



VITAMIN D STATUS & ITS RELATION WITH BONE PAIN IN ADULT BANGLADESHI WOMEN: A STUDY AT A JCI ACCREDITED HOSPITAL IN BANGLADESH

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ABSTRACT

The present cross-sectional study was designed to evaluate the vitamin D status in adult Bangladeshi women by using serum 25 hydroxyvitamin D (S-25-OHD) and its relation with bone pain; a study in a JCI accredited hospital in Bangladesh. We consecutively studied 1013 women belonging to two groups. Apparently healthy women of reproductive age (15-45 years; group A, N = 486 mean $\pm$ SD age 33.3 ± 8.3 years) and postmenopausal women (46-70 years; group B, N = 527 mean $\pm$ SD age 57.7 ± 8.1 years) were the subjects of the study. The subjects in groups A, and B were randomly selected from the Apollo Hospitals Dhaka whose are came to the doctor in different illness. The mean value of S-25-OHD in group A was significantly different compared with groups B (50.4 ± 23.6 nmol/l vs. 39.6 ± 18.1 nmol/l, respectively; $P = 0.04$). No subject showed a serum 25 OHD level above the normal range (75-250 nmol/l) and the distribution in two groups was very close to the lower limit of the deficient range (<25 nmol/l). Vitamin D deficiency (S-25-OHD level <25 nmol/l) was Observed in 11.0% in group A & 20.0 % in group B, respectively. The insufficiency (S-25-OHD level 25-74 nmol/l) of Vitamin D was surprisingly very high in two groups; the prevalence was 60.0 % in group A & 75.0 % in Group B, respectively. In case of sufficiency, there is a Remarkable change between two groups. The prevalence was 29.0% in group A & only 5.0 % in group B, respectively. In our present study, the bone pain was extremely high in group B (67%) than group A (35%). In conclusion, the results of this study suggest that deficiency and insufficiency of vitamin D status are common in adult Bangladeshi women that they were equally at risk of developing vitamin D deficiency. Hypovitaminosis D could be an important public health problem in adult women of Bangladesh. In case of bone pain, the postmenopausal women are sigificantly high risk than the reproductive women due to lack of serious vitamin D insufficiency.

KEYWORDS: Vitamin D-25 dihydroxy, Bone Pain, Hypovitaminosis D.

INTRODUCTION

Vitamins are a category of nutrients that are essential for human health and development and generally must be obtained from the diet. Vitamins are required by the body in very small quantities and are, therefore, considered to be "micronutrients". Vitamins include niacin, riboflavin, thiamin, folic acid, vitamin B₁₂, vitamin B₆, pantothenic acid, biotin, vitamin C, vitamin K, vitamin E, vitamin A, and vitamin D. Vitamin D may be considered unique among vitamins because it is not, by definition, a true vitamin. Although the body can obtain vitamin D through a few natural food sources (including oily fish, eggs, and beef liver) and fortified foods in some developed countries (including commonly-consumed items such as milk and orange

juice), humans actually have the ability to synthesize vitamin D under certain conditions. Endogenous synthesis of vitamin D is a major contributor to an individual's vitamin D status.^[1]

Vitamin D refers to a group of fat-soluble secosteroids responsible for increasing intestinal absorption of calcium, iron, magnesium, phosphate, and zinc. In humans, the most important compounds in this group are vitamin D₃ (also known as cholecalciferol) and vitamin D₂ (ergocalciferol)^[2]. Cholecalciferol and ergocalciferol can be ingested from the diet and from supplements. Very few foods contain vitamin D such as fish-liver oils, fatty fish, mushrooms, egg yolks, and liver. Synthesis of vitamin D (specifically cholecalciferol) in the skin is the

major natural source of the vitamin. Dermal synthesis of vitamin D from cholesterol is dependent on sun exposure (specifically UVB radiation) (wikipedia, the free Encyclopedia). Vitamin D₃ is photosynthesized in the skin of vertebrates by the action of solar ultraviolet (UV) B radiation on 7 dehydrocholesterol.^[3]

