Vitamin D Deficiency and Insufficiency in Healthy Pregnant Women Living In Dhaka, Bangladesh

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Abstract
Background/Objectives: Optimal level of 25-hydroxy-vitamin D (25OHD) in serum (concentration above 30 ng/ml) is essential for protecting the health of the mother and the developing fetus. Vitamin D plays an important role in maintaining proper bone structure, preventing infections, reducing the risk of premature birth and gestational diabetes. The aim of the study was to verify whether healthy pregnant residents of Dhaka, Bangladesh were low (deficient and insufficient) in vitamin D. Material and methods: The material consisted of 140 serum samples of different trimesters of pregnancy. The concentration of 25OHD was measured using the vitamin D total assay on Snibe Maglumi 1000 CLIA system (Diamond Diagnostics Inc., China). Results: The average serum 25OHD concentrations was 19.3ng/ml, with no statistically significant differences (chi-square value, χ² =0.562). The optimal levels of 25OHD (30-76ng/ml) were found in 5.7% of samples, insufficient amount or hypovitaminosis (20-30ng/ml) occurred in 31.4%, deficiency (less than 20ng/ml) in 60.7% and severely deficiency (less than 10ng/ml) in 2.1%. Conclusions: Vitamin D below the optimal level is a common occurrence during pregnancy and Bangladesh Government need to set the level of supplementation among pregnant women appears to be deficient and insufficient. Our data suggest that special attention should be paid to the problem of low vitamin D in pregnant women.

Keywords: Vitamin D, Deficiency, Insufficiency, Pregnant Woman.

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I. Introduction
Vitamin D is a group of fat-soluble secosteroids responsible for increasing intestinal absorption of calcium, magnesium, and phosphate, and multiple other biological effects. In humans, the most important compounds in this group are vitamin D3 (also known as cholecalciferol) and vitamin D2 (ergocalciferol). Cholecalciferol and ergocalciferol can be ingested from the diet and from supplements. Only a few foods contain vitamin D. The major natural source of the vitamin is synthesis of cholecalciferol in the skin from cholesterol through a chemical reaction that is dependent on sun exposure (specifically UVB radiation).1

Vitamin D deficiency, or hypovitaminosis D, most commonly results from inadequate sunlight exposure (in particular sunlight with adequate ultraviolet B rays). Vitamin D deficiency can also be caused by inadequate nutritional intake of vitamin D, disorders limiting vitamin D absorption, and conditions impairing vitamin D conversion into active metabolites-including certain liver, kidney, and hereditary disorders. Deficiency impairs bone mineralization, leading to bone softening diseases as rickets in children. It can also worsen osteomalacia and osteoporosis in adults, leading to an increased risk of bone fractures. Muscle weakness is also a common symptom of vitamin D deficiency, further increasing the risk of fall and fracture in adults.2

Vitamin D has an increasingly recognized repertoire of nonclassical actions, such as promoting insulin action and secretion, immune modulation and lung development. It therefore has the potential to influence many factors in the developing fetus.

Vitamin D and its active metabolite 1,25-dihydroxyvitamin D (1,25(OH)2D) have classical actions on calcium balance and bone metabolism. Without sufficient 1,25(OH)2D, the intestine cannot absorb calcium and phosphate adequately, which leads to secondary hyperparathyroidism and a lack of new bone mineralisation (rickets in children and osteomalacia in adults). Rickets is a childhood vitamin D insufficiency and usually

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develops many months after delivery. However, the neonate is at risk of hypocalcaemic tetany consequent on maternal hypovitaminosis D. Calcium levels are normal in utero when maternal vitamin D is insufficient. However, when maternal calcium delivery is interrupted at birth, the neonate may develop hypocalcaemia. While the developing fetus requires approximately 30 g of calcium, the maternal gut adapts and can overcome some vitamin D insufficiency with increased calcium transport.

Vitamin D deficiency is common in northern Europe, especially in women with pigmented skin. Vitamin D deficiency is three times more common in the winter and spring compared to the summer and autumn in the UK. In a London antenatal population, a vitamin D level of less than 25 mmol/l or 10ng/ml was found in 47% of Indian Asian women, 64% of Middle Eastern women, 58% of black women and 13% of Caucasian women. In the general adult population, reduced vitamin D concentrations are found in obese subjects. Prepregnancy obesity has been associated with lower levels of vitamin D in both pregnant women and their neonates.

