Role of vitamin D: current evidence in Gynecology

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Vitamin D (calcitriol) is a hormone, long known to be a key modulator of bone metabolism, involved in the maintenance of calcium homeostasis by intestinal and renal calcium absorption. The vast majority of the effects of vitamin D is mediated by the receptor found to bind 1,25 dihydroxyvitamin D. The vitamin D receptor is expressed in almost all body cells, including immune, vascular, ovary and pituitary gland. This evidence suggests the implication of vitamin D in diverse physiological processes as immune function, vascular health, placental function, and led to extensive research on vitamin D as a potential influencing factor in the pathogenesis of a number of non-skeletal diseases, including gynecologic conditions and disorders.

The biologic evidence regarding a role for vitamin D in reproductive outcomes and gynecology has grown during the last years. Several studies suggest a link between low level of serum 25-hydroxyvitamin D and increased risk of adverse pregnancy outcomes for mothers (i.e. pre-eclampsia and gestational diabetes), and for newborns (i.e. low birth weight, rickets and increased incidence of autoimmune diseases). Some recent studies have looked also at the association of vitamin D status, sexual maturation and female reproduction. However, even if cholecalciferol supplementation is usually prescribed to pregnant women, no consensus recommendation has yet defined the optimum vitamin D supplementation dosage. This narrative review therefore pursues two aims: (i) summarizing the main current evidence on the role of vitamin D in pregnancy, perinatal health and disorders such as early menarche and dysmenorrhea, in order to shed lights on vitamin D phy...
Vitamin D metabolism and mechanism of actions

The actions of vitamin D are exerted by its active metabolite, namely 1,25 di-hydroxyvitamin D or calcitriol, which is produced through a series of enzymatic steps starting from cholecalciferol or vitamin D₃ (VD₃). The ergocalciferol, or vitamin D₂, follows the same metabolic stages. Vitamin D₃ is produced at skin level, when UV light interacts with a cholesterol derivatives or ingested in the diet (fish oils, fatty fish, cheese, and egg yolks). Vitamin D₂ is derived from plant sterols.

Nowadays, it has become a platitude to affirm that humans naturally obtain 90% of vitamin D from sunlight exposure and 10% from diet (1). Exposure to sunlight, especially ultraviolet B photons, at skin level, initiates conversion of provitamin D₃ (7-dehydrocholesterol) to VD₃ (cholecalciferol). VD₃ in turn binds to vitamin D binding protein, to reach the bloodstream, and be rapidly stored in fat or metabolized in the liver (2).

Both VD₂ and VD₃ have to be hydroxylated to become active. The first hydroxylation undergoes in the liver by 25-hydroxylase. The resulting metabolite, 25-hydroxyvitamin D [25(OH)D or calcifediol], is very stable respect to calcitriol and is therefore most commonly used to measure vitamin D status.

The second hydroxylation bringing to the active form 1,25 di-hydroxyvitamin D [1,25(OH)2D, calcitriol or active vitamin D] occurs mostly in the kidneys in a process tightly regulated by calcium, phosphorus and parathyroid hormone (PTH) levels (3).

After the second hydroxylation, 1,25(OH)2D binds to the VD Receptor (VDR). VDR is a member of the superfamily of nuclear hormone receptors including receptors for steroid, thyroid hormones and retinoic acid. Two type of VDR have been identified:

- the nuclear receptor that modulates gene transcription or a signal transduction pathway for ex-novo protein synthesis (genomic mechanism)
- the membrane receptor that acts by inducing for-

mation of second messengers (such as cyclic AMP-cAMP, diacylglycerol, inositol triphosphate, arachidonic acid) or phosphorylating some cellular proteins.

This non genomic mechanism of action is able to modulate quickly the cell response to various stimuli (4). Four are the main effects mediated by 1,25(OH)2D:

- increasing the efficiency of intestinal calcium absorption,
- ensuring adequate levels of calcium and phosphorus for metabolic function and bone mineralization,
- stimulating osteoblasts to mobilize skeletal calcium stores and (iv) promoting tubular calcium reabsorption and phosphate excretion.