Vitamin D₂ is produced by UV irradiation of ergosterol, which occurs in molds, yeast, and higher-order plants. Under conditions of regular sun exposure, dietary vitamin D intake is of minor importance. However, latitude, season, aging, sunscreen use, and skin pigmentation influence the production of vitamin D₃ by the skin.^[4] Most of the dietary intake of vitamin D comes from fortified milk products and other fortified foods such as breakfast cereals and orange juice. Both vitamin D₂ and D₃ are used in nonprescription vitamin D supplements, but vitamin D₂ is the form available by prescription in the United States. As cholecalciferol is synthesized in the skin by the action of ultraviolet light on 7-dehydrocholesterol, a cholesterol derivative, and vitamin D does not fit the classical definition of a vitamin. Nevertheless, because of the numerous factors that influence its synthesis, such as latitude, season, air pollution, area of skin exposed, pigmentation, age, etc., vitamin D is recognized as an essential dietary nutrient.^[5]

Bone pain is pain coming from the bone. It occurs as a result of a wide range of diseases and/or physical conditions and may severely impair the quality of life for patients who suffer from it^[6]. Bone pain belongs to the class of deep somatic pain, often experienced as a dull pain that cannot be localized accurately by the patient. This is in contrast with the pain which is mediated by superficial receptors in, e.g., the skin. Bone pain can have several possible causes ranging from extensive physical stress to serious diseases such as.^[7] For many years it has been known that bones are innervated with sensory neurons. Yet their exact anatomy remained obscure due to the contrasting physical properties of bone and neural tissue.^[8] More recently, it is becoming clear what types of nerves innervated which sections of bone.^[9] The periosteal layer of bone tissue is highly pain-sensitive and an important cause of pain in several disease conditions causing bone pain, like fractures, osteoarthritis, etc.

However, in certain diseases the endosteal and haversian nerve supply seems to play an important role, e.g. in osteomalacia, osteonecrosis, and other bone diseases. Thus there are several types of bone pain, each with many potential sources or origins of cause.

Methodology

Area of Study: This Study was conducted to clinical Biochemistry Laboratory, Apollo Hospitals, Dhaka, Bangladesh.

Study Populations: In this present study, the survey conducted as cross-selection. Patients from the different

places of Bangladesh go to the physicians of Apollo Hospitals, Dhaka, and then physicians suggest investigating vitamin D and others according to their present conditions. Patients give the blood sample in sample collection room for analysis. The selection of patients was performed by the following manner for this study.

Inclusion criteria

- i. Age between 18-70 years.
- ii. Patient with bone pain.
- iii. One sib of female IHD.
- iv. Respondents consented voluntarily.

Exclusion criteria

- i. Patients/sibs not consenting.
- ii. Acute or chronic diseases that can affect outcome.
- iii. Patient with severe illness as cancer, edema, allergy, known case of chronic diseases and other chronic disease will be excluded.
- iv. Pregnant & Lactating mother.

Time Frame: Study period was one year commencing from September, 2015 to August, 2016. To complete the study in time, a work schedule was prepared depending on different tasks of the study. One month was spent for selection of topic, development of the protocol. Others month were spent on official correspondence, data collection, data analysis, report writing and submission of report.

Sample Collections: Between September, 2015 to August, 2016 "Phlebotomists" from the Apollo Hospitals, Dhaka collected blood samples from the respective patients. Four milliliter of venous blood was drawn from each subject by vein puncture. Drawn blood was allowed to clot in a red tube. Then the sample was transferred to the clinical.

Biochemistry laboratory of Apollo Hospitals, Dhaka by Patients care attendance (PCA). After 20 minutes, samples were centrifuged at 4000 rpm for 10 minutes. Separated serum will be aliquoted in microcentrifuge tubes, labeled and preserved at -30°C for future biochemical analysis.

Statistical analysis: The statistical analysis was carried out by using the X² test and two-way analysis of variance (ANOVA) of IBM SPSS Statistics²⁰. *Post-hoc* analyses between the groups were carried out with two sided *t*-test. The results are expressed as mean ±SD, ranges, and percentiles. The minimum significance level used was <0.05. Then data were entered into computer and results were calculated with Microsoft® Excel 2016. The results were shown in Column and Scatter diagrams.

BIOCHEMICAL METHODS

Determination of vitamin D: Serum 25(OH) vitamin D concentrations were determined using commercially available radioimmunoassay kits from Beckman Coulter

(Access-2) and DiaSorin Liaison XL (MN, USA) by chemiluminescent immunoassay (CLIA) Method.

