Internet Journal of Medical Update

Journal home page: http://www.akspublication.com/ijmu

Review

Calcium homeostasis during pregnancy and lactation: role of vitamin D supplementation

R K Marya[Ψ]

Unit of Physiology, Faculty of Medicine, AIMST University, Bedong, Malaysia

ABSTRACT: Pregnancy and lactation cycle is a period of considerable strain on maternal calcium homeostasis. A number of adaptive mechanisms involve increased intestinal calcium absorption, renal calcium conservation and changes in bone metabolism. These adaptations are mediated through changes in the secretion of various calciotropic hormones [1,25(OH)2 D3, parathormone, and calcitonin]. In all of these adaptive mechanisms, vitamin D is involved directly or indirectly. Now, it is being realized that not only dark-skinned but also even Caucasian women tend to go into vitamin D deficiency during pregnancy. Adverse health outcomes such as preeclampsia, low birth-weight, neonatal hypocalcemia, poor postnatal growth, bone fragility, and increased incidence of autoimmune diseases have been linked to low vitamin D levels during pregnancy and infancy. Vitamin D deficiency seems to be a public health problem even in 2018. Most of the experts in the field are convinced that women need extra amounts of vitamin D during pregnancy and lactation. The amount of vitamin D that is required for optimum calcium homeostasis in this phase of life is still controversial. Studies are under way to establish the recommended daily doses of vitamin D in pregnant and lactating women.

KEY WORDS: *Vitamin D deficiency; Pregnancy; Lactation; Vitamin D supplementation*

INTRODUCTION

The study of calcium homeostasis in pregnancy and lactation was prompted by the observations of osteomalacia in pregnant and lactating women in India and China in early decades of 20th century. About 25-30 g of calcium is transferred mother to the fetus during pregnancy and almost similar amount of is transferred to the neonate in milk during lactation. The adaptive mechanisms involved in the maintenance of homeostasis during these periods of increased calcium demands on the mother involve greater intestinal calcium absorption, decreased urinary calcium losses and increased bone resorption. These adaptations are mediated by increased secretion of the various calciotropic hormones [1,25(OH)$_2$D parathormone and calcitonin. In all of these adaptive mechanisms, vitamin D is involved directly or indirectly. Now, it

is being realized that not only dark-skinned but also even Caucasian women tend to go into vitamin D deficiency during pregnancy and lactation and need to be supplemented with vitamin D[1]. Although, both in pregnancy and lactation, a woman faces similar amount of calcium demand, the adaptive mechanisms during these two phases of reproduction differ in many ways[2]. In this review, these adaptive mechanisms shall be discussed separately, followed by a discussion on the reported benefits of vitamin D supplementation during pregnancy.

CALCIUM HOMEOSTASIS DURING PREGNANCY

Intestinal calcium absorption in pregnancy

Greater calcium demands of the body during pregnancy and lactation can be met with by increasing the dietary calcium intake, or by increasing efficiency of intestinal calcium absorption mechanism or decreasing calcium losses in the urine. Increased appetite for food has been

---

[Ψ]**Correspondence at:** Professor and Head, Unit of Physiology, Faculty of Medicine, AIMST University, Bedong, Kedah, Malaysia; Email: rkumarmarya@yahoo.com

observed in pregnancy, but in developing countries, financial constraints limit the actual increase in intake of calcium-rich food. Obviously, increased efficiency of intestinal calcium absorption remains the chief mechanism of increasing availability of calcium to the mother. A marked increase in the efficiency intestinal calcium absorption in later months of pregnancy has been observed in humans[3]. It has been attributed to vitamin D as well as some other factors. Halloran *et al*[4] estimated intestinal calcium transport ratio (serosal Ca^{++}/ mucosal Ca^{++} ratio) in vitamin D-replete and vitamin D-deficient pregnant rats. The ratio was 6.0 in vitamin D-replete pregnant rats as compared to 3.0 in vitamin D-replete non-pregnant control rats.

