

Original Research Article

Serum calcium level among school-going adolescent girls in Bangladesh and their correlation with vitamin D level

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ABSTRACT

Background: Calcium and vitamin D are essential for bone health during adolescence, a period of rapid bone mass accrual. Adolescent girls in Bangladesh are at increased risk of deficiency due to dietary factors and limited sunlight exposure. This study evaluated serum calcium levels among urban and rural school-going adolescent girls and examined the association between serum calcium and vitamin D status.

Methods: This cross-sectional study was conducted at the Department of Biochemistry, Sylhet MAG Osmani Medical College, Sylhet, from July 2017 to December 2018 among 140 school-going adolescent girls aged 10–19 years (70 urban, 70 rural). Sociodemographic, anthropometric, dietary and lifestyle data were collected using structured questionnaires. Data were analyzed using SPSS version 26.

Results: Urban girls had significantly higher mean serum calcium levels than rural girls (9.12 ± 0.48 vs. 8.86 ± 0.52 mg/dl; $p=0.003$) but lower vitamin D levels (19.4 ± 7.2 vs. 22.8 ± 8.1 ng/ml; $p=0.010$). Low serum calcium was observed in 11% of urban and 24% of rural girls. Vitamin D deficiency was more prevalent among urban girls (55.7%) than rural girls (40.0%). Vitamin D status was significantly associated with serum calcium levels ($p=0.012$), with sufficient vitamin D linked to normal serum calcium. Rural girls had greater sunlight exposure (43.8 ± 18.7 vs. 21.6 ± 12.4 min/day; $p<0.001$).

Conclusions: Hypocalcemia and vitamin D deficiency are common among Bangladeshi adolescent girls, with significant urban–rural differences. Improved nutrition, dietary counselling and promotion of outdoor activities are needed.

Keywords: Adolescent girls, Serum calcium, Vitamin D

INTRODUCTION

years), characterized by accelerated growth, hormonal changes and accelerated bone mass acquisition, which necessitate sufficient nutrition during this period for lifelong bone health.¹ Calcium and vitamin D are essential nutrients at this time. Bone is the main mineral in which calcium is found and vitamin D increases the assimilation of calcium and controls bone mineralization. The deficiencies of either nutrient at the adolescent age can negatively affect the acquisition of peak bone mass and the development of fracture risk and osteoporosis later in

life.^{2,3} Calcium deficiency continues to be a major public health problem globally, especially in low- and middle-income countries where it can often be problematic due to inadequate dietary intake.⁴ Bangladesh is particularly at risk, as calcium consumption from national surveys was found to be significantly below the recommended 1,300 mg/day for adolescents.^{5,6} This is because the physiological needs of adolescent girls are high during growth and their intake of calcium-rich foods, like dairy products and eggs, is low.⁷ However, vitamin D deficiency is also very common in the South Asian region, where vitamin D is produced in abundance. There is a widespread

hypovitaminosis D due to factors like indoor lifestyle, cultural practices with clothes, air pollution and poor food intake.⁸ There has been substantial evidence in the past that Bangladeshi children and adolescents suffer a significant problem of vitamin D deficiency and those in the city have been shown to have worse vitamin D status, resulting from a lack of physical activity and sun exposure.^{9,10} Adolescent girls are a vulnerable group as they suffer losses due to menstruation, are also less active in the outdoor environment and are affected by social and dietary habits that can limit nutrient intake. Adolescent girls in South Asian countries have been found to have lower levels of calcium and vitamin D than boys, indicating a greater nutritional risk for them.^{11,12}

Vitamin D is well known to work in concert with calcium in the body. Vitamin D supports gastrointestinal calcium absorption via the active form 1,25-dihydroxy-vitamin D (calcitriol) and deficiency causes a significant decrease in gastrointestinal calcium absorption, which triggers compensatory hormonal reactions and decreases bone mineralization.^{2,3} Thus, low vitamin D status could lower serum calcium levels, especially in those who have already been at risk for inadequate calcium intake in their diets.¹³ This is compounded by urban-rural differences in nutritional status. While outdoor activity and exposure to sunlight may be advantageous for rural adolescents, urban adolescents may have more sedentary lifestyles, even though their socioeconomic status is relatively good. Thus, dietary, lifestyle behavioral and environmental differences could affect calcium and vitamin D status in various settings.^{7,14}

Although such micronutrients are important to health, information on serum calcium levels and their association with vitamin D in adolescent girls in Bangladesh is scarce, especially when compared to other urban and rural groups. Therefore, this study aimed to evaluate the serum calcium level among school-going adolescent girls, their vitamin D status and the correlation between serum calcium level and vitamin D status in urban and rural Bangladesh.