A. Instruments & Reagents: Both the Instrument & Reagent are manufactured by Beckman coulter Inc, Brea, California, USA.

Principles of the Procedure for Access 25(OH)

The Access 25(OH) Vitamin D Total assay is a two-step competitive binding immunoassay. In the initial incubation, sample is added to a reaction vessel with a DBP releasing agent and paramagnetic particles coated with sheep monoclonal anti-25 (OH) vitamin D antibody. 25 (OH) vitamin D is released from DBP and binds to the immobilized monoclonal anti-25 (OH)

Vitamin D on the solid phase. Subsequently, a 25(OH) vitamin D analogue-alkaline phosphatase conjugate is added which competes for binding to the immobilized monoclonal anti-25 (OH) vitamin D. After a second incubation, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of 25 (OH) vitamin D in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Vitamin D Status	25 (OH) Vitamin D Concentration Range (ng/mL)	25 (OH) Vitamin D Concentration Range (nmol/L)
Deficient	< 20	< 50
Insufficient	20 to < 30	50 to < 75
Sufficient	30 – 100	75 – 250
Upper Safety Limit	>100	> 250

B. Principles of the Procedure for Liaison XL

The role of vitamin D in bone and mineral metabolism was recognized from its first identification as a factor that could cure rickets. However, vitamin D is now recognized as a prohormone that has multiple roles in maintaining optimal health.

Vitamin D₃ (cholecalciferol) and Vitamin D₂ (ergocalciferol) are the most abundant forms of Vitamin D in the body. Vitamin D₃ is synthesized in the skin from 7-dehydrocholesterol in response to sunlight. The best nutrition sources of D₃ are oily fish primary salmon and mackerel. Vitamin D₂'s nutrition sources are from some vegetables, yeast, and fungi.

Vitamin D (D₃, D₂, and metabolites) is converted to 25-hydroxy vitamin D in the liver. The measurement of 25-OH vitamin D concentration in the serum or plasma is the best indicator of vitamin D nutritional status.

Expected Values: 20-50 ng/ml

25-OHD values < 20 are considered to be insufficient and values < 10 - 12 are associated with vitamin D deficiency and risk for osteomalacia. Although values of 20 or more are generally considered to be sufficient, values in the range of 20 - 30 may be insufficient in certain high risk patient subgroups. There is no known benefit of values > 50, and values > 100 should be avoided because of possible risk of vitamin D toxicity. Normal range cutoffs for screening are based on the Institute of Medicine (IOM) Committee's 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D. For a discussion of the controversy regarding normal range cutoffs and the IOM recommendations, see Rosen C, et al. J Clin Endocrinol Metab 97: 1146–1152, 2012.

RESULTS

The mean value of S-25-OHD in group A was significantly different compared with groups B (50.4 ± 23.6 nmol/l vs. 39.6 ± 18.1 nmol/l, respectively; $P = 0.04$). In group A, the value of S-25-OHD concentrations was ranging from 10.0 to 116.0 nmol/L & in group B, the range was 10.0 to 108.0 nmol/l, respectively. In the population we studied, no subject showed a serum 25 OHD level above the normal range (75-250 nmol/l) and the distribution in two groups was very close to the lower limit of the deficient range (<25nmol/l) (Fig-11) & (Fig-12). The observed median value of S-25-OHD was low in all two groups. The median values of S- 25OHD in groups A and B were 46.0 & 37.5 nmol/l, respectively. Vitamin D deficiency (S-25-OHD level <25 nmol/l) was Observed in 15.6 % of subjects of this study and the prevalence were 11.0% in group A & 20.0 % in group B, respectively. In this present study, the insufficiency (S-25-OHD level 25-74 nmol/l) of Vitamin D was surprisingly very high in two groups. Vitamin D insufficiency was observed in 67.8 % of subjects of this study and the prevalence was 60.0 % in group A & 75.0 % in Group B, respectively. On the other hand, in case of sufficiency, there is a Remarkable change between two groups. The sufficiency of Vitamin D (S-25 OHD level 75-250 nmol/l) was observed in 16.5 % of subjects of this study and the prevalence were 29.0% in group A & only 5.0 % in group B, respectively. In our present study, the bone pain was observed in 51.7% of total populations and this is extremely high in group B than group A. The prevalence was 35% in group A & 67% in group B.