There are two forms of vitamin D. Vitamin D₃ (cholecalciferol) is produced from the conversion of 7-dehydrocholesterol in skin and vitamin D₂ (ergocalciferol) is produced in mushrooms and yeast. The biologically active form of vitamin D is 1,25(OH)₂D. This requires hydroxylation of vitamin D in the liver to 25(OH)D (25-hydroxyvitamin D), which then undergoes renal hydroxylation to form 1,25(OH)₂D. Although 25(OH)D has low biological activity, it is the major form of circulating vitamin D. Serum 25(OH)D concentrations are generally thought to reflect nutritional status. Production of 1,25(OH)₂D in the kidney is tightly regulated by plasma parathyroid hormone (PTH) as well as serum calcium and phosphate levels.

The interaction of 1,25(OH)₂D with nuclear vitamin D receptors influences gene transcription. Nuclear receptors for 1,25(OH)₂D are present in a range of tissues including bone, intestine, kidney, lung, muscle and skin. Similar to steroid hormones, 1,25(OH)₂D acts via signal transduction pathways linked to vitamin D receptors on cell membranes. Major sites of action include intestine, bone, parathyroid, liver and pancreatic beta cells. Its biological actions include increases in intestinal calcium absorption, transcellular calcium flux and opening gated calcium channels allowing calcium uptake into cells such as osteoblasts and skeletal muscle. The biological effects of 1,25(OH)₂D are diverse. It inhibits PTH secretion and adaptive immunity, while promoting insulin secretion and innate immunity. It also inhibits cell proliferation and stimulates their differentiation.

The largest source of vitamin D in adults is synthesis from solar radiation; half an hour of sunlight delivers 50000 iu or 1.25mg of vitamin D with white-complexioned skin. Dietary intake of vitamin D makes a relatively small contribution to overall vitamin D status as there is little vitamin D that occurs naturally in the food supply. Melanin absorbs ultraviolet B (UVB) from sunlight and diminishes cholecalciferol production by at least 90%. Dietary vitamin D is absorbed from the intestine and circulates in plasma bound to a vitamin D binding protein. Pre-eclampsia and neonatal hypocalcaemia are the most prevalent complications of maternal hypocalcaemia and are clearly associated with substantial morbidity. A statistical association of glucose intolerance and hypovitaminosis D has been demonstrated. Maternal vitamin D is important to fetal bone development. Fetal lung development and neonatal immune conditions such as asthma may relate in part to maternal vitamin D levels. Although it is not clear whether maternal vitamin D supplementation will prevent these conditions, a strategy for supplementation and treatment of maternal vitamin D deficiency is proposed.

There is conflicting evidence whether hypovitaminosis D in pregnancy is associated with hypertension and pre-eclampsia. In three studies, women who developed pre-eclampsia were found to have lower levels of vitamin D than women who did not, with levels less than 50 nmol/l (20ng/ml) associated with a five-fold increased risk of severe pre-eclampsia. Low levels in the first half of pregnancy were related to the risk of developing pre-eclampsia and the neonates of these mothers had a two-fold increased risk of having vitamin D levels < 37.5 nmol/l or 15ng/ml (vitamin D deficient). In a case–control study, women with severe pre-eclampsia before 34 weeks of gestation had reduced levels of vitamin D compared to control women. Furthermore, women with early-onset severe pre-eclampsia and a small-for-gestational-age (SGA) infant had significantly lower vitamin D levels than those with early-onset severe pre-eclampsia but non-SGA infants. However, many studies have shown a weak or no relationship between vitamin D and hypertensive disorders in pregnancy. A Canadian study showed that women with low circulating maternal vitamin D levels are more likely to have hypertension in pregnancy in the univariate analysis, but not the multivariate analysis. Another study failed to show any association between vitamin D levels and the development of pre-eclampsia, gestational hypertension or preterm birth. A similar study from the USA also failed to demonstrate an association between maternal first trimester vitamin D levels and the subsequent development of preeclampsia after controlling for BMI. However, two meta-analyses, including a meta-analysis of 31 studies, demonstrated that vitamin D insufficiency was associated with pre-eclampsia and SGA infants.

Maternal vitamin D levels have been shown to positively correlate with birthweight centile. In a study from Holland, women with vitamin D deficiency had a 2.4-fold increased risk of having an SGA baby. Another study found that maternal vitamin D levels of < 37.5 nmol/l (15ng/ml) in the first half of pregnancy were associated with an adjusted odds ratio of 7.5 for SGA infants in white women, but not in black women.
Australian researchers found that mean birthweight was 200g lower (P < 0.001) in babies of vitamin D deficient mothers. However, other studies demonstrated no relationship between maternal vitamin D levels in the first trimester and birthweight but did demonstrate that low vitamin D levels in late pregnancy were associated with reduced intraterine long bone growth and lower gestational age at delivery.

