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Original Article

Profile of 25-Hydroxyvitamin D in Individuals Attending Armed Forces Institute of Pathology (AFIP), Dhaka

Mimi Parvin[1], Mreenal Kanti Sarkar[2], Debashis Saha[3]
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Abstract

***Background**: In the last two decades, there has been increasing interest in the biology of vitamin D and a growing recognition is that vitamin D deficiency is common in tropical countries despite having ample sunshine. Myth of 'routine exposure to sunshine does not require extra vitamin D' results in severe asymptomatic vitamin D deficiency. Several factors like socio-economic condition, dietary insufficiency, inadequate exposure to sunlight due to use of sun-screen, clothes, environmental pollution, skin pigmentation may contribute to vitamin D deficiency. **Objective**: The study was designed to evaluate the vitamin D status of patients of different age groups reporting to Armed Forces Institute of Pathology (AFIP), Dhaka. **Materials and Methods**: This cross-sectional, observational study was conducted from October 2015 to March 2017, using serum 25-hydroxyvitamin D (25-OHD) levels to classify patients of different age groups into deficient, insufficient and sufficient categories. Individuals up to 70 years of age of all religions, genders and occupations who gave blood specimens at AFIP were included in the study. Patients with chronic renal failure, primary hyperparathyroidism, on vitamin D supplementation with established diagnosis of osteomalacia and rickets, taking phenytoin or glucocorticoids were excluded. Total 25-OH vitamin D level in serum was estimated by electro-chemiluminescence on Cobas Elecsys e411 fully automated system on the day of collection. This method has been standardized against LC-MS/MS which in turn has been standardized to the NIST (National Institute of Standards and Technology, USA). The functional sensitivity was determined to be 4.01 ng/mL (CV 18.5%). Vitamin D deficiency is defined as 25(OH) D <50 nmol/L, insufficiency as 50–75 nmol/L and sufficiency as ≥75 nmol/L and toxicity >375 nmol/L. The definition of vitamin D status was based on the recommendation of the US Institute of Medicine (IOM) for vitamin D. **Results**: A total of 2867 specimens were enrolled in the study, comprising 1949 (67.98%) female and 918 (32.02%) male. Maximum number (1270) was in the age range of 41−60 years. According to vitamin D status 1640 (57.20%) out of 2867 were found deficient, among which female were 1175 (71.65%) and male 465 (28.35%) with 1:2.5 male and female ratio. There were 255 male (37.83%) and 419 (62.17%) female in the insufficient group with 1:1.64 male-female ratio. Lowest value of <7.5 nmol/L was found in 36 subjects where 80.55% were female. Female were also found to be predominant (62.75%) in highest concentration of vitamin D (>175 nmol/L). Female in all age groups predominated in both deficiency and insufficiency groups. No toxic values were found in any age group among male or female. **Conclusion**: Vitamin D deficiency is pandemic; it has been assumed that one billion people worldwide have vitamin D deficiency or insufficiency. Laboratory professionals are confronted with substantial increase in laboratory testing due to growing clinical interest in vitamin D status. At the same time avoidance of overscreening of vitamin D by physicians should also be considered.*

***Key words**: Vitamin D; Vitamin D deficiency; 25-Hydroxy vitamin D; Vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol)*

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1. Professor, Department of Biochemistry, Armed Forces Medical College, Dhaka Cantonment, Dhaka
2. Graded Specialist in Pathology, Armed Forces Institute of Pathology (AFIP), Dhaka Cantonment, Dhaka
3. Classified Specialist in Pathology, AFIP, Dhaka Cantonment, Dhaka
***Correspondence** Mimi Parvin, Email: drmimi708@yahoo.com*

