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Study of Vitamin D levels in Mothers and Their New Borns.

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Abstract:
Background: *Adequate vitamin D concentrations during pregnancy are necessary to neonatal calcium homeostasis, bone maturation and mineralization. The aim of study is to evaluate vitamin D status in mothers and their newborns.*
Methods: *100 pregnant women were recruited from M.S.Ramaiah hospitals for the study and maternal blood was collected at the time of delivery. After the delivery of the new borns to the respective mothers, cord blood samples were collected. The samples were assayed for 25-hydroxyvitamin D3, calcium, phosphorus and alkaline phosphatase levels.*
Results: *In the present study it is observed that vitamin D concentration in new borns is lower than their level in mothers (P<0.001). Moreover with the decrease in maternal vitamin D level, there is likewise decrease in its level in new borns also (P<0.001). However with the increase in new born vitamin D level, there is preceding rise in its level in their mothers. There is likewise increase in calcium and phosphorus levels in both new borns and their mothers.*
Conclusion: *The new borns vitamin D level is appropriately influenced by the maternal vitamin D status. Calcium level in new borns is influenced by both maternal vitamin D and calcium status along with new borns vitamin D level. Adequate calcium and vitamin D intake during pregnancy is essential. It is necessary to reconsider the recommendation for vitamin D supplementation for women during pregnancy.*
Keywords: *Calcium, cord blood, immunity, new borns, vitamin D.*

I. Introduction

Vitamin D deficiency is linked with long- latency diseases, with the implication that vitamin D can affect all organ systems including metabolism in bone.There is increase in prevalence of low circulating 25-hydroxyvitamin D (25(OH) D) concentrations linked to restricted sunlight exposure and inadequate vitamin D intake in women of child-bearing age and in children in many countries [1]. In addition to rickets and other possible consequences of disturbed calcium homeostasis, epidemiological evidence indicates lack of vitamin D supplements in infancy and early childhood may increase the incidence of type 1 diabetes mellitus [2].

Vitamin D encompasses a number of steroid-like vitamers: vitamins D2–D7. Vitamin D2 and D3 have known physiological significance in humans, with both undergoing hydroxylation steps to become active hormones and are involved in calcium and phosphorus metabolism. Vitamin D2and D3 differs only by the nature of their side chains. The 2 forms of vitamin D can be obtained from the diet but predominantly, vitamin D is obtained in the D3 form and subsequently by the action of UV light on a vitamin D precursor in the skin [3].Vitamin D3 undergoes two hydroxylation steps before becoming an active hormone: the first step occurs in the liver and results in the production of 25-hydroxyvitamin D (25(OH) D). This form of vitamin D must undergo a further hydroxylation step to become physiologically active in the form of 1-α, 25-hydroxyvitamin D (1α, 25(OH) D). The second hydroxylation step takes place mainly in kidneys and also to some extent in innate immune cells such as monocytes and macrophages [4]. With the discovery of vitamin D receptors (VDRs) in macrophages, the role of 1α, 25(OH) D as an immune modulator has become increasingly apparent [5].

Vitamin D is important for multiple physiologic processes, including calcium absorption. In recent years, the role of vitamin D in regulation of cell growth, immunity and cell metabolism has received prominence [6]. Maternal 25(OH) D is believed to freely cross the human placenta. The placenta expresses vitamin D receptors (VDR) and also produces the enzyme CYP27B1 to convert 25(OH) D to its active form. The most widely appreciated role of vitamin D in the human body is to maintain normal levels of calcium and phosphate in the blood which in turn facilitate other essential processes such as bone mineralization, contraction of muscles, nervous system activities and cellular function. Adequate vitamin D status is critically important for the neonate, with neonatal hypocalcaemia and rickets being major consequences of deficiency. In areas where vitamin D deficiency is endemic, rickets may be diagnosed soon after birth. In general, breast-milk is thought to

be a relatively poor source of vitamin D, making maternal vitamin D status during pregnancy important for vitamin D status of the child during early infancy.The present study was undertaken to study vitamin D level in term pregnant mothers and their new borns. It would help us to decipher relation of vitamin D, calcium, phosphorus and alkaline phosphatase levels between mothers and their new borns.

II. Materials and Methods

The study was conducted over a period of 6 months at M. S. Ramaiah hospitals. 100 consecutive women with full-term, uncomplicated pregnancy in early stages of labor and their live birth neonates were recruited for the study. Pregnant women with chronic liver disease, renal disease or treatment with antitubercular or antiepileptic drugs in the previous 3 months were excluded from the study. The study protocol was approved by the institutional ethics committee. After taking an informed consent, all the details relating to mother like age, obstetric history and other relevant details were recorded.