Hypovitaminosis D is associated with impaired glucose tolerance and diabetes in the general population. However, the evidence for an association between low vitamin D levels and gestational diabetes mellitus (GDM) is conflicting. Low concentrations of 25(OH)D have been related to the risk of developing type II diabetes mellitus (T2DM) through effects on insulin secretion and insulin sensitivity. However, not all studies support these findings. The Third National Health and Nutrition Examination Survey (NHANES III) did not demonstrate an association between 25(OH)D levels and diabetes or insulin resistance in African Americans, in contrast to Caucasians and Mexican Americans.

In another study of European Caucasian subjects, insulin secretion and action were not associated with levels of 25(OH)D. It is vital that such studies are controlled for obesity, a risk factor itself for vitamin D deficiency. GDM is considered to share the same pathogenesis as T2DM and similar associations between 25(OH)D and the development of GDM have been sought. Maternal 25(OH)D concentrations have been related to the risk of developing GDM in various cohorts.

Depending on the diagnostic criteria used, it has been suggested that GDM complicates up to 16% of pregnancies, although the true incidence can be much greater in some ethnic groups. There are some data to suggest that the association between 25(OH)D levels and GDM risk is specific to ethnicity. In a majority non-Hispanic white population, 25(OH)D concentrations at 16 weeks of gestation were significantly lower in GDM subjects than in controls, whereas no association was found in Indian mothers where 25(OH)D concentrations were measured at 30 weeks of gestation. Some studies have investigated more than one ethnic group using statistical techniques to correct for the effect of ethnicity, but none have been designed to describe the association in specific ethnic populations. Conversely, a well-conducted study has found no association between maternal 25(OH)D and the development of GDM. A meta-analysis of 31 studies demonstrated vitamin D insufficiency was associated with a higher risk of GDM.

Vitamin D deficiency (<37.5 nmol/l or 15mg/ml) has been associated with a four-fold increased risk of primary caesarean section (caesarean section performed for the first time), although this has not been demonstrated in all studies. Vitamin D deficiency is also associated with bacterial vaginosis in pregnant women.

In conclusion, hypovitaminosis D may be associated with hypertension, pre-eclampsia and increased caesarean section rates. There are no randomised trials showing that vitamin D supplementation alters these putative risks.

Neonatal vitamin D levels are correlated with those of their mother, with maternal vitamin D deficiency increasing the risk of neonatal vitamin D deficiency. In an Australian study, hypovitaminosis D was found in 15% of pregnant women and 11% of neonates. Vitamin D deficiency is a major cause of hypocalcaemic seizures in neonates and infants. Hypocalcaemia is not uncommon in neonates and is a potentially severe problem. Mothers of babies who suffer hypocalcaemic seizures are more likely to be vitamin D deficient (85%) than mothers of babies who do not (50%). In another study from Egypt, all mothers of babies with hypocalcaemic seizures had severe vitamin D deficiency.

Maternal vitamin D deficiency is a common, and potentially preventable, cause of neonatal hypocalcaemia. This is especially common in South Asian women.

Hypovitaminosis D is associated with impaired growth and bone development in the fetus. Evidence is accruing to show that less profound maternal 25(OH)D insufficiency may lead to suboptimal bone size and density after birth without overt rachitic change. This is likely to lead to an increased risk of osteoporotic fracture in later life. A retrospective cohort study showed that children who had received supplements with vitamin D in the first year of life had a significant increase in femoral neck bone density at the age of 8 years compared to the group that did not receive supplements. In a UK mother–offspring cohort, 31% of the mothers had circulating concentrations of 25(OH)D in late pregnancy of 27–50 nmol/l or 10.8–20mg/ml. There was a positive association between maternal 25(OH)D concentration in late pregnancy and whole body bone mineral content and density, assessed using dual energy X-ray absorptiometry (DEXA), in the offspring at 9 years of age. Furthermore, maternal UVB exposure and vitamin D supplementation were associated with the bone mass of the child (P < 0.05), while lower levels of umbilical-venous calcium were also associated with lower childhood bone mass, suggesting a possible role for placental calcium transport in this process. Additionally, maternal UVB exposure during pregnancy was positively associated with whole body bone mineral content in the offspring at the age of 9 years in the Avon Longitudinal Study of Parents and Children, although later analysis does not confirm these data. Similar findings have come from another UK cohort, the Southampton Women’s Survey, in which neonatal bone area and bone mineral content were reduced in the
female offspring of mothers who had 25(OH)D concentrations < 33 nmol/l (13.2ng/ml) in late pregnancy. These findings of altered neonatal bone mass have been confirmed by a Finnish mother-offspring cohort in which babies born to mothers with circulating 25(OH)D status below the median (42.6 nmol/l or 17.04ng/ml) had reduced tibial bone mineral content and cross-sectional area, measured by peripheral quantitative computed tomography (pQCT). In a follow-up study, a deficit in tibial cross-sectional area was still observed at 14 months' follow-up,44 despite the low vitamin D group catching up with the other group for the bone mineral content.