METHODS

This study was conducted at the Department of Biochemistry, Sylhet MAG Osmani Medical College, Sylhet, from July 2017 to December 2018. There were 140 participants for this study, comprising 70 girls from each of the urban and rural areas, using a purposive sampling method based on the inclusion and exclusion criteria. The study included girls aged 10-19 years, apparently healthy, enrolled in a formal school and with written informed consent from their parents/guardians. Exclusion criteria were: girls with any chronic disease (renal, hepatic or endocrine), girls already taking calcium and/or vitamin D supplements at the time of inclusion and pregnancy or lactation. The outcome measures were serum calcium level (mg/dl) and serum 25(OH)D concentration (ng/ml); other secondary measures included age, BMI, menarche status, family type, socioeconomic status, daily dietary patterns

(milk, egg, fish, green leafy vegetables), the use of calcium and vitamin D supplements, duration of sun exposure and outdoor activity habits. Blood samples were obtained from the fasting state by conventional venipuncture and serum was isolated for biochemical analysis. Arsenazo III colorimetric method was used to measure serum calcium and chemiluminescent immunoassay was used to measure serum 25(OH)D. Calcium levels <8.5 mg/dl were considered low and 8.5-10.5 mg/dl were considered normal. Vitamin D levels were classified as deficient (<20 ng/ml), insufficient (20-29 ng/ml) and sufficient (≥30 ng/ml) according to the endocrine society guidelines.

Anthropometric data such as height and weight were collected with standardized instruments and BMI was calculated and classified as underweight, normal, overweight and obese for the age and sex according to the WHO reference standards for adolescents. The data were entered and analyzed using SPSS version 26. Descriptive data (mean, standard deviation, frequency and percentage) were calculated. Chi-square tests were used to compare associations among categorical variables, such as vitamin D status and serum calcium level and an independent samples t-test to compare continuous variables between urban and rural groups. Statistically significant was defined as a p value of <0.05.

RESULTS

Table 1 shows the sociodemographic information of 140 adolescent girls in school in urban (n=70) and rural (n=70) areas. The 13–15 years age group is the largest in both settings (48.6% urban; 51.4% rural). Nuclear family structure was more prevalent in urban (68.6%) compared to rural (51.4%) girls. There was a significant difference in economic status, as the rural girls were mainly from low-income groups (52.9%) and the urban girls were mostly from middle-income families (61.4%).

The anthropometric and clinical characteristics of the subjects are described in Table 2. Urban girls had a slightly higher mean age (14.2 ± 2.1 years), height (151.6 ± 6.8 cm), weight (45.8 ± 7.4 kg) and BMI (19.9 ± 3.1 kg/m²) compared to rural girls. More girls in rural areas were underweight (31.4%) compared to urban girls (18.6%) and higher prevalence of overweight and obesity among urban girls. 84.3% and 78.6% of the urban and rural girls, respectively, had reached menarche, while 25.7% and 30% reported menstrual irregularity, respectively.

The dietary intake and lifestyle behaviours of the participants are summarized in Table 3. Daily milk consumption was more common among urban girls (34.3%) compared to rural girls (17.1%). At least three days per week, 54.3% of urban and 38.6% of rural girls had eggs and 58.6% of urban and 62.9% of rural girls had fish, respectively. The consumption of green leafy vegetables was higher among the rural girls (70%) than among the urban girls (51.4%). Rural girls were significantly more likely to be exposed to at least 30

minutes of sunlight per day (65.7%) than their urban counterparts (25.7%) and were more likely to participate in regular outdoor activity (68.6% rural vs. 30% urban).

The serum biochemical profiles of participants are given in Table 4. Urban girls had a higher mean serum calcium level (9.12 ± 0.48 mg/dl) than rural girls (8.86 ± 0.52 mg/dl) and rural girls had a higher mean serum level of vitamin D (22.8 ± 8.1 ng/ml) than urban girls (19.4 ± 7.2 ng/ml). The prevalence of low calcium in rural girls (24.3%) was higher compared to the prevalence of low calcium in urban girls (11.4%). Urban girls were more likely to have vitamin D deficiency (<20 ng/ml) (55.7%) than rural girls (40.0%), while sufficient vitamin D ≥ 30 ng/ml was more common in rural girls (24.3%) than in urban girls (12.9%).