Renal conservation of calcium

Renal conservation of calcium is another mechanism, which may be utilized by the body for improving the availability of calcium to the fetus. However, due to increased GFR in later months of pregnancy, the excretion of many urinary constituents such as amino acids, glucose and calcium increases[5]. Calcium conservation during pregnancy may be observed in in those with vitamin D-deficiency when the affected women tend to develop hypocalciuria[6]. An association between hypocalciuria and pregnancy-induced hypertension has also been reported[7].

Bone metabolism in pregnancy

The effect of pregnancy on bone metabolism is not entirely clear. It has been suggested that minerals accumulate in the bone during pregnancy in anticipation of calcium demands during lactation[3]. In vitamin D-deficient rat, there is roughly 25% increase in femoral bone mineral content by the end of pregnancy. In vitamin D-replete rat, however no change in mineral content could be demonstrated during pregnancy[8]. Many workers have investigated the changes in bone mineral content in human pregnancy but results remain inconclusive.

Placental calcium transport

To meet the fetal demand, calcium is actively pumped across the placenta[9]. This view is supported by the observation of 1-2 mg/dL higher serum calcium level in fetus than in mother. In the later months of pregnancy, increased rate of calcium transport in placenta is accompanied by appearance of calcium-binding protein in placenta. This protein has properties similar to those of intestinal calcium-binding protein associated with vitamin D induced transport of calcium in the intestine. Thus, vitamin D may have a role in improving placental calcium transport. The active transport of calcium across the placenta may not be dependent on vitamin D alone. It seems, parathyroid hormone related peptide (PTHrP) is also involved in placental calcium transport[10].

Calciotropic hormones during pregnancy

Vitamin D
25-Hydroxyvitamin D: In an extensive review of literature, Ritu *et al*[11] concluded that subclinical vitamin D deficiency is highly prevalent in India, both in urban and rural settings and across all socioeconomic and geographical strata. Numerous reports have highlighted the widespread prevalence of low 25(OH)D levels during pregnancy and lactation in Indian women[12-17]. Actually, hypovitaminosis D during pregnancy and lactation seems to exist worldwide. Ponsonby *et al*[1] reviewed literature on vitamin D status in pregnancy published between 1997 and 2006. They concluded that vitamin D deficiency as shown by low 25(OH)D levels was common in darkly pigmented mothers particularly those who have migrated to regions with low UV radiations. Vitamin D deficiency was also found to be prevalent in Caucasian women, especially who had not taken vitamin D supplements during pregnancy. Dror *et al*[18] have also confirmed a high prevalence of maternal vitamin D inadequacy during pregnancy and at delivery in various ethnic populations living at different latitudes.
1,25-dihydroxyvitamin D3: Several longitudinal and cross-sectional studies have shown that, when compared to non-pregnant young women, pregnant women have high serum $1,25(OH)_2D3$ concentration from early pregnancy. Serum $1,25(OH)_2D3$ concentration rises steadily throughout gestation and at term it reaches levels about double of those in non-pregnant women[19]. It seems more probable that the $1,25(OH)_2D3$ in the maternal circulation originates mainly from the maternal kidneys rather than placenta[10].

Parathormone (PTH)
Earlier reports had suggested increased levels of PTH during pregnancy. However, recent sensitive assays techniques have revealed low-normal PTH levels in pregnant women in all the three trimesters. In contrast to humans, rats develop physiological hyperparathyroidism in late pregnancy in response to a fall in serum calcium.

Calcitonin
Serum calcitonin levels are elevated during pregnancy in humans and animals. The increase is attributed to both thyroidal and extrathyroidal synthesis of the hormone in the placenta and breast. However, the possible role of calcitonin in protection of the maternal skeleton from increased resorption is not clear.