Introduction

Vitamin D deficiency has long been recognized as a cause of rickets in children and osteomalacia in adults. Preclinical phase of vitamin D deficiency, known as vitamin D insufficiency, increases the risk of osteoporotic fractures. Low levels of vitamin D metabolites are associated with malabsorption of calcium, which results in bone loss.[1] Complementing the well-known role of vitamin D in mineral metabolism, research has demonstrated an increasing list of "nonclassic" vitamin D actions, including effects on immune modulation, cell cycle proliferation, and brain health. Vitamin D deficiency has now been associated with increased risk of multiple sclerosis, certain cancers (especially breast, colorectal, and prostate), and chronic respiratory infections (eg, tuberculosis). Consequently, vitamin D deficiency has health implications that extend well beyond skeletal abnormalities.[2] Doctors in tropical countries like Bangladesh hardly felt that our population could be vitamin D deficient. Increasing concern rose for our urban population with modern amenities and lifestyle barring from exposure to sunlight. But as the technology to measure vitamin D became inexpensive and widely available, more and more studies were done.[3]

In the last two decades, there has been increasing interest in the biology of vitamin D and a growing recognition that vitamin D insufficiency is common throughout the world. One consequence of the growing clinical attention to vitamin D has been a substantial increase in laboratory testing for vitamin D. In the United States, many clinical laboratories have experienced increases in vitamin D testing of 100% or more in the last five years.[2] Laboratory professionals are often confronted with challenges related to vitamin D testing, including variable reference ranges across marketed assays and inappropriate ordering of 1, 25-dihydroxyvitamin D testing. Two possible reasons for misordering include confusion of the two forms of vitamin D or a mistaken notion that monitoring the "active" form of vitamin D should be done to assess nutritional status. In addition, clinicians may request measurement of both 25-hydroxyvitamin D and 1, 25-dihydroxyvitamin D (perhaps thinking more information from this "panel" is helpful) when measuring only 25-hydroxyvitamin D alone is all that is needed for routine assessment of nutritional stores.[2]

In our country there are very few studies regarding vitamin D status in our population. So we have designed to observe the vitamin D status in different age groups of male and female genders attending Armed Forces Institute of Pathology (AFIP) of Dhaka cantonment, Bangladesh.

Materials and Methods

This cross sectional observational study was conducted at Armed Forces Institute of Pathology (AFIP) from October 2015 to March 2017. The study was conducted on 2867 collected samples in AFIP from subjects by physicians' referral. Subjects from all age range, religion, gender and occupation who gave blood sample at AFIP were eligible to participate. Informed consent was obtained and then participants were subjected to laboratory investigations. Patients with chronic renal failure, primary hyperparathyroidism, on vitamin D supplementation, those with established diagnosis of osteomalacia and rickets, those taking phenytoin or glucocorticoids were excluded. After obtaining consent, 3 mL of blood sample was collected by phlebotomy in dry glass test tube (red top vacutainer) and was transferred in cool-box to the laboratory. After clotting, serum was separated by complete centrifuge of blood sample.

Total (25 OH) Vitamin D level in serum was estimated by electro-chemiluminescence on Cobas Elecsys e411 fully automated system on the day of collection. This method has been standardized against LC-MS/MS which in turn has been standardized to the NIST (National Institute of Standards and Technology, USA). The functional sensitivity was determined to be 4.01 ng/mL (CV 18.5%).

Vitamin D deficiency is defined as 25(OH) D <50 nmol/L, insufficiency as 50–75 nmol/L and sufficiency as ≥ 75 nmol/L and toxicity >375 nmol/L.[4] The definition of vitamin D status was based on the recommendation of the US Institute of Medicine (IOM) for vitamin D.[5]

Results

Total 2867 subjects were enrolled in this study. Out of them 1949 (67.98%) were female and 918 (32.02%) were male. Maximum numbers (1270) of subjects were in the age range of 41−60 years (Table I). According to vitamin D status, out of 2867, 1640 (57.20%) were found deficient, among which female were 1175 (71.65 %)

and male 465 (28.35%) (Table II). There were 255 male (37.83%) and 419 (62.17%) female in the insufficient group (Table II). Lowest value of <7.5 nmol/L was found in 36 subjects with female predominance (80.55%) (Table II). Female were found predominant (62.75%) also in highest range of vitamin D (>175 nmol/L) concentration. Female in all age groups predominated in both deficiency and insufficiency group (Table III−VI). No toxic values were found in any age groups among male and female (Table II).