Comparisons between the two groups of key biochemical and lifestyle parameters are statistically provided in Table 5. Serum calcium was significantly higher and BMI significantly lower in urban girls (mean difference of 0.26 mg/dl; $p=0.003$ and 1.1 kg/m²; $p=0.031$, respectively). Rural girls, however, had significantly higher vitamin D levels (mean difference: -3.4 ng/ml; $p=0.010$) and daily sunlight exposure (mean difference: -22.2 minutes; $p<0.001$), indicating that there are significant lifestyle differences between the rural and urban settings.

Vitamin D status was significantly correlated with serum calcium levels ($p=0.012$), as shown in table 6. A minority of girls (26.9%) had low serum calcium, in those with vitamin D deficiency, while only 3.8% of girls had sufficient vitamin D levels and 12.8% were vitamin D insufficient. The data show that there is a clear gradient,

such that the higher the vitamin D status, the smaller the number of girls with hypocalcemia.

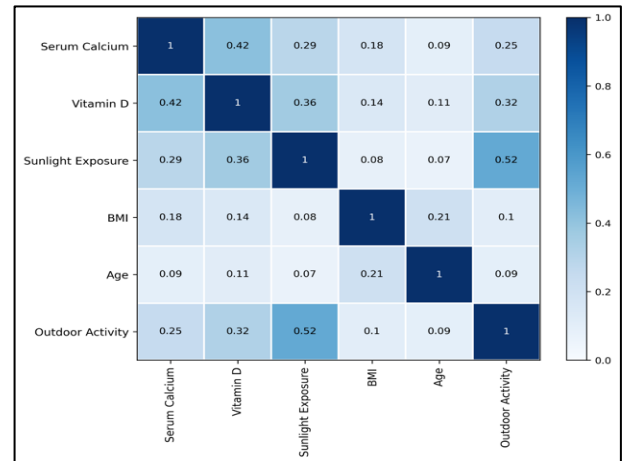


Figure 1: Correlation between serum calcium and selected variables.

The correlation between serum calcium and some of the most important selected variables is illustrated in Figure 1. Serum calcium showed the strongest positive correlation with vitamin D level ($r=0.42$), indicating that higher vitamin D concentrations were associated with higher serum calcium levels. Positive correlations were also observed with daily sunlight exposure ($r=0.29$), outdoor activity duration ($r=0.25$) and BMI ($r=0.18$). Age showed a weak and statistically nonsignificant correlation with serum calcium ($r=0.09$).

Table 1: Sociodemographic profile of the study population.

Variables	Category	Urban, N (%)	Rural, N (%)
Age group (in years)	10-12	16 (22.9%)	19 (27.1)
	13-15	34 (48.6%)	36 (51.4)
	16-19	20 (28.6%)	15 (21.4)
Family type	Nuclear	48 (68.6%)	36 (51.4)
	Joint	22 (31.4%)	34 (48.6)
Economic status of the family	Low	14 (20.0%)	37 (52.9)
	Middle	43 (61.4%)	29 (41.4)
	High	13 (18.6%)	4 (5.7)

Table 2: Anthropometric and clinical profile.

Variables	Category	Urban, N (%) / Mean $\pm$ SD	Rural, N (%) / Mean $\pm$ SD
Age (in years)		14.2 $\pm$ 2.1	13.8 $\pm$ 2.0
Height (cm)	Mean $\pm$ SD	151.6 $\pm$ 6.8	149.8 $\pm$ 6.5
Weight (Kg)		45.8 $\pm$ 7.4	42.3 $\pm$ 6.9
BMI (kg/m ²)		19.9 $\pm$ 3.1	18.8 $\pm$ 2.8
BMI category	Underweight	13 (18.6%)	22 (31.4%)
	Normal	47 (67.1%)	43 (61.4%)
	Overweight	8 (11.4%)	4 (5.7%)
	Obese	2 (2.9%)	1 (1.4%)
Menarche status	Attained	59 (84.3%)	55 (78.6%)
Menstrual irregularity	Present	18 (25.7%)	21 (30.0%)

Table 3: Dietary and lifestyle characteristics.

