Original Article

Hypovitaminosis D is common in both veiled and nonveiled Bangladeshi women

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The present cross-sectional study was designed to evaluate the vitamin D status in three groups of women in Bangladesh by using serum 25-hydroxyvitamin D (S-25-OHD), alkaline phosphatase (S-ALP), calcium (S-Ca) and phosphate (S-P). Sampling was undertaken at three locations in the city of Dhaka, Bangladesh. Representative subjects of three groups of women aged 18-60 years were studied. Study subjects included nonveiled young women = group A (N = 36, mean ± SD age 22.3 ± 1.9 years), veiled women = group B (N = 30, mean ± SD age 47.7 ± 9.4 years) and nonveiled diabetic women = group C (N = 55, mean ± SD age 50.2 ± 5.9 years). The mean value of S-25-OHD was not significantly different in the groups. The distribution of S-25-OHD concentration in all groups was shifted overall toward the lower limit of the normal range. Vitamin D deficiency (serum 25-OHD level <25 nmol/l) was detected in 39% of young women (university students), 30% in veiled women and 38% in diabetic women, respectively. Vitamin D insufficiency defined as serum 25-OHD concentration $\leq$40 nmol/l was detected in 78% of group A, 83% in group B and 76% in group C, respectively. As indicated, prevalence of vitamin D insufficiency was a bit higher in group B compared with the other groups studied although it was not statistically significant (P >0.05). In the present study, there were several independent predictors of serum 25-OHD, i.e. both increasing parity (r = 0.286; P <0.005) and increasing time spent outdoors (r = 0.515; P <0.001) were associated with significant increase in serum 25-OHD. A strongly significant inverse correlation between serum ALP and 25-OHD (r = - 0.303; P <0.001) was observed. The results showed that women in Bangladesh, regardless of different age-groups, lifestyle and clothing, were at risk of developing hypovitaminosis D. The results emphasize the appropriate health message for vitamin D needs in Bangladeshi women, since vitamin D insufficiency significantly affects bone integrity.

Key Words: vitamin D deficiency, veiled women, Bangladesh

Introduction

Vitamin D is important for the maintenance of normal blood levels of calcium and phosphorus. It promotes bone mineralization by increasing the absorption of calcium and aids in maximizing skeletal health from birth to death. Vitamin D prevents rickets in children and osteomalacia in adults. Bones can become thin, brittle, soft or misshapen in subjects with vitamin D deficiency. The first symptoms caused by vitamin D deficiency are usually muscle pain, fatigue, muscular weakness and gait disturbances. Sunlight and diet are the two major sources of vitamin D in humans. Hypovitaminosis D is associated with limited exposure to sunlight, inadequate dietary intake of vitamin D, increased skin pigmentation, problems with kidney functions in converting vitamin D to its active form as well as lack of vitamin D supplementation.

The need for appropriate amount of vitamin D intake in young and elderly women is well established. A decrease in the vitamin D status with advancing age has been observed in several studies in different populations. A high prevalence of sub-clinical vitamin D deficiency was reported in veiled women.[1-4] The role vitamin D deficiency in both type 1 and type 2 diabetes is well recognised. In addition, the high prevalence of hypovitaminosis D, osteomalacia and osteoporosis were reported in earlier studies in diabetic subjects.[5-7] Dietary vitamin D supplementation was found to be associated with reduced risk of type 1 and type 2 diabetes.[8,9] However, the information regarding vitamin D status of diabetic subjects in Bangladesh is scarce.

Vitamin D deficiency is common worldwide irrespective of its occurrence in both high and low-latitude countries. In addition to the European countries, vitamin D deficiency has been reported in many countries in the Middle East, Africa and Asia. Surprisingly, it is more frequent in the sunny Mediterranean countries than in certain northern countries such as Norway. Despite the abundant sunlight, vitamin D deficiency is prevalent in South Asian countries as well as other neighbouring countries such as

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Turkey, China, Japan and Thailand.[10-16] A high prevalence of vitamin D deficiency, low dietary intake of calcium was observed in premenopausal Bangladeshi women of both high and low socioeconomic status.[17,18] Their lifestyle largely confining them to the home and the traditional clothing that Bangladeshi women wear exposes very little of their skin to sunlight. Under these conditions women in different groups are at risk of developing vitamin D deficiency.