The LIASION 25 OH Vitamin D assay is a direct competitive chemiluminescence immunoassay (CLIA) for quantitative determination of total 25 OH vitamin D in serum. During the first incubation, 25 OH Vitamin D

is dissociated from its binding protein and binds to the specific antibody on the solid phase. After 10 minutes the tracer, (vitamin D linked to an isoluminol derivative) is added. After second 10 minute incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added to initiate a flash chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLU) and is inversely proportional to the concentration of 25 H vitamin D present in calibrators, controls, or samples.

1. Distribution of Serum 25-hydroxy vitamin D concentration in Reproductive Woman.

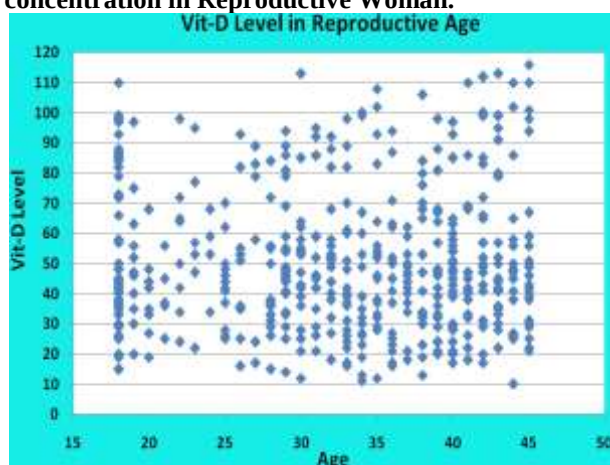


Fig-1: Distribution of Serum 25-hydroxyvitamin D concentration of woman in Reproductive Age.

2. Distribution of Serum 25-hydroxyvitamin D concentration in Postmenopausal Woman.

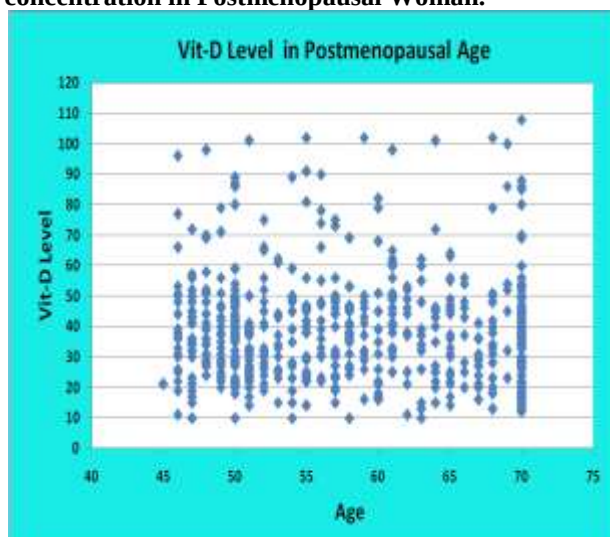


Fig-2: Distribution of Serum 25-hydroxyvitamin D concentration of woman in Postmenopausal Age.

3. Status of Serum 25-hydroxyvitamin D concentration in Reproductive Woman

Total Population	486
Deficient	53
Insufficient	292
Sufficient	141

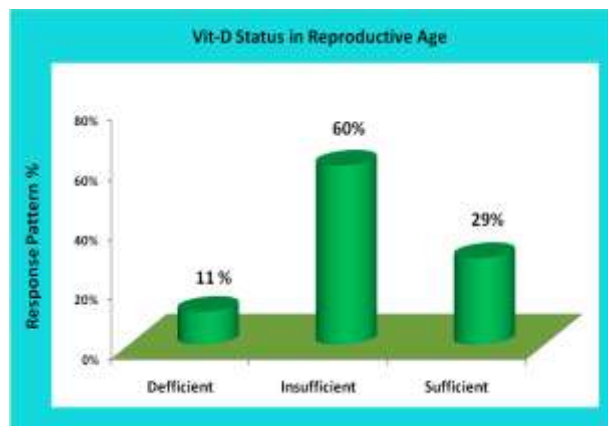


Fig-3: Status of Serum 25-hydroxyvitamin D concentration of woman in Reproductive Age.