Table I: Distribution of study population according to age range

Age range	Number	Percentage
Up to 20 years	290	10.12
21−40 years	719	25.08
41−60 years	1270	44.30
>60 years	588	20.50
Total	2867	100

Table II: Distribution of subjects according to vitamin D concentration (N=2876)

Sex	Deficient <50 nmol/L	Insufficient 50−75 nmol/L	Sufficient >75 nmol/L	Toxicity >375 nmol/L
Male (n=918)	465 (28.35%)	255 (37.83%)	198 (35.80%)	0
Female (n=1949)	1175 (71.65%)	419 (62.17%)	355 (64.20%)	0
Total	1640	674	553	Nil
Lowest value: <7.5 nmol/L in 36 individuals [Male: 7 (19.44%), Female: 29 (80.55%)]				
Highest value: >175 nmol/L in 51 individuals [Male: 19(37.25%), Female: 32(62.75%)]				

1 nmol/L = 0.4 ng/mL

Table III: Distribution of study population according to vitamin D status in subjects up to 20 years (n=290)

Sex	Deficient <50 nmol/L	Insufficient 50−75 nmol/L	Sufficient >75 nmol/L
Male (n=104)	52 (29.21%)	29 (46.03%)	23 (46.94%)
Female (n=186)	126 (70.79%)	34 (53.97%)	26 (53.06%)
Total	178	63	49

Table IV: Distribution of vitamin D status in subjects from 21−40 years (n=719)

Sex	Deficient <50 nmol/L	Insufficient 50−75 nmol/L	Sufficient >75 nmol/L
Male (n=165)	94 (19.83%)	47 (30.13%)	24 (26.97%)
Female (n=554)	380 (80.17%)	109 (69.87%)	65 (73.03%)
Total	474	156	89

Table V: Distribution of vitamin D status in subjects of 41−60 years (n=1270)

Sex	Deficient <50 nmol/L	Insufficient 50−75 nmol/L	Sufficient >75 nmol/L
Male (n=430)	232 (31.82 %)	120 (40.54%)	78 (31.84%)
Female (n=840)	497 (68.18 %)	176 (59.46 %)	167 (68.16%)
Total	729	296	245

Table VI: Distribution of vitamin D status in subjects of >60 years (n=588)

Sex	Deficient <50 nmol/L	Insufficient 50−75 nmol/L	Sufficient >75 nmol/L
Male (n=219)	87 (33.59 %)	59 (66.41%)	73 (42.94%)
Female (n=369)	172 (37.11 %)	100 (62.89 %)	97 (57.06 %)
Total	259	159	170

Discussion

Recent studies carried across different countries in South and South East Asia showed, with few exceptions, widespread prevalence of hypovitaminosis D, irrespective of age, gender, race and latitude of residence, or dietary practices.

In Bangladesh (24ºN), hypovitaminosis D is common in women regardless of age, lifestyle and clothing.[6] Prevalence of hypovitaminosis defined as 25(OH) D levels below 37.5 nmol/L was 38% in Bangladeshi women from high income group and increased to 50% in women from low income groups.[7] Reports indicate that 26–84% of women in Lebanon, Saudi Arabia, the United Arab Emirates (UAE), Bangladesh, Japan and Finland have serum 25(OH)D concentrations <25 nmol/L (10 ng/mL).[8] In one study in Pakistan it was found that 92% had vitamin D deficiency (70−97% in Karachi and 81−92% in Lahore).[9] Another study in Pakistan also showed high levels of deficiency among all age groups, genders, income levels, and locations. Amongst the selected citizens, 53.5% had vitamin D deficiency, 31.2% had insufficient vitamin D, and only 15.3% normal vitamin D.[10] High rates of biochemical vitamin D deficiency or insufficiency among healthy individuals have been reported in large-scale studies from all parts of the world (the United States, Canada, South America, Europe, Australia, the Middle East, South Asia, and Africa). Severe vitamin D deficiency is common in China, India, South America, and the Middle East. In India, studies from different parts of the country have reported a prevalence of vitamin D deficiency varying from 30% to 100%. The cut-offs to define vitamin D deficiency, however, varies among the studies. Studies utilizing higher cut-off (75 nmol/L or 30 ng/mL) to diagnose vitamin D deficiency have reported a near-universal presence of vitamin D deficiency among diverse age groups, including pregnant and lactating women, newborns, adolescents, and healthy professionals.[9] The study conducted by the Department of Rheumatology of BSMMU in 2014 revealed only one percent people in Bangladesh have sufficient vitamin D level of 30−50 nanogram, 97% have vitamin D level of 10−29 nanogram.[11] These findings are similar to our findings which revealed that majority of the both sex groups were hypo-vitamin D in all ages.