In Bangladesh, there are very few published studies on vitamin D status of different groups of women. The present study was carried out in three groups of Bangladeshi women. We chose these groups on the assumption that veiled women would show a lower vitamin D status than nonveiled women. In addition, diabetes (type 2) is becoming more common in Bangladeshi women, possibly due to the low 25-OHD levels; thus we also included this group.

Subjects and Methods

Subjects

The study was conducted in three locations in the capital city (Dhaka) of Bangladesh during June to 1st week of July in 2001. Bangladesh lies within a tropical to subtropical monsoon climate zone which extends from Latitude $20^0$43´ to $26^0$36´N and Longitude $88^0$3´ to $92^0$40´E. We consecutively studied 121 women belonging to three groups. Apparently healthy young adult women (group A, N = 36), veiled women (group B, N = 30) and diabetic women (group C, N = 55) were the subjects of the study. The subjects in groups A, B and C were randomly selected from the Institute of Nutrition and Food Science, University of Dhaka, women activists of a nonpolitical Muslim group (Tablig), Nakhalpara and the Ibrahim Memorial Diabetic Hospital, Shahbag, respectively. Group A subjects were in first to fifth year of their university studies and came mainly from affluent families of high educational level. Group B consisted of women who cover themselves with a thick black cloak in addition to their normal clothes when they go outdoors. Their heads, arms, and legs are covered and faces left uncovered or covered with a semitransparent cloth. These subjects traditionally held weekly gatherings at a site for religious discussion during recent years. Group C subjects were registered diabetic patients of the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIR-DEM). The subjects of this study were mainly house-wives, except the women in group A. The authorities at all these three locations were supportive of the study and the subjects participated enthusiastically. We explained the purpose of the study to potential subjects through verbal as well as written communications and asked their consent to participate by signing the paper. The response rate was more than 90% in each location, although not all women agreed to undergo phlebotomy. The exclusion criteria included were pregnancy or lactation within the previous 3 years. The characteristics of the subjects are presented in Table 1. The study was approved by the Ethics Committee of the Faculty of Agriculture and Forestry, University of Helsinki, Finland. The ethics guidelines of the University of Dhaka were also followed during the field study in Bangladesh.

Laboratory measurements

Blood samples were drawn in the morning (between 8:30 and 10:00am) by disposable syringe through venepuncture and a maximum of 5ml of blood was taken after overnight fasting of the subjects. The serum was separated after complete centrifugation of blood samples within two hours of venepuncture, collected in tubes and preserved at a temperature of −20°C at the Institute of Nutrition and Food Science, University of Dhaka. Finally, the serum samples were transported to Helsinki on dry ice supplied by Bangladesh Oxygen and preserved at −20°C at the Division of Nutrition, Department of Applied Chemistry and Microbiology, University of Helsinki until analysed.

There is no difference in sunshine during the period of field study that could substantially affect vitamin D status. The serum 25-OHD level was used to evaluate the vitamin D status. The serum 25-OHD concentration was measured by radioimmunoassay method using kits from DiaSorin (Stillwater, Minnesota, USA). The reference range for 25-OHD was 25-120 nmol/l and vitamin D deficiency was defined as serum 25-OHD levels <25 nmol/l. The intra- and interassay CVs were 12% and 13%, respectively. The serum levels of calcium (S-Ca), phosphate (S-P) and alkaline phosphatase activity (S-ALP) were measured using routine laboratory methods to get additional information about vitamin D status. The food composition database used in Bangladesh does not contain vitamin D. Due to this limitation, estimation of dietary intake of vitamin D was not possible.

Anthropometry and other information

For anthropometric measurements the height, weight, mid-upper arm circumference (MUAC), waist and hip were measured. Body weight was measured to the nearest 0.5 kg, with the subjects wearing no shoes, having light clothing and standing on a portable weighing scale. The standing height was measured without shoes using a wall-mounted scale to the nearest 0.5 cm. The height and weight were used to calculate the Body Mass Index BMI [weight (kg)/height (m^2)]. The classifications of BMI applied in this study were recommended by the World Health Organization[19]. BMI values of <18.5 kg/m^2 and ≥25kg/m^2 represented thinness and overweight, respectively. A normal weight was considered to fall within these two extremes. A questionnaire exploring the subjects socioeconomic situations, lifestyles and demographics was developed.