16

Plasma calcium level

A few decades ago, it was accepted that plasma calcium level shows a significant decrease during pregnancy and it was attributed to the fetal drain of calcium. Later, in contrast to total serum calcium level estimated earlier, it became possible to estimate both ionic calcium and albumin-bound calcium levels in the serum. Numerous cross-sectional and longitudinal studies confirmed that serum ionic calcium levels remain normal during pregnancy[2,10]. The decrease in total calcium level was because of hemodilutional decrease in plasma albumin and hence in concentration of albumin-bound calcium fraction. It needs to be stressed that maintenance of normal plasma ionic calcium level during pregnancy would be possible only with the adequacy of various adaptive mechanisms discussed above. True ionic hypocalcemia may occur in pregnant women with vitamin D deficiency.

CALCIUM HOMEOSTASIS DURING LACTATION

Lactation presents a calcium challenge to the mother similar to that experienced during pregnancy. In some species, e.g. rat, the stress on the calcium homeostatic mechanisms is far greater in lactation than in pregnancy. In 21 days of lactation, the rat transfers to her litter over 2.5 g of calcium, equal to 60 % of calcium content of her skeleton. The daily loss of calcium in the milk in lactating rat usually exceeds 100 mg, which is 100 times more than daily urinary calcium excretion. In human female, calcium loss in milk (350 mg/day) is only marginally greater than the 24-h urinary calcium excretion.

Intestinal calcium absorption

In the rat, there is some evidence of increased calcium absorption during lactation, but is has not been proved in humans. The fractional intestinal absorption of calcium (FA-Ca) was measured using a dual non-radioactive Ca isotope technique in 26 control women, 49 women in the last trimester (36 weeks) of pregnancy and 31 of these women in established (20 weeks) lactation. It was concluded that that FA-Ca is significantly elevated in late pregnancy but not in established lactation, when compared with control women[20]. In contrast to the high 1, 25-dihydroxy-vitamin D3 levels of pregnancy, maternal free and bound 1, 25-dihydroxyvitamin D levels fall to normal within days of parturition and remain there throughout lactation. Consequently, the intestinal absorption of calcium is equal to the non-pregnant state and decreased from that in pregnancy. This change coincides with the fall in 1, 25-dihydroxyvitamin D levels to normal.

While scanning the literature on intestinal calcium absorption in lactation, it would be pertinent to note the species difference in calcium metabolism. In the rat, calcium requirements of the fetus are almost negligible as compared to calcium requirements during lactation. During the last two months of human pregnancy, daily fetal requirement of calcium is greater than calcium secreted in milk[21]. This fact may explain why in humans, firm evidence of enhanced intestinal calcium absorption is available during pregnancy but not during lactation.

Urinary calcium excretion

In non-pregnant, non-lactating women, almost 200 mg calcium is lost daily in the urine. In lactating women, a similar amount is lost in the milk. Hence a small change in urinary calcium excretion may make an important contribution to calcium balance in lactating women. The GFR falls during lactation to a level below the pregnant and non-pregnant value, and the renal excretion of calcium is typically reduced to levels as low as 50 mg per 24 h[10].

Bone metabolism in lactation

In human lactation, serial measurements of bone density have shown a fall of 3-10.0% in bone mineral content after 2–6 months of lactation. Trabecular sites (lumbar spine, hip, femur, and distal radius) are affected more than cortical sites[18]. Loss of bone mineral from the maternal skeleton seems to be a normal consequence of lactation and may not be preventable by raising the calcium intake above the recommended dietary allowance. Several recent studies have demonstrated that calcium supplementation does not significantly reduce the amount of bone density lost during lactation[22]. Not surprisingly, the lactational decrease in bone mineral density correlates with the amount of calcium lost in the breast milk output[23].