During the early stages of labor, under aseptic precautions, about 5 ml of venous blood was drawn from mother. After the delivery of the baby, about 5 ml of cord blood from the baby born to the respective mother was collected. Maternal sample and newborn cord blood samples were centrifuged at 3000 rpm for about 8 minutes and the separated sample was analyzed for 25 hydroxy Vitamin D, calcium, phosphorus level and alkaline phosphatase activity. Calcium was estimated according to Schwarzenbach with o-cresolphthalein complexone method, phosphorus by Molybdate UV method and alkaline phosphatase by p-nitrophenyl phosphate method colorimetrically in Roche Cobas 6000. 25 hydroxy Vitamin D levels were analyzed using ELISA kit (Diagnostika, GmbH, Hamburg, Germany) [7]. The plate was read at 450 nm in an ELISA reader (CPC diagnostics, Stat Fax 4700, microstrip reader).

Statistical analysis: The results were expressed as mean ± SD. Significance was assessed at 5% level of significance. Student "t" test (two tailed, independent) was used to find the significance of study parameters. Pearson correlation was used to study the relation between the various parameters. Statistical analysis was performed using SPSS 20.0 software.

III. Results

Table 1- Demographic details and Biochemical profiles in new borns and their mothers (mean ±SD).

Profiles	Mothers	New borns
Age	25.32+4.17 years	Full Term baby
N =	100	100

Table 2- Demographic details and Biochemical profiles in new borns and their mothers (mean ±SD).

Profiles	Mothers (serum)	New borns (cord blood)
Calcium (mg/dl)	9.25±0.83	9.90±1.24
Phosphorus (mg/dl)	4.53±1.48	6.49±1.69
Alkaline phosphatase (IU/ml)	173.43±54.06	157.68±44.32
Vitamin D (ng/ml)	16.35±8.0	15.26±7.30

There is no significant difference in serum calcium level between mothersand theirnew born as shown in Table 2. There is increase in serum phosphorus level in new borns when compared to mothers. There is increase in serum alkaline phosphatase level in mothers ascompared to their new borns, this rise may be due to increased release of alkaline phosphatase synthesized from placenta (Table1). Serum vitamin D levels in mothers and new borns are shown in Table 2.

The result is further analysed based on cord blood vitamin D level in new borns.
Group A- Vitamin D level ≤5 ng/ml (new borns).
Group B- Vitamin D level between 5.1- 15 ng/ml (new borns).
Group C- Vitamin D level between 15.1-25 ng/ml (new borns).
Group D- Vitamin D level ≥25.1 ng/ml (new borns).

Table 3- Biochemical profiles of mother and their new borns in Group A(≤ 5 ng/ml).

Profiles	Mother (serum)	New borns (cord blood)	P value
Vitamin D (ng/ml)	7.73±0.97	3.37±1.0	<0.001***
Calcium(mg/dl)	8.87±0.41	7.52±1.04	= 0.00067
Phosphorus(mg/dl)	3.50±0.88	6.47±0.99	<0.001***
N	10	10	

There is significant difference in serum Vitamin D levels between mother and new born in group A. There is increase in vitamin D level in mother as compared to new borns (Table 3).

Table 4- Biochemical profiles of mothers and their new borns in Group B (between 5- 15 ng/ml).

Profiles	Mother (serum)	New borns(cord blood)	P value
Vitamin D (ng/ml)	10.56±3.4	9.46±2.56	=0.121
Calcium(mg/dl)	8.84±0.97	9.48±1.09	=0.013
Phosphorus(mg/dl)	4.14±0.81	6.01±0.95	<0.001***
N	36	36	-

There is not much of a difference between serum Vitamin D and serum calcium level in Group B. However there is increase in serum phosphorus level in new borns as compared to mother in this group (Table 4).

Table 5- Biochemical profiles of mothers and their new borns in Group C (between 15.1- 25 ng/ml).

Profiles	Mother (serum)	New borns (cord blood)	P value
Vitamin D (ng/ml)	21.76±6.76	20.77±2.48	=0.172
Calcium(mg/dl)	9.59±0.65	10.50±0.76	<0.001***
Phosphorus(mg/dl)	5.02±1.83	6.45±1.31	<0.001***
N	48	48	-

In Group C, the mean level of serum Vitamin D is greater than 20 ng/ml in both mother and their new borns. There is increase in calcium and phosphorus level in new borns in this group.