Statistical analyses

The statistical analysis was carried out using the χ^2 test and two-way analysis of variance (ANOVA). *Post-hoc* analyses between the groups was carried out with two-sided t-test. The results are expressed as mean ± SD. The Mann-Whitney U-test was used for skewed distribution of variables. The Pearson correlation coefficient was used to estimate the relationship between biochemical and other variables. Logistic regression models were fitted with vitamin D deficiency as the outcome variable. Multivariate logistic regression models were fitted to determine the risk factors that were significant and independently predictive of vitamin D deficiency after adjustment for other factors. The minimum significance level used was <0.05. The analyses were carried out with Stata Statistical Software.[20]

Results

Serum 25-hydroxyvitamin D concentration

The mean value of S-25-OHD in group A was not significantly different compared with groups B (30.3 ± 22.6 nmol/l vs 31.0 ± 11.0 nmol/l, respectively; *P* = 0.81) and C (30.3 ± 22.6 nmol/l vs 31.5 ± 13.5 nmol/l, respectively; *P* = 0.67). In the population we studied, no subject showed a serum 25-OHD level above the normal range (25–120 nmol/l) and the distribution in all three groups was very close to the lower limit of the normal range (Fig. 1). The observed median value of S-25-OHD was similar and very low in all three groups. The median values of S-25OHD in groups A, B and C were 30.6, 29.2 and 28.3 nmol/l, respectively. Vitamin D deficiency (S-25-OHD level <25 nmol/l) was observed in 36.4% of subjects of this study and the prevalences were 38.9% in group A, 30% in group B and 38.2% in group C, respectively. Several thresholds are commonly used to identify the prevalence of vitamin D insufficiency. Serum 25-OHD concentrations ≤ 40 nmol/l resulting in increase in serum parathyroid hormone concentrations, corresponding to hypovitaminosis D was observed in 77.7% of our subjects.[21] The prevalence of hypovitaminosis D was high in all three groups (77.9% in group A, 83% in group B and 74.5% in group C, respectively).

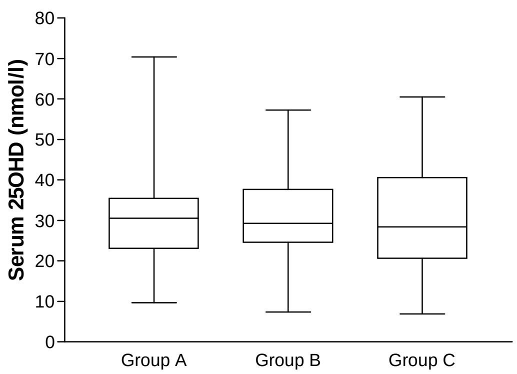

Figure 1. Box plot presentation of the distribution of serum 25-OHD by groups categories.

Other variables

The S-Ca concentration was significantly higher in group A than in groups B and C (A *vs* B, *P* <0.001; A *vs* C, *P*<0.001). The S-P concentration showed significant differences between group A and B (1.28 ± 0.20 vs 1.17 ± 0.14, respectively; *P* <0.05), whereas the difference was not statistically significant between groups A and C. None of the subjects were found to have hypocalcaemia (total calcium level below 2.10 nmol) and hypophosphataemia (S-P level below 0.9 mmol) was observed in five subjects (distributed as one each in groups A and B, three in group C) of subjects with severe hypovitaminosis D. Throughout the population, S-25-OHD was not correlated with total calcium (*r*=0.132; *P*=0.149) or phosphate (*r*=-0.023; *P*=0.801). The S-ALP levels were not significantly different between groups A and B (*P* = 0.95) but the difference was statistically significant between groups A and C (*P* = 0.02, Fig. 2). None of the subjects in groups A or group B showed S-ALP activity above the reference limit (>350 U/l). Two subjects in group C had markedly high levels of S-ALP (304 & 400 U/l) with very low serum 25-OHD level (19.4 & 12.2 nmol/l). The S-ALP was significantly correlated with serum 25-OHD level inversely (r=- 0.303; *P* <0.001).