Several factors may contribute to our observational findings of hypovitaminosis D. The first is decreased endogenous production of vitamin D_3 from 7-dehydrocholesterol in skin by UV-B rays in sunlight. In practical term, optimal endogenous vitamin D3 production in response to sunlight in northern latitudes requires sunlight exposure in the warmer months and during peak sunlight hours (approximately 10:00 am to 3:00 pm). People who reside predominantly indoors during peak daylight hours produce very little vitamin D endogenously. Sunscreen use also dramatically decreases vitamin D production. Sunscreen with sun protection factor (SPF) 15 or higher blocks 99% or more of vitamin D production. Skin pigment acts as natural sunscreen, so people with darker skin i.e. increased melanin content requires 10 times more sun exposure to produce same amount of vitamin D as a person with pale skin. The ability to produce vitamin D also decreases as part with aging process, mainly related to decreased availability of 7-dehydrocholesterol[2], at the same time, kidneys become less efficient at converting to active vitamin D. Obese, a body mass index greater than 30 requires more vitamin D as body fat acts as a "sink" by collecting fat soluble vitamin D, does not allow it to be released into the bloodstream.[3,12]

The higher prevalence of hypovitaminosis D as well as vitamin D deficiency in our subjects may be due to inadequate sun exposure to produce sufficient amounts of vitamin D endogenously, with the exception of those who perforce need to work outdoors in the sun. Our social and/or religious norms related to public modesty dictate that most parts of an individual's body, irrespective of gender, be covered. Dhaka city is one of the most highly polluted cities as well as ranks 8th among most densely populated country in the world (44,500 people/km^2) as per UN habitat urban data, a majority of people are perforced to live in overcrowded tenements, which are closely packed and 6–7 stories high. Consequently, direct sunlight does not reach inside most parts of the dwellings, thereby disallowing any sun exposure to an individual in the privacy of one's home. Additionally, lack of space offers limited options for outdoor activities. Reduction of outdoor activities and space leads to confinement to indoor for longer time which leads to addiction of our young ones to computer games, facebooking and fast food consumption resulting in obesity and vitamin D deficiency. Atmospheric pollution of metropolitan Dhaka also factors with respect to vitamin D status, a higher degree of air pollution containing ozone leads to efficient atmospheric absorption of ultraviolet-B (UV-B) photons, thereby reducing the skin photosynthesis of vitamin D. Use of sunscreen creams and umbrellas do not help either. The extreme discomfort of the scorching heat associated with most sunny days of summer and (not to mention) the undying desire of most of us to attain a fairer skin complexion instantly extinguish any desire for sun exposure, and a person's primary focus is on finding ways to avoid the sun, at all cost. In the blazing heat of hot Bangladeshi summer these two concerns score very high and the quest for vitamin D sufficiency takes a backseat, always. Therefore, in our scenario, vitamin D sufficiency cannot be attained by depending on adequate sun exposure.