Vitamin D-mediated biochemical pathways in non-skeletal conditions

The effect of vitamin D has been described in several organ systems within human body. A study based on gene array found that 1,25(OH)2D carries out a wide variety of biologic functions involving more than 160 pathways. Interestingly, these pathways are not only linked to bone-related conditions, but also to autoimmune disorders, cardiovascular disease and cancer, thus explaining the extra-skeletal benefits of vitamin D (5). In addition, the receptors for 1,25(OH)2D are widespread in a plethora of tissues (placenta, prostate, breast, colon, lung, bone, parathyroid, pancreas, immune system, and vascular wall), which also express 1α-hydroxylase and are able to transform 25(OH)D to its active hormonal form (calcitriol) (6-8). This finding has led to the concept that maintenance of adequate 25(OH)D levels in the blood is required for vitamin D regulation of a large number of physiologic functions, beyond that of the classic actions involved in bone mineral metabolism.

During pregnancy, serum levels of 1,25(OH)2D increase up to 2-fold starting at 10-12 weeks of gestation, reaching a maximum in the third trimester (9). The increased 1,25(OH)2D synthesis depends on the
acceleration of 1α-hydroxylation in maternal kidneys, and possibly enhances placental and decidual 1α-hydroxylase activity (10). The stimulus to increase the synthesis of 1,25(OH)2D is not clear. A potential signal could be mediated by the PTH-related peptide (PTHRP), which is produced in fetal parathyroid glands and placental tissues, and is known to increase placental synthesis of active vitamin D. Other signals that regulate 1,25(OH)2D synthesis during pregnancy include prolactin, placental lactogen, calcitonin and osteoprotegerin. Prolactin and placental lactogen contribute to increase calcium absorption from the intestine, to decrease urinary calcium excretion, and to stimulate the production of PTHrP and 1,25(OH)2D (11).

Interestingly, two studies conducted 30 years later from each other, converge to indicate that calcitonin and osteoprotegerin may be able to protect the maternal skeleton from excessive reabsorption of calcium (12, 13). Given the increase in the active form of vitamin D, pregnant women likely have a higher cellular exposure to vitamin D during the second and third trimesters, suggesting a role for vitamin D in obstetric wellbeing. Perinatal outcomes supposed to be related to vitamin D include preeclampsia, gestational diabetes, low birth weight, preterm delivery, disturb of skeletal development (rickets) (14).

In addition, because VDR and 1α-hydroxylase are expressed in the human uterus, a beneficial effect of vitamin D in the pathophysiology of uterus disorders is also possible (15).

Maternal effects of vitamin D deficiency in pregnancy

Gestational hypertension and pre-eclampsia

Pre-eclampsia (PE) is a disease affecting 2-8% of pregnancies, characterized by the combined presentation of hypertension and proteinuria. Along with the other hypertensive disorders, it is one of the major causes of maternal and perinatal mortality and morbidity worldwide (16). PE is thought to originate in early pregnancy, when the maternal immune system limits placental invasion in mothers vulnerable to cardiovascular diseases. Compared to normal pregnancies, vitamin D metabolism is markedly altered in PE. This may be due to reduced placental 1α-hydroxylase activity, resulting in lower circulating 1,25(OH)2D concentrations compared to normotensive or chronically hypertensive pregnant women.

Although there is no consensus on an optimal level serum concentration of 25(OH)D, most agree that a serum level of 32 ng/mL is adequate. Vitamin D insufficiency and deficiency are diagnosed at levels of less than 32 ng/mL and less than 20 ng/mL 25(OH)D, respectively (17).

Vitamin D status is reportedly lower in pre-eclamptic mothers at the time of diagnosis (18) and even at disease onset according to a nested case control study (19).

In a study, conducted on 150 patients, the plasma 25(OH)D was significantly decreased among patients with early onset of severe PE compared to healthy controls (18 ng/mL vs 32 ng/mL; p < 0.001) (18).

In another study, conducted on a cohort of 3,992 women, the median maternal serum 25(OH)D concentration at midgestation was 23% lower in women who subsequently developed severe PE compared with healthy women delivering at term (19).

A recent systematic review and meta-analysis, which incorporates data from two large scale epidemiological studies (the Hungarian Case-Control Surveillance of Congenital Abnormalities-HCCSCA and the Avon Longitudinal Study of Parents and Children-ALSPAC) into meta-analysis of previous published prospective and intervention studies, has deepened the association between vitamin D supplementation and vitamin D status with PE risk. Results from meta-analysis, obtained using separately two large scale epidemiological studies with published studies, which considered more than 40,000 cases in total, indicated that mothers receiving vitamin D supplementation earlier in pregnancy had lower odds of PE, in both analyses [pooled odds ratios (OR) 0.81 and 95% confidence interval (CI) 0.75-0.87 and pooled OR 0.52 and 95% CI 0.30-0.89] (20).