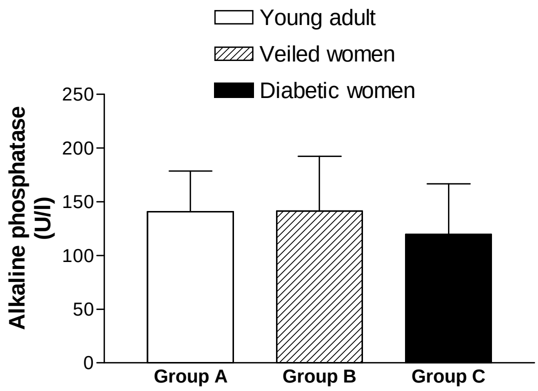

Figure 2. Serum alkaline phosphatase concentrations in three groups (bars showing the standard deviation).

No subject reported using any calcium and vitamin D supplementation and more than 80% of the subjects reported their attitude of avoidance of direct sunshine exposure. There was no significant relationship between S-25OHD, BMI and waist:hip ratios. Probable variables that influence the vitamin D levels were studied, using multi-linear regression analysis; age, professions, veiling, parity and lifestyle were considered in this case. Our study found no importance of age as a determinant of vitamin D status (r = 0.004; *P* = 0.96). Regardless of age, a great majority of the subjects in this study have vitamin D insufficiency. In the present study, parity positively correlated with S-25-OHD.

Anthropometric characteristics

Physical characteristics, such as height, weight, MUAC, BMI and waist:hip ratios (WHR) of three groups is presented in Table 1. Low BMI values (BMI<18.5 kg/m^2) were prevalent and underweight percentage (17%) was higher in group B compared with group A (0%) and group C (1.8%). High BMI values (BMI >25.0 kg/m^2) were more prevalent and overweight percentage (45.5%) was significantly higher in group C than in group A (14%) and group B (23%).

Discussion

In line with our earlier study, the present study showed that more than one-third of a young adult and elderly Bangladeshi women are vitamin D-deficient.[17] In addition, we found that S-ALP was high in all groups studied which is a usual finding in osteomalacia. Serum 25-OHD negatively correlated with S-ALP indicating an osteomalacic effect on bone. The subjects could be more prone to seasonal vitamin D insufficiency because sunlight in winter may not promote conversion of the vitamin D precursor in the skin due to usual foggy weather in winter. In contrast to expectations, lower mean values of serum 25-OHD concentration were seen in normal healthy young adult women rather than in veiled and diabetic

Table 1. Characteristics of the subjects, standard deviation or SD is given in parentheses

Physical, haematological and other data	Group A *N*=36	Group B *N* =30	Group C *N* =55	*P value* [a]GrA vs GrB	*P value* [b]GrA vs GrC
Age (y)	22.3 (1.9)	47.7 (9.4)	50.2 (5.9)	<0.001	<0.001
Weight (kg)	53.8 (5.4)	50.1 (8.1)	57.5 (10.3)	<0.05	<0.05
Height (cm)	155.5 (4.6)	150.6 (4.9)	150.6 (5.7)	<0.001	<0.001
MUAC (cm)	25.2 (1.9)	25.2 (2.2)	26.1 (2.7)	NS	NS
BMI (kg/m^2)	22.2 (2.1)	22.0 (3.1)	25.2 (3.7)	NS	<0.001
Waist circ (cm)	73.1 (5.8)	79.3 (9.2)	88.8 (9.7)	<0.001	<0.001
Hip circ (cm)	92.4 (4.8)	89.2 (7.0)	98.3 (9.6)	<0.05	<0.001
WHR (cm/cm)	0.79 (0.05)	0.88 (0.05)	0.90 (0.06)	<0.001	<0.001
S-25-OHD (mmol/l)	30.5 (11.6)	31.0 (11.0)	31.5 (13.5)	NS	NS
S-ALP (U/l)	140.9 (37.9)	141.5 (50.8)	132.2 (60.2)	NS	NS
S-Ca (mmol/l)	2.64 (0.15)	2.50 (0.09)	2.80 (0.13)	<0.001	<0.001
S-P (mmol/l)	1.28 (0.20)	1.17 (0.14)	1.23 (0.19)	<0.05	NS
*TSO (min)	49 (22)	83 (52)	113 (52)	<0.001	<0.001
Parity	-	5.3 (2.5)	4.4 (2.0)	<0.0014	<0.001
**MFI (USD)	349 (240)	158 (141)	190 (116)	<0.001	<0.001

* Time spent outdoors; ** Monthly family income; [a] Group A vs group B; [b] Group A vs Group C.