4. Status of Serum 25-hydroxyvitamin D concentration in Postmenopausal Woman:

Total Population	527
Deficient	105
Insufficient	395
Sufficient	26

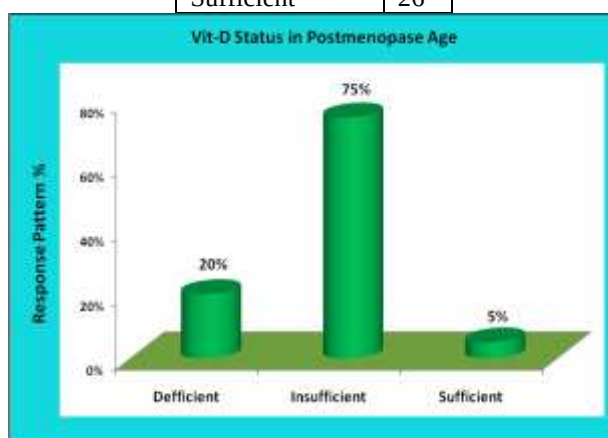


Fig-4: Status of Serum 25-hydroxyvitamin D concentration of woman in postmenopausal age.

5. Deficient level of Vit-D between Reproductive & Postmenopausal Woman:

Total Population	1013
Reproductive	53
Postmenopausal	105

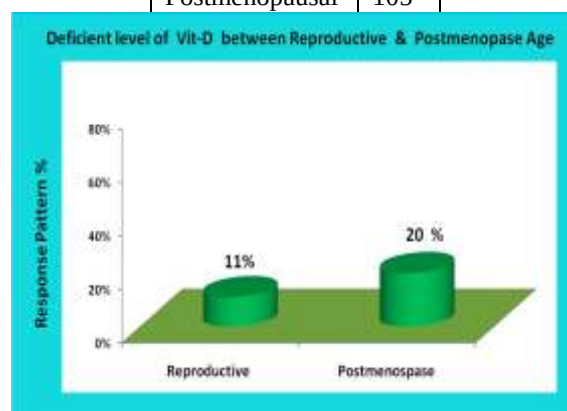


Fig-5: Deficient level of Vit-D between Reproductive & Postmenopausal Age.

6. Insufficient level of Vit-D between Reproductive & Postmenopausal woman.

Total Population	1013
Reproductive	292
Postmenopausal	395

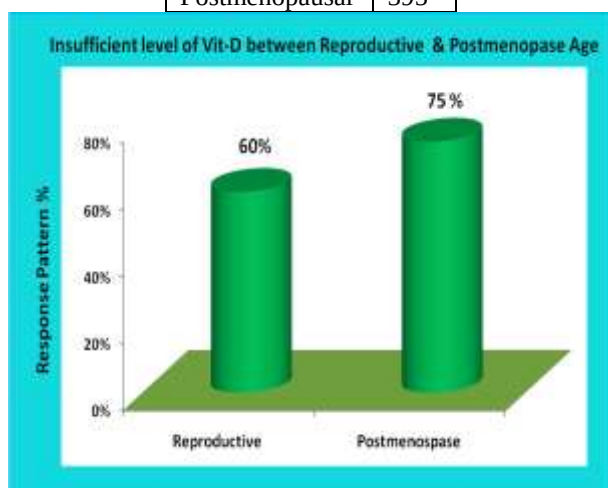


Fig-6: Insufficient level of Vit-D between Reproductive & Postmenopausal Age.

7. Sufficient level of Vit-D between Reproductive & Postmenopausal Woman:

Total Population	1013
Reproductive	141
Postmenopausal	26

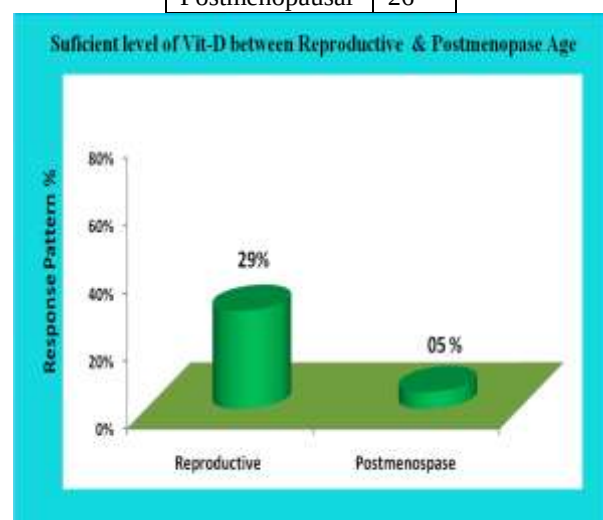


Fig-7: Sufficient level of Vit-D between Reproductive & Postmenopausal Age.