Another recent meta-analysis showed a significant association between PE and insufficient serum level of 25(OH)D respect to the comparison group (pooled OR 1.79 and 95% CI 1.25-2.58) (21).

Despite studies and meta-analysis evidence a consistent association between lower vitamin D level and higher PE incidence, the exact role that vitamin D plays in the development of PE is still unclear.

A potential mechanism could be related to placental defects responsible for a decreased synthesis of active vitamin D. Vitamin D reduction in turn could represent a key event in the development of this disease, by contributing to decrease calcium levels. It has been supposed that the reduction of calcium levels induces mechanisms known to be associated with hypertension, such as the stimulation of renin release from the kidney, ultimately leading to the development of PE (22).

In addition to vitamin D-mediated calcium level decrease, another mechanism, through which vitamin D might be related to maternal hypertension, could involve the maintenance of immune homeostasis and tolerance.
Vitamin D receptors on immune cells express key enzymes involved in the hormonal activation and catabolism of vitamin D metabolites, suggesting that the availability and effectiveness of 1,25(OH)2D can be directly regulated by the cells of the immune system. In vitro studies have demonstrated that 1,25(OH)2D administration leads to an up-regulation of regulatory T cell responses (and a down-regulation of pro-inflammatory ones), which protect the pregnant from PE (23). On the contrary, a defective control of T cells can be related to poor placental invasion, which in turn leads to the release of placental-derived vasoconstrictor factors, thus causing hypertension.

All these findings support the important role of vitamin D in PE, in cardiovascular and immune changes during pregnancy, suggesting the possible beneficial role of vitamin D supplementation, especially in pregnancy women exhibiting low plasmatic level of 25(OH)D.

**Gestational diabetes mellitus**

Vitamin D deficiency has been associated with higher risk of Type 2 Diabetes. Vitamin D is known to influence insulin secretion by pancreatic β cells and thereby affecting circulating glucose level, although the mechanism is not yet completely clarified.

A possible mechanism can be mediated by calbindin-D, a vitamin D-dependent calcium-binding proteins, present in pancreatic cell. Studies using calbindin-D28K null mice have suggested that this protein can modulate insulin release by regulating intracellular calcium. Furthermore calbindin-D, by buffering calcium, can protect against cytokine mediated destruction of β pancreatic cells (24).

Vitamin D deficiency during early pregnancy increase the risk of Gestational Diabetes Mellitus (GDM) by enhancing insulin responsiveness to glucose transport. However, studies that examined circulating concentrations of 25(OH)D in relation to diabetes risk are limited and sometimes provide discordant results.

For instance, a nested case-control study conducted on 171 women in USA showed that, among women who developed GDM, maternal plasma 25(OH)D concentrations at an average of 16 weeks of gestation were significantly lower than controls (24.2 vs 30.1 ng/ml, P<0.001) (25). Another case control study conducted on 248 women in UK showed no association between first-trimester maternal levels of 25(OH)D and subsequent development of GDM, even if the study showed that 25(OH)D was positively associated with fasting glucose and hemoglobin A1c at 28 weeks (26).

Nevertheless a systematic review and meta-analysis, including 10 studies, showed that pregnant women with gestational diabetes had significantly lower 25(OH)D level than the comparison group (21).

Literature data support the association of 25(OH)D insufficiency with gestational diabetes, suggesting a benefic role of vitamin D supplementation. However, it remains a need for large, well designed randomized controlled trials to determine whether strategies to optimize maternal 25(OH)D levels are effective in reduce risk of this disorder.

**Gestational vitamin D deficiency effects on newborns**

**Birth weight**

Regarding 25(OH)D levels, the fetus depends on the maternal supply, which is believed to cross easily through the placenta. Vitamin D deficiency during pregnancy affects the fetus and the newborn. Birth weight is decreased, bone mineralization is impaired and neonatal hypocalcemia is frequent (27).

Several studies have reported an association between infant size and vitamin D. A systematic review and meta-analysis showed a significant association between small for gestational age infants and 25(OH)D insufficiency in respect with the comparison group (21). The association between small for gestational age infants and 25(OH)D insufficiency remained significant after adjustments for critical confounders, suggesting the robustness of these results.