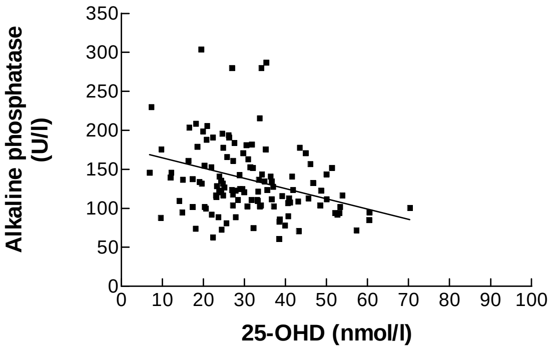

Figure 3. The inverse correlation between serum alkaline phosphatase and 25-OHD.

and diabetic women. Our results indicated that hypovitaminosis D is highly prevalent in the population studied, whether young, elderly or veiled. The aetiology of vitamin D deficiency in our subjects could be multifactorial.

The higher prevalence of hypovitaminosis D as well as vitamin D deficiency in Bangladeshi women could generally be explained by reduced sunshine exposure (traditional avoidance of sunlight, clothing habits, less performance of outdoor activities), inadequate dietary intake and no use of supplementation. Homebound lifestyle, spending little time outdoors in the sunlight is probably the main reason for this situation. Secondly, Dhaka City is one of the most highly polluted cities in the world, which could affect sunlight exposure and thus skin synthesis of vitamin D. As in large cities, a higher degree of air pollution containing ozones leads to efficient atmospheric absorption of ultraviolet-B (UV-B) photons, thereby reducing the skin photosynthesis of vitamin D.[22] Vitamin D formation in the skin is affected by both intensity and duration of exposure to UV light. Since the intensity is lower in winter, the prevalence of hypovitaminosis D could be even higher in winter.

Many studies have reported the influence of clothing on vitamin D status in women. In different countries, a high prevalence of vitamin D deficiency as well as lower level of serum 25-OHD were observed in veiled women compared with nonveiled women.[1,2,4,23,24] In contrast to these findings, the nonsignificant difference in serum 25-OHD observed in our study between veiled and nonveiled subjects may have been caused by lower dietary intake of vitamin D as well as inadequate sunlight exposure to the skin due to a homebound lifestyle.

In the present study, healthy nonveiled young women with high income and educational levels reported that they usually spent very little time daily in direct sunlight compared with veiled and diabetic women. The serum 25-OHD levels could be significantly higher in nonveiled young women by increasing their outdoor activities with adequate sunlight exposure to the skin. In the present study, parity positively correlated with S-25-OHD which is in contrast to earlier findings.[3,23] The reason could be that Bangladeshi women with high parity (parity $\geq$1) have more responsibilities pertaining to childcare and other household activities that require their spending more time outdoors in the sunlight, which probably accounted for this situation. We observed that the median value of S-25-OHD was high in diabetic women. The time spent out-doors was significantly high in this group (Table 1) which could be the reason of this situation.

The prevalence of hypovitaminosis D has been observed in different degrees in several countries. Substantial studies in the European countries reported a high prevalence of hypovtaminosis D in young adults and the elderly population. Van der Wielen *et al.*, (1995) showed that hypovitaminosis D was surprisingly more common in elderly people living in sunny countries such as Italy, Spain and Greece than among those living in Scandinavian countries such as Norway.[25] Despite having enough sunlight, the prevalence of hypovitaminosis D was up to 83% of elderly Greek women compared with only 18% of the elderly population in Norway. A high intake of fish, vitamin D fortification of food, and vitamin D supplementation could explain this difference. The healthy young adult population in France and northern Italy was also found to have surprisingly lower levels of serum 25-OHD.[26,27] In a study among Australian Muslims healthy women reported a high prevalence (68%) of vitamin D deficiency.[28]