Table 6- Biochemical profiles of mothers and their new borns in Group D (≥25.1ng/ml).

Profiles	Mother (serum)	New borns (cord blood)	P value
Vitamin D (ng/ml)	24.50±2.73	25.78±0.63	=0.29
Calcium(mg/dl)	9.76±0.28	11.01±0.64	=0.0013
Phosphorus(mg/dl)	4.73±0.48	6.83±1.27	=0.004
N	6	6	-

Both mother and new borns have similar serum Vitamin D level.There is rise in serum calcium and phosphorus level in new borns as compared to mother but the rise is not very significant.

Table 7- Biochemical profiles of new borns in Group A, B & C (cord blood).

Profiles	Group A	Group B	Group C	P value
Vitamin D (ng/ml)	3.37±1.0	9.46±2.56	20.77±2.48	<0.001***
Calcium(mg/dl)	7.52±1.04	9.48±1.09	10.50±0.76	<0.001***
Phosphorus(mg/dl)	6.47±0.99	6.01±0.95	6.45±1.31	=0.207
N =	10	36	48	-

There is gradual rise in serum Vitamin D level from group A to group C. There is rise in serum calcium level from group A to group C with the increase in serum Vitamin D level. There is no significant difference in serum phosphorus levels between various groups. The rise in vitamin D and calcium level is observed from group A to C.

Table 8: Distribution of Vitamin D levels in mothers and their new borns:

Vitamin D levels	Number of cases-Mothers	Number of cases-New borns
Less than 5 ng/ml	02 (2%)	10 (10%)
Between 5.1-15 ng/ml	49 (49%)	36 (36%)
Between 15.1-25ng/ml	35 (35%)	48 (48%)
Greater than 25.1 ng/ml	14 (14%)	06 (6%)

In the present study the distribution of study population in depending on vitamin D level is shown in Table 8. As the table shows only 2% of the mothers have vitamin D level less than 5 ng/ml, whereas 10% of the new borns have vitamin D level less than 5 ng/ml. Similarly 14 % of mothers have vitamin D level greater than 25.1 ng/ml, however 6% have vitamin D level greater than 25.1 ng/ml.

Table 9: Correlation between profiles in new borns in Group A (cord blood).

Biochemical profiles	P value
Vitamin D and Calcium	0.165
Vitamin D and Phosphorus	-0.205
Calcium and Phosphorus	-0.433

Table 10: Correlation between profiles in new borns in Group B (cord blood).

Biochemical profiles	P value
Vitamin D and Calcium	0.012
Vitamin D and Phosphorus	-0.801
Calcium and Phosphorus	-0.468

Table 11: Correlation between profiles in new borns in Group C (cord blood).

Biochemical profiles	P value
Vitamin D and Calcium	0.0205
Vitamin D and Phosphorus	-0.205
Calcium and Phosphorus	-0.433

Correlation between vitamin D, calcium and phosphorus in new borns under various groups are shown in Table 9, 10 & 11. There is no significant correlation in the present study as new born biochemical profile is dependent to a larger extent on their maternal levels and due to transplacental transport of these molecules.

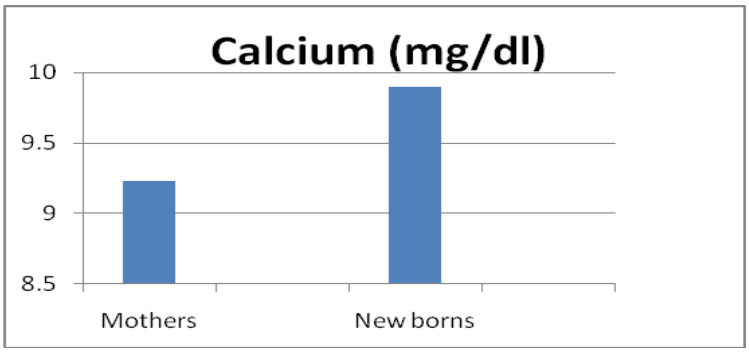

Fig 1: Calcium level in Mothers and their new borns.

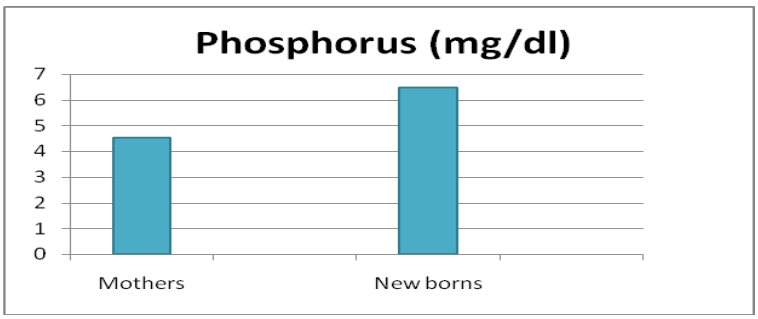

Fig 2: Phosphorus level in Mothers and their new borns.