Variable	Urban, N (%)	Rural, N (%)
Daily milk intake	24 (34.3)	12 (17.1)
Egg intake ≥ 3 days/week	38 (54.3)	27 (38.6)
Fish intake ≥ 3 days/week	41 (58.6)	44 (62.9)
Green leafy vegetables ≥ 3 days/week	36 (51.4)	49 (70.0)
Calcium supplement use	6 (8.6)	2 (2.9)
Vitamin D supplement use	5 (7.1)	1 (1.4)
Sunlight exposure ≥ 30 min/day	18 (25.7)	46 (65.7)
Regular outdoor activity	21 (30.0)	48 (68.6)

Table 4: Serum calcium and vitamin D status among participants.

Variable	Category	Urban, N (%) / Mean $\pm$ SD	Rural, N (%) / Mean $\pm$ SD
Serum calcium, mg/dl	Mean $\pm$ SD	9.12 $\pm$ 0.48	8.86 $\pm$ 0.52
Serum calcium status	Low, <8.5 mg/dl	8 (11.4)	17 (24.3)
	Normal, 8.5-10.5 mg/dl	62 (88.6)	53 (75.7)
Vitamin D, ng/ml	Mean $\pm$ SD	19.4 $\pm$ 7.2	22.8 $\pm$ 8.1
Vitamin D status	Deficient, <20 ng/ml	39 (55.7)	28 (40.0)
	Insufficient, 20–29 ng/ml	22 (31.4)	25 (35.7)
	Sufficient, ≥ 30 ng/ml	9 (12.9)	17 (24.3)

Table 5: Comparison of biochemical parameters between urban and rural girls.

Parameter	Urban Mean $\pm$ SD	Rural Mean $\pm$ SD	Mean difference	P value
Serum calcium, mg/dl	9.12 $\pm$ 0.48	8.86 $\pm$ 0.52	0.26	0.003
Vitamin D (ng/ml)	19.4 $\pm$ 7.2	22.8 $\pm$ 8.1	-3.4	0.010
BMI (kg/m ²)	19.9 $\pm$ 3.1	18.8 $\pm$ 2.8	1.1	0.031
Daily sunlight exposure, minutes	21.6 $\pm$ 12.4	43.8 $\pm$ 18.7	-22.2	<0.001

Table 6: Association between vitamin D status and serum calcium level.

Vitamin D status	Normal calcium N (%)	Low calcium N (%)	P value
Deficient, <20 ng/ml	49 (73.1)	18 (26.9)	0.012
Insufficient, 20–29 ng/ml	41 (87.2)	6 (12.8)	
Sufficient, ≥ 30 ng/ml	25 (96.2)	1 (3.8)	

DISCUSSION

The serum calcium and vitamin D levels of school-going adolescent girls in rural and urban areas of Bangladesh were evaluated in this study, which demonstrated that there was a significant difference between both areas and a significant association between the vitamin D level and serum calcium level. The results provide valuable evidence to a relatively sparse age-group/country context body of evidence. The net prevalence of low serum calcium level was 11.4% in urban and 24.3% in rural areas, which reflects the nutritional vulnerability of adolescent girls in Bangladesh. Similar dietary inadequacies and reliance on low calcium staple foods of the adolescent girls were reported by Oyibo et al in rural secondary schools in Nigeria and with a high prevalence of hypocalcemia among these girls.¹⁵ Hypocalcemia was also recorded in healthy young girls during the pubertal growth phase by Omidvar et al who suggested a higher requirement for calcium and a lower intake.¹⁶ The increased incidence of hypocalcemia in rural girls in this study reflects reduced

milk consumption and higher prevalence of underweight in this group, which are known to be associated with decreased calcium reserves.¹⁷ Notably, the mean serum vitamin D levels in the rural girls were significantly higher (22.8 $\pm$ 8.1 ng/ml) than the urban girls (19.4 $\pm$ 7.2 ng/ml). This finding is supported by significantly higher amounts of sun exposure among the rural subjects (43.8 $\pm$ 18.7 min/day vs. 21.6 $\pm$ 12.4 min/day; $p < 0.001$) and by a higher percentage of outdoor activity (68.6% vs. 30.0%). The main source of vitamin D in the Bangladeshi population is cutaneous synthesis upon exposure to UVB rays, which is not that much available in their diet.¹⁸ Mahfuz et al showed that residence in urban areas and an indoor lifestyle were independent risk factors for vitamin D deficiency in children, while duration of sun exposure was a significant protective factor.¹⁹ Similarly, hypovitaminosis D was reported to be significantly more common in urban children of Bangladesh compared to rural children and children exposed to less sunlight were over 2.5 times more likely to have hypovitaminosis D than those who were exposed more.²⁰ Although girls in a rural population had higher vitamin D, their calcium serum levels were

depleted, indicating that vitamin D deficiency coexists with the presence of calcium inadequacy, which is a highly vulnerable population. Vitamin D levels for the rural girls were at the mean of 22.8 ng/ml, which is still in the insufficient range (<30 ng/ml) and their dietary calcium intake, which is low due to low levels of dairy foods (17.1%) and low economic status, may not be adequate to overcome the suboptimal calcium absorption resulting from insufficient vitamin D.