The studies of pregnant women suggest that the fetal calcium demand is met in large part by increased intestinal calcium absorption, which more than doubles from early in pregnancy and possibly the maternal skeleton does contribute calcium to the developing fetus. In comparison, the studies in lactating women suggest that skeletal calcium resorption is a dominant mechanism by which calcium is supplied to the breast milk, while renal calcium conservation is also apparent. These observations indicate that the maternal adaptations to pregnancy and lactation have evolved differently over time, such that dietary calcium absorption dominates in pregnancy, whereas the temporary borrowing of calcium from the skeleton appears to

dominate during lactation. Lactation seems to program an obligatory skeletal calcium loss irrespective of maternal calcium intake, but the calcium is completely restored to the skeleton after weaning[10].

Calciotropic hormones during lactation

Vitamin D
Though plasma $1,25(OH)_2D3$ levels are elevated during pregnancy, within days of parturition, levels fall to normal and remain so throughout lactation[10].

Parathormone
Many cross-sectional and longitudinal studies have confirmed that serum PTH levels remain low throughout lactation. Thus, the earlier view that lactation is a period of secondary hyperparathyroidism has not been substantiated in human. Part of the confusion arose from the studies in lactating rats in whom, serum PTH levels were found to be elevated.

Calcitonin
Unlike pregnancy, serum calcitonin levels remain normal during lactation.

Plasma calcium level

Most of the recent studies have shown that serum total and ionic calcium levels are elevated during lactation. Moreover, mothers nursing twins have been found to have significantly higher total serum calcium levels than mothers nursing a singleton.

EFFECTS OF VITAMIN D DEFICIENCY DURING PREGNANCY AND LACTATION

The clinical, biochemical, radiological and histopathological features of osteomalacia in pregnant and lactating women with severe vitamin D deficiency are too well known to warrant repetition. In babies born to such mothers, neonatal hypocalcemia and even congenital rickets have been reported.

In human pregnancy, even mild deficiency of vitamin D seems to reduce the fetal growth. In Asian immigrants in the UK, due to low dietary intake of vitamin D and reduced solar exposure, low serum 25(OH)D levels as well as low serum calcium and phosphate levels have been reported in mothers as well as in newborn[24,25]. Cockburn et al[25] reported results of a trial on 1139 Scottish women who attended two different obstetric wards. In one ward, 506 women received 400IU/d vitamin D from 12th week of pregnancy onwards, whereas those attending the other ward (n=633) received placebo containing no vitamin D. In the supplemented group, plasma 25(OH)D concentration, plasma calcium and phosphate concentrations were higher than the control group at 24th and 34th week of pregnancy as well as in cord blood and in infants at day 6 of age. In another study in the UK, Brooke et al[26] conducted a double-blind trial of vitamin D supplementation among pregnant immigrant Asian women. Fifty-nine women received 1000 IU/d of vitamin D beginning at 28-32 week of pregnancy, whereas 67 women received placebo. Though the birth-weight of infants in the two groups was not different, the incidence of small for gestational age infants in the control group (28.6%) was significantly greater than that in the supplemented group (15.3%). Maxwell et al[27] reported improvement in the nutritional status of Asian immigrant pregnant women by vitamin D supplementation. Results of a randomized study in 120 North Indian women investigated at delivery were reported in 1981[28]. Of these, 75 women had taken no vitamin D supplements, 25 had been given 1200 IU/d vitamin D (with 375 mg/d calcium) during the third trimester of pregnancy, and 20 had been given 600,000 IU vitamin D orally in both the seventh and eighth months of pregnancy. It was estimated that the usual daily vitamin D intake among this population was <30 IU/d. 75 women who did not take any vitamin D supplements during pregnancy showed statistically significant hypocalcaemia, hypophosphatemia and elevation of HLAP. Hypocalcaemia and hypophosphatemia were present in cord blood, too. Twenty-five women, who had received 1,200 IU vitamin D/day throughout the 3rd trimester, showed significantly lower HLAP levels and increased fetal birth-weight but there was no other improvement in maternal or cord blood chemistry. Administration of vitamin D in two large doses of 600,000 IU each in the 7th and 8th months of pregnancy in 20 women proved more efficacious. Statistically significant improvement was observed in all the three-biochemical parameters in maternal as well as cord sera. Fetal birth-weight was also significantly greater with this mode of therapy. Another trial on vitamin D supplementation involved 200 north Indian pregnant women[29]. The women were randomly assigned to receive either 600,000 IU of vitamin D twice during the last trimester (seventh and eighth months of gestation, n=100) or no supplement (n=100). Serum calcium concentrations were higher and alkaline phosphatase concentrations were lower for mothers who were treated with vitamin D, compared with those who were not. Similar findings were observed for cord samples. Infants of mothers who received vitamin D had greater intrauterine growth, with greater birth-weight, crown-heel length, head circumference, arm circumference, and skinfold thickness, compared with infants of mothers who did not receive vitamin D.