The second main factor for underlying vitamin D deficiency in our cases is low dietary intake. It is evident that most of the food items rich in vitamin D are of animal origin. Milk and milk products are unaffordable to the socioeconomically underprivileged Bangladeshi population. Not only that milk and milk products are rampantly diluted and/or adulterated. Intake of caffeine from tea is quite high in Bangladesh and the proportion of milk is very low in tea. Thus calcium intake through these beverages is low. Vitamin D is stable during cooking up to 200^{0}C. However, thermal stability of vitamin D is an inverse function of both temperature and time. In Bangladesh, milk is boiled for several minutes before consumption. Before the same lot of milk is consumed in entirety, it is subjected to two-three rounds of boiling. Our cooking practice dictates that tea has to be boiled for several minutes to right flavor. This boiling may reduce the content of any vitamin D that there may have been left after boiling of the milk itself. We are in general adhering to traditional cooking styles and practice. Consumption of uncooked fresh products, especially vegetables, milk etc., is generally considered ill-advised. Here slow cooking is widely practiced resulting thermal destruction of many vitamins. Pertaining shallow and deep-frying of food, most cooking fats and oils have smoke points above 180^{0}C. Shallow and deep frying of foods is very popular in our country. When foods are fried, vitamin D in the food comes out into the cooking medium and is thermally degraded.

With more studies, there are reports from many countries of a high prevalence of vitamin D deficiency in women of child-bearing age and during pregnancy as well as in nursing mothers. Breast milk contains low concentrations of vitamin D, especially in lactating women who themselves are vitamin D insufficient with likely adverse consequences for women, the fetus and growing infants and children. In more recent reports, 18% of pregnant women in the UK, 25% in the UAE, 80% in Iran, 42% in northern India, 61% in New Zealand and 60–84% of pregnant non-Western women in the Netherlands have been shown to have serum 25(OH)D concentrations <25 nmol/L. These studies raise the concern that infants are entering the world with a vitamin D deficit that begins in utero. This concern is based on the strong relationship between maternal and fetal (cord blood) circulating 25(OH) D levels, and is supported by recent studies from many countries, including the UAE, Iran, the United Kingdom, Greece and the US that have demonstrated a high prevalence of vitamin D deficiency in mother-infant pairs at birth. Even infants born to vitamin D replete mothers become vitamin D deficient after 8 weeks if not supplemented with vitamin D, while an unsupplemented infant born to a vitamin D deficient mother will reach a state of deficiency more quickly than an infant whose mother was replete during pregnancy.[8] These are similar to our findings.

The third main factor that can contribute to vitamin D deficiency in all age groups is malabsorption. Since vitamin D is a fat soluble vitamin, gastrointestinal condition that affects fat absorption may lower absorption of fat soluble vitamin D. Conditions that can reduce vitamin D absorption include use of bile acid sequestrants (eg, cholestyramine, colesevelam), cystic fibrosis, celiac disease and non-celiac gluten sensitivity, Whipple disease, Crohn's disease, inflammatory bowel disease and gastric bypass surgery.[2, 3]

Among multiple reasons for hypovitaminosis D, alteration in the vitamin D metabolism has been postulated after finding increased 24 hydroxylase activity in skin fibroblasts of South Asians.[13] Environmental agents such as betel nut chewing have been shown to be an independent determinant of increased 24 hydroxylase expression and of decreased serum calcitriol levels.[14]

Vitamin D deficiency is pandemic. It has been estimated that one billion people worldwide have vitamin D deficiency or insufficiency.[15] Vitamin D deficiency is multi-factorial in origin, and some of the risk factors are poor sunlight exposure, skin pigmentation, overpopulation, inadequate playing field, and dietary insufficiency. Atmospheric pollution has also been attributed to vitamin D deficiency in children. Intriguingly, despite ample sunshine, vitamin D deficiency is reported in widely prevalent tropical countries. Cultural and social practices including lifestyle pattern such as clothing (Burkha or veil including black socks covering hand and feet) that limit the exposure to sunlight impact the level of vitamin D in the body.[15] Consequently, subclinical vitamin D deficiency is widely prevalent. Our study is to create awareness among physicians about vitamin D deficiency as it remains asymptomatic or due to its vague nature of symptoms, it remains undetected in a majority of individuals. Clinical detection of symptomatic cases is the tip of iceberg of vitamin D deficiency. Laboratory professionals are confronted with substantial increase in laboratory testing due to growing clinical interest in vitamin D deficiency but avoidance of overscreening vitamin D remains important.

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