women [2]	Not measurable
Vitamin D and functional impairment	None	Only analgesic use as an outcome in trials [13,16,17]	Not measurable

Improving vitamin D status could therefore reduce menstrual pain and its effects on daily life among Pakistani adolescent girls, but each step in this chain still has to be tested in this population.

4.5 Implications for Pakistan

The near-universal inadequacy of both vitamin D and calcium intake in this sample matters regardless of any effect on menstrual pain, because adolescence is when peak bone mass is built, and it is consistent with the high national prevalence of vitamin D deficiency [20]. Primary dysmenorrhea is a major cause of impaired daily functioning and school absence among Pakistani girls [3–5], so identifying modifiable factors is a priority. Given that supplementation reduced pain in deficient adolescents and young women elsewhere [8,13–19], trials of vitamin D in deficient Pakistani adolescent girls are justified, ideally with school absenteeism and functional outcomes measured alongside pain.

Even the girls with the most varied diets consumed well under 1 µg/day, and dietary advice by itself will not close a gap this large. Approaches that do not depend on individual dietary change are likely to be more effective [26]. Pakistan has expanded large-scale food fortification since 2016, including fortification of edible oil and ghee with vitamins A and D, and investigators in rural Sindh have recommended direct micronutrient supplementation for adolescent girls together with poverty alleviation [29,30]. Ensuring that fortified foods reach poor rural households, and extending supplementation programmes to unmarried adolescent girls, could improve vitamin D status in the population studied here.

4.6 Strengths and limitations

The main strengths are the population-based sample of rural adolescent girls, who are rarely studied, and the analysis itself: we adjusted intake for energy, used Firth regression to handle the strong separation by family history, examined the dose–response with splines, tested effect modification and calculated E-values. Because the data are openly available, others can reproduce every result. The study also has important limitations. The cross-sectional design cannot establish causality. Serum 25(OH)D was not measured, and dietary intake does not capture sunlight synthesis. Dysmenorrhea was recorded as a binary variable, so pain severity and functional impairment could not be analysed directly and were addressed only through the evidence synthesis. The public dataset lacks sampling weights and cluster identifiers, so estimates are unweighted and confidence intervals may be too narrow. Dietary intake was self-reported and subject to recall error. The age-group findings were exploratory, and multiple subgroup comparisons increase the chance of a false-positive result. Only 28 girls had low or medium dietary diversity, so that estimate is imprecise. The serum vitamin D data came from a separate cohort in a different rural district of Sindh and could not be linked to individual dysmenorrhea status. Finally, the evidence synthesis was targeted rather than systematic and may have missed relevant studies.

4.7 Future research

Future studies in Pakistani adolescents should measure serum 25(OH)D, sun exposure, pain severity on a validated scale and functional outcomes such as school days missed, with attention to age after menarche. These should be followed by randomised trials of vitamin D supplementation in deficient girls.

5. Conclusion

Among adolescent girls in rural Pakistan, dietary vitamin D intake was far below requirements in virtually all participants, and was lowest among girls from households with low dietary diversity, underweight girls and girls without schooling. Lower intake was associated with primary dysmenorrhea in unadjusted analysis, but not independently of family history and other risk factors, although an inverse association may exist among older adolescents. The nutritional deficit alone justifies measures to improve vitamin D status across this population. Studies measuring serum vitamin D, pain severity and functional impairment, and trials of supplementation, are needed to establish whether improving vitamin D status could also reduce the burden of dysmenorrhea in Pakistani girls.

Abbreviations

25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; CI, confidence interval; EAR, Estimated Average Requirement; IQR, interquartile range; OR, odds ratio; SD, standard deviation; SES, socioeconomic status; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology; VAS, visual analogue scale.

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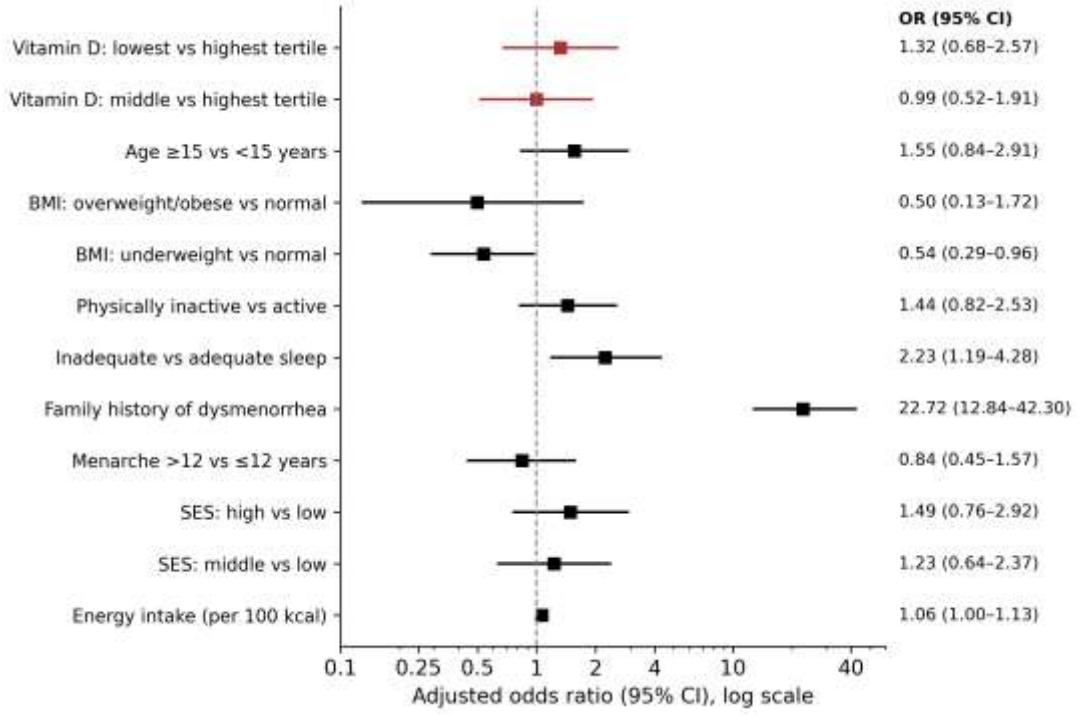
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Supplementary Material

Supplementary Table S1. Association of vitamin D intake with dysmenorrhea by age group and calcium intake (adjusted OR, 95% CI)‡

Subgroup	n	Dysmenorrhea, %	Per 1-SD increase in vitamin D	Lowest tertile vs higher two	p for interaction§
Age <15 years	124	54.8	1.18 (0.71–1.96)	0.91 (0.30–2.76)	0.051 / 0.191
Age ≥15 years	271	63.8	0.67 (0.46–0.97)	1.85 (0.88–3.92)	
Calcium below median	197	60.4	—	1.28 (0.51–3.23)	0.631 / 0.774
Calcium at or above median	198	61.6	—	1.61 (0.70–3.67)	

‡Standard logistic regression adjusted for the covariates in Table 4, excluding age group in age-stratified models. §Likelihood ratio test for the continuous / lowest-tertile exposure.



Supplementary Figure S1. Adjusted odds ratios (95% CI, log scale) for primary dysmenorrhea from Firth penalised logistic regression (n = 395). Vitamin D tertile estimates are shown in red, with the highest tertile as reference.