Of the four studies on birth weight included in the meta-analysis, infants of mothers with 25(OH)D concentrations less than 37.5 nmol/L during pregnancy had lower birth weight (random weighted mean difference -130.92 g, 95% confidence interval -186.69 to -75.14 g) (21).

A cross-sectional study conducted in Iran on 449 pregnant women, healthy at the time of delivery, and their newborns, showed that the incidence of low birth weight was significantly lower in newborns from mothers who received the recommended doses of calcium and vitamin D (28).

In a prospective study on 2,251 pregnant women on effects of maternal nutrition and growth in healthy pregnant women, total intake of vitamin D was a significant predictor of infant birth weight. In addition, pregnant women with vitamin D intakes <200 IU/day had infant with birth weight that were 60 g below women with vitamin D intakes at or above 200 IU/day (29).

A recent systematic review focused on vitamin D supplementation in pregnancy supports a positive relationship between maternal vitamin D status and offspring birth weight. However, the review underlines the heterogeneity between studies in terms of design, ethnicity, sunlight exposure, dose and treatment, as potential confounding factors (30).

Although the biological basis for the association
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between 25(OH)D deficiency and birth weight is already unclear, it is possible that 25(OH)D deficiency may affect fetal growth due to its effect on fetal bone development. Literature data shows the association between vitamin D insufficiency and low offspring birth weight, suggesting a preventative role of vitamin D supplementation.

However, large observational studies based on administrative data and electronic medical records to address the association between vitamin D and birth weight are necessary in order to account for confounding variables.

Skeletal development and rickets

The importance of VD for infant skeletal development and the manifestation of infantile rickets has long been recognized.

The maternal serum concentration of 1,25(OH)2D synthesized by the deciduous cells of placenta are elevated during pregnancy, providing a source of active vitamin D and increased calcium absorption for fetus. With the abrupt cessation of the placenta source of calcium at birth, the newborns serum calcium levels decreases. In situations of maternal vitamin D deficiency, babies might born with poor stores of calcium. In addition, due to cessation of transplacental calcium pump after birth, they are unable to absorb calcium from breast milk, which is vitamin D deficient. This triggers the cascade of events which start with hypocalcaemia followed by secondary hyperparathyroidism, leading to mobilization of calcium from bones in favour of vital and needier organs like heart and brain (31).

A recent observational study and literature review confirm the hypothesis that maternal vitamin D deficiency is a major factor in the development of rickets in the breastfeeding infants. The study has showed in both newborns with active rickets and their mothers a significant reduction of 25(OH)D level and a significant increase in PTH level, with respect to infant with healed rickets and their mothers (32). A review on fetal and newborn effects of gestational vitamin D deficiencies evidences that poor skeletal mineralization in uterus, induced by vitamin D deficiency, may manifest as rickets in newborn infants (33).

In addition, results from MAVIDOS double-blind study suggested that daily administration of 1,000 IU to pregnant women with low levels of vitamin D, starting from the fourteenth week of pregnancy until childbirth, can improve bone mineral density in children (34).

Because poor vitamin D storage of the mother may impair vitamin D status in infants, some Medical Society suggests the cholecalciferol supplementation.

Considering that vitamin D deficiency in mother is related to the increased risk of rickets, a consensus about proper 25(OH)D serum levels and vitamin D dosage in women and newborns who need it, is pivotal.

Role of vitamin D in early menarche and dysmenorrhea

Early menarche

Genetic factors play a major role in determining the age of menarche; thus, there is a high concordance within ethnic groups as well as between age of menarche in mothers and daughters. Other factors are postulated to affect the age of menarche, including socioeconomic conditions, energy expenditure and state of health.

There are few studies looking at the association of vitamin D status and sexual maturation and female reproduction. One of the previous study in humans indicated that the VDR gene polymorphism at the Apal site is significantly associated with the earlier age at menarche (35). In a recently published prospective study, Villamor et al. found increased risk of early menarche in vitamin D-deficient girls compared to vitamin D-sufficient girls in Bogota, Colombia (36). The Authors found that the relationship between earlier menarche and lower concentrations of vitamin D last also after controlling for BMI; this adjustment is strongly needed since obese individuals tend to have lower serum concentrations of vitamin D as well as earlier menarche. Going into further details, researchers reported that biochemical pathways might involve adipose-derived hormones, showing that early puberty was associated with increased leptin levels, which were inversely correlated with 25-(OH)D. Another cross-sectional study on precocious puberty in girls showed a significant difference in the mean serum 25(OH)D concentration between the precocious puberty group versus the control group (37). The Authors indicate that a possible biological mechanism could involve insulin-like growth factor-1 (IGF-I), one of the growth factors believed to modulate the onset of puberty by stimulating the gonadotropin-releasing hormone (GnRH) pulse. Vitamin D showed inverse correlation with IGF-I, which in turn modulates the onset of puberty and pubertal progression by stimulating the GnRH (38). So, it is conceivable that vitamin D-mediated effects may influence IGF-1 levels and pubertal onset through an effect on gonadotropin and sex hormone.