8. Bone Pain of Reproductive woman in relation with vit-D

Total Population	486
Yes	170
No	316

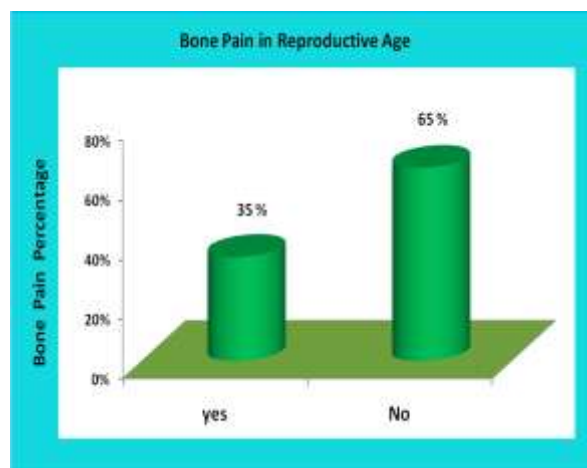


Fig-8: Bone Pain in Reproductive Age.

9. Bone Pain of Postmenopausal woman in relation with vit-D

Total Population	527
Yes	354
No	173

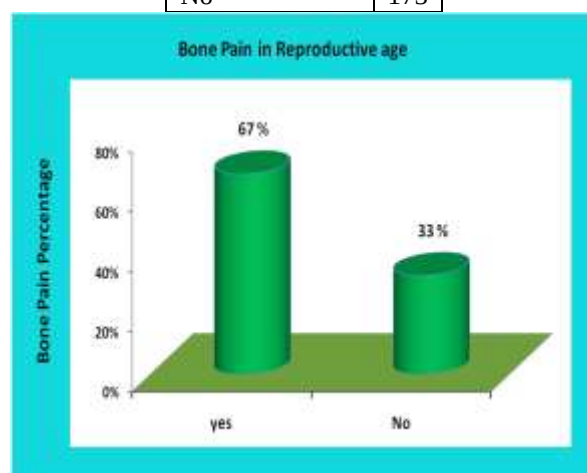


Fig-9: Bone Pain in Postmenopausal Age.

10. Comparison of Bone Pain between Reproductive & Postmenopausal woman in relation with vit-D:

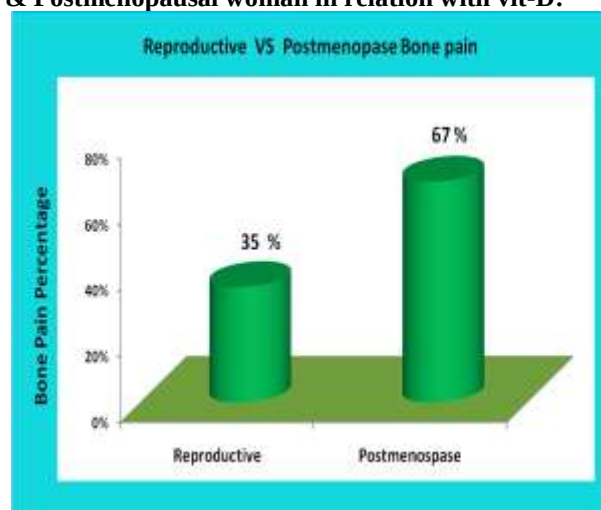


Fig-10: Comparison of Bone Pain between Reproductive & Postmenopausal age.