Evidence that 25(OH)D-related changes may be detectable early in gestation has come from the Southampton Women's Survey. In this cohort, fetal distal femoral metaphyseal cross-sectional area was increased relative to femur length at 19 and 34 weeks of gestation in those babies whose mothers had low levels of circulating 25(OH)D, changes reminiscent of those seen in postnatal rickets. These findings suggest that the adverse consequences of maternal vitamin D deficiency for the offspring are manifest early in pregnancy. There are no data from randomised controlled trials to show benefit from maternal vitamin D supplementation in terms of fetal or longer term growth of the child.

Low maternal vitamin D intake in pregnancy is associated with wheeze and asthma in the offspring. Low cord blood 25(OH)D concentrations have been associated with respiratory syncytial virus bronchiolitis and respiratory infections. There are plausible physiological mechanisms for an association between prenatal vitamin D status and immune development. The metabolite 1,25(OH)2D has been shown in animal and in vitro models to have an immune-modulatory role and low levels of neonatal vitamin D have been linked to childhood asthma. Maternal vitamin D supplementation is associated with cord blood gene expression of tolerogenic immunoglobulin such as immunoglobulin-like transcripts 3 and 4 (ILT3 and ILT4). Cord blood 25(OH)D is correlated with mononuclear cell release of IFN-γ and hence Th1 cell development.

The aim of the study was to assess whether randomly selected healthy pregnant residents of Dhaka were low in vitamin D and to measure the extent of that deficit.

II. Materials And Methods

Study group
The material consisted of serum samples from 140 healthy volunteers of different trimesters of pregnancy. All women were non-smokers and healthy.

Study Place and Time
The study took place in Crescent Hospital, Uttara, Dhaka, Bangladesh from January 2017 to March 2018.

Vitamin D Assay
MAGLUMI 25-OH Vitamin D (CLIA) kit (Shenzhen New Industries Biomedical Engineering Co., Ltd., China) was used for the quantitative determination of 25-OH Vitamin D in human serum. The method, competitive immunoluminometric assay, can be used for samples over the range of 3-150ng/ml.

Competitive immunoluminometric assay: Used a purified 25-OH Vitamin D antigen to label ABEI, and used 25-OH Vitamin D monoclonal antibody to label FITC. Sample, Calibrator, or Control, Displacing reagent, FITC Label and magnetic microbeads coated with anti-FITC were mixed thoroughly and incubated at 37 °C, forming antibody-antigen complexes; After sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time. Then added ABEI Label, incubation and washing for the 2nd time, sample antigen and ABEI labeled antigen compete to combine with FITC labeled monoclonal antibody, forming antibody-antigen complexes. Subsequently, the starter reagents were added and a flash chemiluminescent reaction was initiated. The light signal was measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of 25-OH Vitamin D present in controls or samples. 100μl sample, calibrator or control is mixed with 100μl FITC Label, 50μl Displacing reagent, 20μl Nano magnetic microbeads, 20 min Incubation. 400μl each time Cycle washing. Add 200μl ABEI Label. Incubation for 10 min. 400μl each time Cycle washing and finally Measurement.

Vitamin D status
Vitamin D supply in adults, based on serum 25OHD concentration, can be divided into the following categories: severe deficiency (<10 ng/ml), medium deficiency (10-20 ng/ml), hypovitaminosis D (20-30 ng/ml), recommended or optimum level (30-76 ng/ml) and toxic level (>100 ng/ml). These categories differ slightly from the World Health Organization and American and Canadian classification, where levels below 10 ng/ml (USA) or below 20 ng/ml (Canada) were considered deficient and levels below 30 ng/ml were classified as insufficient. An optimal level is typically defined as serum 25OHD level between 30-76 ng/ml.
Statistical Analysis

Data were assessed using the Statistical Package for Social Science (IBM SPSS Statistics, version 18, IBM Corporation, SPSS Inc. Chicago, III, USA) and Microsoft Office Excel 2007.