Although the mechanism underlying the effect of its deficiency on pubertal progression is unclear, vitamin D seems to influence sexual maturation in girls. Stronger evidence on this phenomenon would be welcome.
Dysmenorrhea

Dysmenorrhea is a common disorder characterized by painful uterine cramping, just before or during menstruation, in the absence of any pelvic pathologic conditions.

Few evidences suggest that vitamin D supplementation was favorably associated with the discomfort caused by dysmenorrheal episodes.

A recent prospective intervention study, conducted on 40 women aged 18 to 40 years, has evaluated the effects of a single-loading oral dose of vitamin D₃ of 300,000 IU on primary dysmenorrhea. The results have showed a significant reduction of pain in the vitamin D group compared with the placebo group over the 2-month study duration, and a reduction of 40% of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) usage (39). The Author has actually indicated prostanoids (PG) excessive production at uterine level as a potential pathogenetic trigger of dysmenorrhea.

As a matter of fact, an in vitro study had previously demonstrated that 1,25(OH)₂D decreases the level of proinflammatory cytokines such as interleukin 6 and tumor necrosis factor, and regulates the expression of several key genes involved in the pathway of PGs, decreasing the biological activity of the latter (40).

These hypotheses has been recently confirmed by a cross sectional study which investigated vitamin D and PTH status among 56 adolescent and young females with severe and very severe dysmenorrhea. The Authors found that about 61% of subjects included experienced very severe dysmenorrhea, with 80% of participants showing insufficient plasmatic vitamin D levels, and 48% of them suffering from hyperparathyroidism (41).

Considering together, these findings suggest a potentially beneficial effect of vitamin D supplementation in the uterus pathophysiology, supporting the use of vitamin D supplementation in these patients, especially when exhibiting low plasmatic levels of 25(OH)D.

Nevertheless, further large observational studies are required to establish the effect of vitamin D status on dysmenorrhea and to support the vitamin D supplementation for these women.

Discussion

The VDR is expressed in almost all body cells, such as immune, vascular cells as well as ovary and human pituitary gland. This has led to extensive research on vitamin D as a potential influencing factor in a large number of non-skeletal physiologic functions.

A number of case-control, prospective observational studies and meta-analyses have investigated so far possible associations between vitamin D and pregnancy outcomes or sexual maturation.

Some studies dealt with the implications of 25(OH)D low serum levels in conditions affecting pregnant women and newborns, such as pre-eclampsia, gestational diabetes mellitus, low birth-weight and rickets.

Furthermore, fewer studies have evidenced that vitamin D might play a role in the dysmenorrhea and early puberty.

Even if a greater number of controlled and randomized interventional studies on the use of vitamin D in these pathologies are still needed to confirm these findings, for some gynecologic conditions, a greater body of evidence has already suggested that closer attention should be given to vitamin D deficiency in the medical practice.

For pregnant women thought to be at increased risk of vitamin D insufficiency or deficiency, the evaluation of serum 25(OH)D level can be considered. In these women, especially when exhibiting high risk of development of gestational hypertension and metabolic disorder, the supplementation of vitamin D could be a preventative practice in order to avoid the adverse maternal and neonatal outcomes. When vitamin D deficiency is identified during pregnancy, most experts agree that 1,000-2,000 IU per day of vitamin D is safe (42).

Both the importance of vitamin D status evaluation and the improvement of the supplementation in pregnancy, newborns and girls who exhibited a low 25(OH)D serum concentration have to represent the pillars for a preventive approach.

In this perspective, the achievement of a shared and unanimous consensus on the optimal supplementation of vitamin D for specific cases, could better support the healthcare specialist in the clinical practice.

In addition, because vitamin D supplementation is well-tolerated and cost-effective, a deep knowledge of mechanisms of action of vitamin D in these and other gynecological conditions could be useful to prevent serious complications for patients and to reduce healthcare expenditure for the system.
References