18

Adverse health outcomes such as preeclampsia, low birth-weight, neonatal hypocalcemia, poor postnatal growth, bone fragility, and increased incidence of autoimmune diseases have been linked to low vitamin D levels during pregnancy and infancy[30].

CURRENT RECOMMENDATIONS FOR VITAMIN D SUPPLEMENTATION IN PREGNANCY AND LACTATION

Vitamin D deficiency is common in pregnant women despite the widespread use of prenatal vitamins, because these are inadequate to maintain normal vitamin D levels. New studies have provided more evidence on the effects of supplementing pregnant women with vitamin D alone or with calcium on pregnancy outcomes. Supplementing pregnant women with vitamin D in a single or continued dose increases serum 25-hydroxyvitamin D at term and may reduce the risk of preeclampsia, low birth-weight and preterm birth. However, The evidence on whether vitamin D supplementation should be given as a part of routine antenatal care to all women to improve maternal and infant outcomes remains unclear[31].

Many of the experts worldwide suggest that women should be given vitamin D supplements during pregnancy although there is no agreement as to the amount useful for assuring optimal pregnancy outcome and neonatal growth. A prominent proponent of increasing vitamin D intake during pregnancy and lactation is Dr. Michael Holick, an endocrinologist at Boston University Medical Center. He recommends administration of 1000-2000 IU/d of vitamin D during pregnancy 31. Hollis et al[32] conducted a randomized, controlled trial, in which women with a singleton pregnancy at 12 to 16 weeks' gestation received 400, 2000, or 4000 IU of vitamin D_3 per day until delivery. Not a single adverse event was attributed vitamin D supplementation. It was concluded that vitamin D supplementation of 4000 IU/d for pregnant women is safe and most effective in achieving sufficiency in all women and their neonates regardless of race. According to these workers, the daily intake of vitamin D, currently advocated, is comparatively ineffective at achieving adequate circulating 25(OH)D concentrations during pregnancy, especially in African Americans.

Endocrinology Clinics of North America published a compendium of articles by research workers who work on vitamin D. In general these authors recommend maintenance of plasma levels of 25(OH)D ≥ 30 ng/mL requiring a daily vitamin D intake of ≥ 1000 IU for most children and adults and twice that amount during pregnancy and lactation[33]. Institute of Medicine (USA) has also recommended higher daily intake of calcium and vitamin D, especially during pregnancy and lactation[34]. According to the American College of Obstetricians and Gynecologists Committee Report[35], recent evidence suggests that vitamin D deficiency is common during pregnancy especially among high-risk groups, including vegetarians, women with limited sun exposure (e.g. those who live in cold climates, reside in northern latitudes, or wear sun and winter protective clothing) and ethnic minorities, especially those with darker skin. Newborn vitamin D levels are largely dependent on maternal vitamin D status. Consequently, infants of mothers with or at high risk of vitamin D deficiency are also at risk of vitamin D deficiency. For pregnant women thought to be at increased risk of vitamin D deficiency, maternal serum 25- OH-D levels can be considered. When vitamin D deficiency is identified during pregnancy, most experts agree that 1000–2000 international units per day of vitamin D are safe. Similarly, Endocrine Society (USA) has suggested that pregnant and lactating women require at least 600 IU/day of vitamin D and at least 1500-2000 IU/d may be needed to maintain a blood level of 25(OH)D above 30 ng/ml[36].