III. Results

Total 140 serum samples were collected from healthy pregnant women. 23 (16.4%) samples were in the age of ≥18 to ≤23 years, 46 (32.9%) samples were in ≥23 to ≤28 years, 30 (21.4%) samples were in ≥28 to ≤33 years, 30 (21.4%) samples were in ≥33 to ≤38 years and 11 (7.9%) samples were in ≥38 to ≤43 years. Highest number pregnant women were found in the age group ≥23 to ≤28 years (Fig. 1).

Table 1 showed that 2.1% pregnant woman suffered from severe vitamin D deficiency (≤10ng/ml). Among them 1.4% in respect to total participants were in the ≥18 to ≤23 years age group and 0.7% were in the ≥33 to ≤38 years age group. 60.7% participants showed vitamin D deficiency (10-20ng/ml). Of the 60.7% participants 7.9% in respect to total participants were in the ≥18 to ≤23 years age group, 20.7% were in the ≥23 to ≤28 years, 15.7% were in the ≥28 to ≤33 years, 12.2% were in the ≥33 to ≤38 years and 4.3% were in the ≥38 to ≤43 years age group. 44% pregnant women were identified with vitamin D insufficiency (20-30ng/ml). In this 44%, 6.4% of 140 participants were in the age group ≥18 to ≤23 years, 8.4% were in the ≥23 to ≤28 years, 5.7% were in the ≥28 to ≤33 years, 7.1% were in the ≥33 to ≤38 years and 3.6% were in the ≥38 to ≤43 years age group. Of the 5.7% participants 2.1% in respect to total participants were in the ≥18 to ≤23 years age group, 2.1% were in the ≥23 to ≤28 years, 0% were in the ≥28 to ≤33 years, 1.4% were in the ≥33 to ≤38 years and 0% were in the ≥38 to ≤43 years age group. In respect to the toxicity of vitamin D (≥100ng/ml) no pregnant woman was found in this category.
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IV. Discussion

Although the optimal serum level of 25OHD for adults is above 30 ng/ml, according to international recommendations, population studies indicate that vitamin D insufficiency is a global epidemic. The prevalence of vitamin D insufficiency in the Bangladeshi population remains largely unknown. Our results, coming from a group of healthy pregnant women living in Dhaka, indicate that low vitamin D in terms of ‘deficiency’ severely affected 2.1%, affected 60.7% and in terms of ‘insufficiency’ affected 31.4% of the studied individuals and average 25OHD serum concentration was 19.3 ng/ml.

During pregnancy vitamin D demand is even higher than for non-pregnant women, due to growing needs of the mother and the child. Thus, it is unsurprising that vitamin D supplementation is highly necessary and vitamin supplements containing vitamin D3 or D2 are most recommended to pregnant women.

Our study reveals a high prevalence of Vitamin D deficiency and insufficiency in pregnant women of Dhaka, Bangladesh. Hypovitaminosis D and osteomalacia among pregnant South Asian women have been widely reported. Vitamin D deficiency has also been reported in pregnant women in tropical countries, but all studies were in Muslim populations, in whom the practice of purdah might have played an important role. Vitamin D deficiency may be due to less sun exposure. Since there is positive correlation between sunlight and Vitamin D status, the observed decrease in Vitamin D concentration and deficiency in pregnant women can be attributed to sedentary indoor lifestyle in which there is less exposure to sun. Another possible reason of Vitamin D deficiency in pregnant women is attributable to diets that are not rich in Vitamin D. Dietary sources consumed by pregnant women are very low in Vitamin D content. Bangladeshis are usually not eating salmon, sardines, tuna, mackerel which are rich in Vitamin D content. The explanation could also lie in prolonged deficiency of dietary calcium intake among Bangladeshi population because of the expensive nature of milk and milk products. Dietary calcium deficiency has been shown to lead a secondary Vitamin D deficiency in rats. Similar findings are also suggested in studies on humans.

V. Conclusion

Serum Vitamin D concentration were observed low in 94.1% of the participated pregnant women. Supplementation of Vitamin D during the entire period of pregnancy is recommended in order to avoid the complications associated with Vitamin D deficiency during pregnancy. Vitamin D below the optimal level is a common finding in pregnant residents of Dhaka. Vitamin D supplementation level during pregnancy through multivitamin tablets (typically containing 400 IU vitamin D3) in Bangladesh need to focus strongly. Our data suggest that special attention should be paid to the issue of vitamin D deficiency and insufficiency in pregnant women.

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