DISCUSSION

A significant proportion of the Bangladeshi women involved in this study had optimal vitamin D status and its relations with bone pain. The mean serum vitamin D level among the reproductive women in the current study was 50.4 nmol/L and 39.6 nmol/L in the postmenopausal women, which is well below the normal value of 75-250 nmol/L defining optimal vitamin D status. Over 67.8% of the women were below the normal (75-250 nmol/L) value, indicating that vitamin D insufficiency is a substantial problem in this population. These findings are consistent with results from other studies conducted among women of reproductive age in Bangladesh^[10,11] Our results indicated that hypovitaminosis D is highly prevalent in the population studied, whether reproductive or postmenopausal. 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 postmenopausal 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. But this condition is low viewed in reproductive women. 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^[12]

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. In the present study, the reproductive woman who comes to the Apollo hospitals Dhaka with high income and educational levels reported that they usually spent many time daily in outside activities and they wear ultra modern dresses not borkha compared with postmenopausal women usually spent many time in house and when go outside wear borkha. The serum 25-OHD levels could be significantly higher in reproductive women by increasing their outdoor activities with adequate sunlight exposure to the skin. Bangladeshi reproductive women 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 reproductive women (46.0 nmol/l). The time spent out-doors activity high in this group 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

hypovitaminosis D in young adults and the elderly population. Van der Wielen *et al.*, (1995)^[13] 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. 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.^[14] In a study among Australian Muslims healthy women reported a high prevalence (68%) of vitamin D deficiency.^[15] The literature showed that reproductive subjects spending more time outdoors in the sunlight wearing ultra modern dresses not borkha 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 vitamin 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^[16]. 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 25-74 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 (Chapuy MC; Dawson-Hughes B; Guillemant J *et al.*, 1999). Based on this definition, nearly 67.8% of the subjects in this study have vitamin D insufficiency, whereas only 16.5% populations were sufficient amount of Vitamin D. So the present study shows that most of the populations are Vitamin D insufficient. Vitamin D status of different population groups in Bangladesh is scarce. A decrease in the vitamin D status with advancing age has been observed in several studies in different populations (Tsai *et al*, 1987; 1991; Dubbelman *et al*, 1993; van der Wielen *et al*, 1995). Therefore, the findings of this study suggest that both the reproductive & postmenopausal women's in Bangladesh could be at substantial risk of inadequate vitamin D status and further study is recommended to detect the severity of vitamin D deficiency in this population group.

The final hypothesis is about hypovitaminosis D induced bone pain. There is evidence for an association between low vitamin D status and pain in the general population^[17]. The pain included chronic back pain, diffuse musculoskeletal pain or chronic widespread pain, and polymyalgia; and vitamin D was suggested as the cause of such nonspecific pain. According to Gregory E. Hicks *et al.*, 2008, there is a unique relationship was

found between vitamin D status and back pain, in those older women with vitamin D deficiency were more likely to have significant back pain. In our present study, the bone pain was observed in 51.7% of total populations and this is significantly high in postmenopausal women (67%) than reproductive women (35%). One plausible explanation is that vitamin D deficiency leads to osteoporosis, which commonly seen in older women^[18]. In osteoporosis, increased bone weakness increases the risk of a broken bone among the elderly. Chronic pain and a decreased ability to carry out normal activities may occur following a broken bone.

CONCLUSION

In conclusion, the results of this study suggest that vitamin D deficiency and insufficiencies of vitamin D status are common in adult Bangladeshi women that they were equally at risk of developing vitamin D deficiency. The prevalence of hypovitaminosis D status was higher in the postmenopausal women (67%) than the reproductive women (35%) of this study. The results indicated that hypovitaminosis D could be an important public health problem in adult women of Bangladesh. In case of bone pain, the postmenopausal women are significantly high risk than the reproductive women due to lack of serious vitamin D insufficiency. Therefore, it is important to pay attention to this rather high prevalence of insufficient or borderline vitamin D status and more active measures need to be taken by giving information to health professionals and the subjects themselves about the importance of vitamin D for health, including the need for out-of-doors exposure to sunlight, adequate dietary intake of vitamin D and implementation of current recommendations to improve their vitamin D status. Recent work in the area of vitamin D status and pain suggest a need for continued research in this area, particularly in postmenopausal women who require greater levels of sunlight exposure to synthesize vitamin D. According to jannatul mawa et al., 2013^[18], the Bangladeshi college going students are not enough knowledge about vitamin D and its supplementation. A comprehensive programme to prevent vitamin D deficiency in Bangladeshi women is recommended. In this case high intake of vitamin D-rich food as well as inclusion of a food-fortification programme could be suggested. The government and policy makers should pay attention about improving this situation by utilizing mass media and print media to increase awareness regarding vitamin D.

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