In contrast to the numerous recommendations for vitamin D supplementation during pregnancy and lactation cited above, both Indian Council of Medical Research (ICMR and WHO have maintained a conservative approach. In the latest report, 37 ICMR has not recommended routine vitamin D supplementation during pregnancy and lactation. According to WHO guidelines[38], "in view of the limited evidence currently available to directly assess the benefits and harms of vitamin D supplementation in pregnancy for improving maternal and infant health outcomes, the use of this intervention during pregnancy as a part of routine antenatal care is not recommended". In view of the reports of highly prevalent vitamin D deficiency in India and other countries, it may be prudent to estimate 25(OH) levels at early-pregnancy and those with 25(OH) D levels below 30 ng/mL be given vitamin D supplements and vitamin D status be monitored every four weeks[33]. This approach would provide vitamin D to those who need it without the possible risk of over-dosage.

REFERENCES

1. Ponsonby AL, Lucas RM, Lewis S, Halliday J. Vitamin D status during pregnancy and aspects of offspring's health. *Nutrients*. 2010;2:389-407.
2. Kovacs CS, Kronenberg HM. Maternal-fetal calcium and bone metabolism during pregnancy, puerperium and lactation. *Endocine Rev*. 1997;18:832-72.
3. Heany RP, Skilman TG. Calcium metabolism in normal human pregnancy. *J Clin Endocrinol Metab*. 1971;33:661-70.

4. Halloran BP, DeLuca HF. Calcium transport in small intestine during pregnancy and lactation. *Am J Physiol*. 1980;239:E64-8.

5. Maikranz P, Holley JL, Parks JH, et al. Gestational hypercalciuria causes pathological calcium oxalate supersaturations. *Kidney Int*. 1989;36:108-13.

6. Marya RK, Rathee S, Manrow M. Urinary calcium excretion in pregnancy. *Gynecol Obstet Invest*. 1987;23:141-4.

7. Donovan AM, Williams A, Gordon L, et al. Hypocalciuria in preeclampsia and gestational hypertension due to decreased fractional excretion of calcium. *Arch Med Sci*. 2009;1:80-85.

8. Halloran BP, DeLuca HF. Skeletal changes during pregnancy and lactation: The role of vitamin D. *Endocrinology*. 1980;107:1923-9.

9. Twardock AR, Austin MK. Calcium transfer in perfused guinea pig placenta. *Am J Physiol*. 1970;219:540-5.

10. Kovacs CS. The role of vitamin D in pregnancy and lactation: insights from animal models and clinical studies. *Annu Rev Nutr*. 2012;32:97-123.

11. Ritu G, Gupta A. Vitamin D deficiency in India: prevalence, casualties and intervention. *Nutrients*. 2014; 6:729-75.

12. Goswami R, Gupta N, Marwaha RK, et al. Prevalence and significance of low 25-hydroxyvitamin D concentrations in healthy subjects in Delhi. *Am J Clin Nutr*. 2000;72:472-5.

13. Sachan A, Gupta R, Das V, Aggarwal A. High prevalence of vitamin D deficiency amonst pregnant women and newborns in northern India. *Am J Clin Nutr*. 2005;81:1060-4.

14. Mehrotra P, Marwaha RK, Aneja S, et al. Hypovitaminosis D and hypocalcemic tetany seizures in Infancy. *Indian Pediatrics*. 2010;47:581-58

15. Marwaha RK, Tandon N, Chopra N, et al. Vitamin D status in pregnant Indian women across trimesters and different seasons and its correlation with neonatal serum 25-hydroxyvitamin D levels. *Br J Nutr*. 2011;106:1383-9.

16. Harinarayan CV, Sachan A, Reddy PA, et al. Vitamin D status and bone mineral density in women of reproductive and postmenopausal age groups: a cross-sectional study. *J Assoc Physicians India*. 2011;59:698-704.