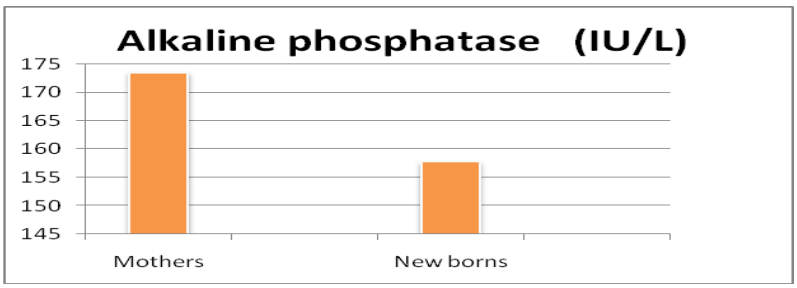

Fig 3: Alkaline phosphatase level in Mothers and their new borns.

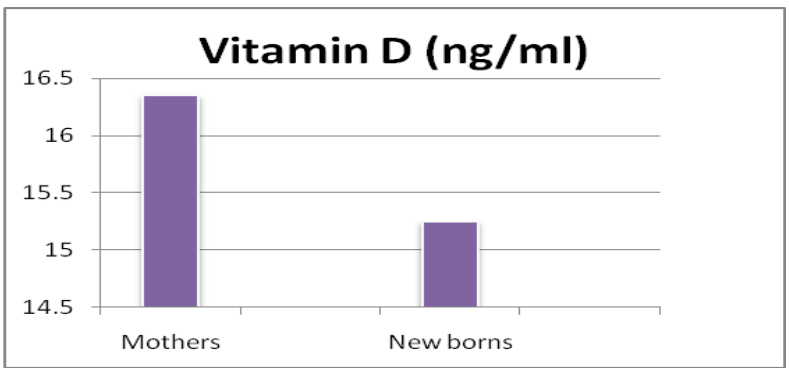

Fig 4: Vitamin D level in Mothers and their new borns.

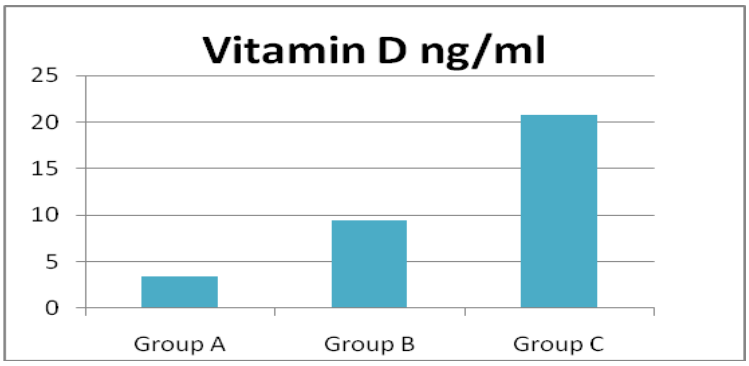

Fig 5: Cord blood Vitamin D level of new borns in various groups.

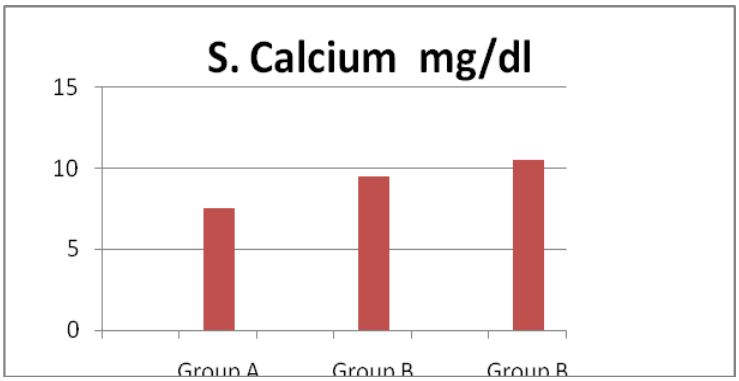

Fig 6: Cord blood calcium level of newborns in various groups.