Harinarayan et al emphasized that even a slight improvement in vitamin D status might not be enough to counteract the effect of the low dietary calcium intake.²¹ A key result of this study was the strong correlation between vitamin D levels and serum calcium levels ($p=0.012$). Girls who had adequate vitamin D levels were much less likely to have hypocalcemia (3.8%) than vitamin D-deficient girls (26.9%). In this gradient relationship, the association between vitamin D and calcium homeostasis is reinforced. Al-Ajlan et al, compared children and adolescents aged 10-19 years and found a positive relationship between serum 25(OH)D and calcium levels, with the greatest number of cases of hypocalcemia in the lowest quartiles of vitamin D.²² Kahwati et al reported that vitamin D supplementation increases serum calcium and decreases parathyroid hormone levels in vitamin D-deficient populations, which was consistent with the biological plausibility of this association.²³

The BMI was higher ($19.9 \pm 3.1 \text{ kg/m}^2$ vs $18.8 \pm 2.8 \text{ kg/m}^2$; $p=0.031$) among the urban girls in this study and the proportion of underweight was lower, indicating a nutrition transition among urban girls in Bangladesh. They did have higher levels of dairy and egg consumption, but this didn't lead to improved Vitamin D levels, as these levels are more dependent on sun exposure than dietary intake.²⁴ There is a correlation with urban girls having higher calcium levels with lower vitamin D levels, but this could be explained by elevated intake of milk in urban girls (34.3% vs. 17.1%) and raised socioeconomic status, which partly offset low exposure to sunlight. This discovery indicates that calcium and vitamin D deficiency are two distinct phenomena that can be affected by different environmental and behavioral factors. Girls living in rural areas ate green leafy vegetables more often than their urban counterparts (70% vs. 51.4%), which is an important source of calcium apart from dairy products. Consumption of fish was also greater in rural areas (62.9% vs. 58.6%), a food that helps provide vitamin D. A study of dietary intakes of adolescents in rural and urban areas of Bangladesh revealed that rural girls had higher levels of vegetable and fish intake than urban girls, but still consumed less than the recommended levels of micronutrients for both groups.²⁵ Moreover, Keats et al showed that adolescent diets in LMICs are generally lower in energy but that they may be rich in a variety of micronutrients from traditional food systems in rural areas.²⁶ The results of this study have a public health implication in the country of Bangladesh. Adolescence is a pivotal period of bone formation, when the skeletal mass is at its maximum; about 50% of the adult skeleton is formed during adolescence, which highlights the

importance of adequate calcium and vitamin D intake during this stage of life.²⁷ There is an urgent need to promote dietary diversity, calcium-rich food intervention and to build the physical activity policy in schools to create more outdoor space, especially for adolescent girls in urban areas. The importance of calcium and vitamin D for adolescent development should be promoted through health education activities targeting parents, caregivers and school officials under the national nutrition policy in Bangladesh.

Limitations

This study was a cross-sectional study and causal inference could not be made due to the nature of the study; and purposive sampling was used, which may restrict the generalizability of the results to the larger population of adolescent girls in Bangladesh.

CONCLUSION

The study demonstrated that hypocalcemia and vitamin D deficiency are widely prevalent among adolescent girls (school-going) in urban and rural areas of Bangladesh, with considerable disparity between them. Taking into account the vitamin D status, low serum calcium was more prevalent in rural girls, suggesting the combined effect of low calcium intake and low economic access to dairy products. Urban girls had higher levels of serum calcium, but had a high prevalence of vitamin D deficiency due to low doses of sunlight and mostly indoor life. Vitamin D status was shown to have a significant relationship with serum calcium level, which highlights the importance of vitamin D in the regulation of calcium during adolescence. The results of this study show the immediate need for integrated public health measures in Bangladesh, such as nutritional supplementation programs in schools, encouraging outdoor physical activity, calcium-rich food promotion campaigns and screening program intervention for adolescent girls who are at risk in both urban and rural areas.

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