17. Lonhey V. Vitamin D deficiency: Indian scenario. *J Assoc Physicians India*. 2011;59:695-6.

18. Dror DK, Allen LH. Vitamin D inadequacy in pregnancy: biology, outcomes, and interventions. *Nutr Rev*. 2010;68:465-77.

19. Papapetrou PD. The interrelationship of serum 1,25-dihydroxyvitamin D, 25- hydroxyvitamin D and 24,25-dihydroxyvitamin D in pregnancy at term: a metaanalysis. *Hormones*. 2010;9:135-44.

20. Specker BL. Nutrition in Pregnancy and Lactation. In: Holick M.F., Dawson-Hughes B. (eds) Nutrition and Bone Health. Nutrition and Health. Humana Press, Totowa, NJ. 2004.

21. Morrison SD. The nitrogen balance of pregnant rats. *J Physiol*. 1956;133:167-70.

22. Kalwarf HJ, Specker BL. Bone mineral changes during pregnancy and lactation. *Endocrine*. 2002;17:49-53.

23. Laskey MA, Prentice A, Hanratty L, et al. Bone changes after 3 mo of lactation: influence of calcium intake, breast-milk output, and vitamin D-receptor genotype. *Am J Clin Nutr*. 1998;67:685-92.

24. Heckmatt JZ, Peacock M, Devies AE, et al. Plasma 25-hydroxyvitamin D in pregnant Asian women and their babies. *Lancet*. 1979;2(8142):546-9.

25. Cockburn F, Belton NR, Purvis RJ, et al. Maternal vitamin D intake and mineral metabolism in mothers and their newborn infants. *B Med J*. 1980;281:11-4.

26. Brooke OG, Brown IR, Bone CD, Carter ND. Vitamin D supplements in pregnant Asian women: effect on calcium status and fetal growth. *Br Med J*. 1980;280:751-4.

27. Maxwell JD, Aug L, Brooke OG, Brown IFR. Vitamin D supplements enhance weight gain and nutritional status in pregnant Asians. *Br J Obstet Gynaecol*. 1981;88:987-91.

28. Marya RK, Rathee S, Lata V, Mudgil S. Effects of vitamin D supplementation in pregnancy. *Gynecol Obstet Invest*. 1981;12:155-61.

29. Marya RK, Rathee S, Dua V, Sangwan K. Effect of vitamin D supplementation during pregnancy on fetal growth. *Indian J Med Res*. 1988;88:488-92.

30. De-Regil LM, Palacios C, Lombardo LK, Peña-Rosas JP. Vitamin D supplementation for women during pregnancy. *Cochrane Database Syst Rev*. 2016;(1):CD008873.

31. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357:266-281.

32. Hollis BW, Johnson D, Hulsey TC, Wbeling M, et al. Vitamin D supplementation during pregnancy: double blind randomized clinical trial of safety and effectiveness. *J Bone Miner Res*. 2011;26:2341-57.

33. Christakos S, Ajibade DV, Dhawan P, Fechner AJ, et al. Vitamin D: metabolism. *Endocrinol Metab Clin North Am*. 2010;39(2):243-53.

34. Institute of Medicine. Dietary reference intakes for calcium and vitamin D. Washington, DC: The National Academies Press; 2011:1015.

35. ACOG Committee on Obstetric Practice. ACOG Committee Opinion No. 495: Vitamin

20

D: Screening and supplementation during pregnancy. *Obstet Gynecol*. 2011;118(1):197-8.

36. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society Clinical Practice Guidelines. *J Clin Endocrinol Metab*. 2011;96:1911-30.

37. Nutrient Requirements and Recommended Dietary allowances for Indians :A Report of the Expert Group of the Indian Council of Medical Research, 2010.

38. WHO Guidelines: Vitamin D supplementation in pregnant women. Geneva, World Health Organization, 2016.