The literature showed that rural subjects working in the field and spending more time outdoors in the sunlight have higher vitamin D levels despite their very low vitamin D intake. In fact, the effect of sunlight on vitamin D status has been well documented and confirms the importance of sunlight exposure in the synthesis of vita-min D. The level of serum 25-OHD is the best available laboratory aid for diagnosing frank vitamin D deficiency, which causes rickets in children and osteomalacia in adults. Vitamin D deficiency is typically associated with 25-OHD levels of <25 nmol/l. Vitamin D insufficiency is likely to be of greater clinical importance in identifying inadequate levels of vitamin D, which affects optimal bone health. Serum level of 25-OHD <40 nmol/l is the most commonly used definition of vitamin D insufficiency, although several authors suggested that the cut-off value for vitamin D insufficiency could be as high as 80 nmol/l.[27,29,30] Based on this definition, nearly 80% of the subjects in this study have vitamin D insufficiency. Virtually all participants in this study would be assessed as having had vitamin D insufficiency if this threshold were raised by 10 nmol/l to 50 nmol/l.[31,32] The prevalence of the deficiency could actually be far worse, since the study was performed in summer, when serum 25-OHD concentrations are typica lly higher.[32-34] A study carried out in winter-time to see the seasonal variation of vitamin D status in Bangladeshi women is needed in order to plan confidently to alleviate this problem.

We conclude that vitamin D deficiency and borderline vitamin D status were so common in different groups of adult Bangladeshi women that they were equally at risk of developing vitamin D deficiency. The prevalence of hypovitaminosis D status is alarmingly high (≅ 80%) in all these groups. The results indicated that hypovitaminosis D could be an important public health problem in adult women of Bangladesh. A comprehensive programme to prevent vitamin D deficiency in Bangladeshi women is recommended. Strategies may include extensive awareness of the importance of sunlight exposure and improved judicio us exposure to sunlight and improved dietary supplies of calcium and vitamin D. In this case high intake of vitamin D-rich food as well as inclusion of a food-fortification programme could be suggested. Integration of the topic of vitamin D deficiency in the popular broadcasting media as well as newspapers could play an important role in this aspect.

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Original Article

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维生素 D 缺乏症在戴面纱和不戴面纱的孟加拉国妇女中很普遍

文中的代表性研究是设计来测量三组服用 S-25-OHD 的孟加拉国妇女血清维生素 D、碱性磷酸酶、钙和磷酸盐水平。样本是从孟加拉国首都达卡城的三个地方选取的。三组 18－60 岁的妇女作为代表性样本进行研究。研究的样本包括未戴面纱的年轻女性为 A 组（n = 36, 年龄 22.3 ± 1.9 岁），戴面纱的妇女 B 组（n= 30, 年龄 47.7 ± 9.4 岁）和未戴面纱的女性糖尿病患者为 C 组（n= 55, 年龄 50.2 ± 5.9 岁)。这些组中 S-25-OHD 平均值并没有显著差异。所有组中 S-25-OHD 浓度的分布总的移到正常范围的下限。在 39％的年轻女性、30％的戴面纱女性以及 38％的女性糖尿病患者中均被检测为维生素 D 缺乏症（血清 25-OHD 浓度≤25nmol/L）。维生素 D 不足被定义为血清 25-OHD 浓度≤40 nmol/L，在 A 组中占 78％、B 组中 83％以及 C 组中 76％。正如所显示的一样，与其它组相比，B 组维生素 D 不足的比例高一些，尽管统计学上并不显著（P>0.05）。在本研究中，有一些血清 25-OHD 的独立预测因子，如增加锻炼(r=0.286; P <0.005)和户外时间(r =0.515; P <0.001)都与血清 25-OHD 的显著正相关。观察到血清 ALP 和 25-OHD 之间呈强显著负相关（r = -0.303; P<0.001）。结果显示孟加拉国妇女存在着发生低维生素 D 血症的风险，与年龄、生活方式以及衣着无关。结果强调了孟加拉国女性维生素 D 需求的适用的健康讯息，因为维生素 D 的不足将影响骨完整性。

关键词： 维生素 **D** 缺乏症、女性、孟加拉国。