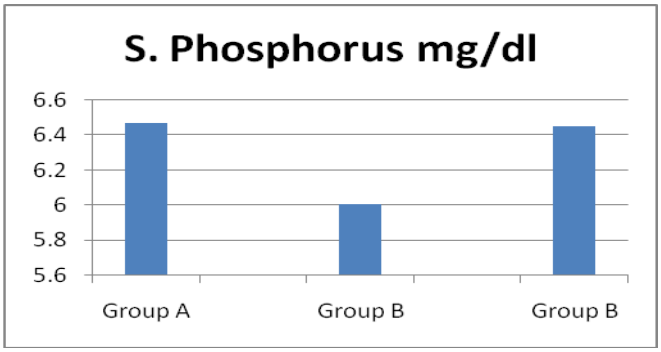

Fig 7: Cord blood phosphorus level of new borns in various groups.

IV. Discussion

Calcium and bone metabolism in adults is dependent on vitamin D level and vitamin D sufficiency would be especially critical during pregnancy and lactation. Vitamin D–deficient or insufficient neonates are at greater risk in predisposing complications including hypocalcemia and rickets [8]. During pregnancy, mothers provide large amounts of calcium to the developing fetus. However, it has been reported about maternal adaptations during pregnancy, lactation and fetal development provides the necessary calcium relatively independently of vitamin D status [9]. It is only after birth that dependency on vitamin D becomes evident, at least with respect to calcium metabolism and skeletal health. During gestation, the human fetus accretes 30 g calcium on average, of which 99% is contained within the skeleton system. More than 150 mg/kg of this calcium is actively transferred each day across the placenta during the third trimester [10]. The present study is designed to determine maternal vitamin D level along with other biochemical profiles. Serum calcium concentrations (which include ionized, protein-bound and complexed fractions) fall early in pregnancy as a result of drop in serum albumin. In the present study there is decrease in serum calcium level below the physiological limit in group A as compared to other groups (Table 3). The levels of other hormones with potential calcium-regulating effects—including estradiol, prolactin, placental lactogen and the calcium-regulating hormone parathyroid hormone-related protein increases during pregnancy. 25-Hydroxyvitamin D [25(OH)D], the storage form of vitamin D, readily traverses the placenta and thereby cord blood 25(OH)D concentrations are nearly equal to maternal concentrations. The cord blood sample in the present study is used

to assess vitamin D and other biochemical profiles in new borns. Vitamin D receptors can be found in most tissues in the body and the impact of vitamin D deficiency on the developing fetus and maternal health is of significant concern.

Adequate maternal vitamin D status could play essential roles in ensuring appropriate fetal and placental development and proper immune response during pregnancy [11]. It also regulates key target genes associated with proper implantation of the placenta. There is no significant difference in maternal vitamin D, calcium and phosphorus level as compared to their levels in new borns especially in Group B, C & D (table 4, 5 & 6). However, under Group A there is significant decrease in new born vitamin D as compared to their maternal level (table 3). Subsequently there is decrease in phosphorus levels along with decrease in vitamin D levels in new borns in this group. This indicates there is increase in influence of vitamin D levels on phosphorus levels as compared to calcium levels.

There are reports from many countries of high prevalence of vitamin D deficiency in women of child-bearing age, during pregnancy and in nursing mothers associated with adverse consequences on women, fetus, growing infants and in children [12]. Maternal age has no association with vitamin D levels. Research studies showed adverse outcomes of vitamin D deficiency such as neonatal rickets and low bone mineral density in childrens. There is increase in phosphorus level in new borns in the study (table 2) as compared to their mother due to increased growth and development in intra uterine life. There was decrease in serum phosphorus level in Group B as compared to Group A & C (table 7). However, the mean value of phosphorus is greater than 6 mg/dl in all the three groups. There was increase in serum alkaline phosphatase level in pregnant mother as compared to their new borns (table 2). There is release of alkaline phosphatase from placenta also, which can contribute to their higher level.

According to modified Kuppuswamy scale, it was observed that as socioeconomic status increases, frequency of vitamin D deficiency decreases [13]. Variation in deficiency of vitamin D levels during pregnancy has been observed from different parts of the country. Study done by Sachan et al.[14] observed that 84 % of pregnant women were deficient of vitamin D taking the cut-off of 22.5 ng/ml and serum mean 25(OH) level of 14 ± 9.3 ng/ml which is comparable with mean serum 25(OH) D level of 15.41 ± 8.97 ng/ml in the present study population. Vitamin D insufficiency is associated with an increased risk of gestational diabetes, pre-eclampsia and small for gestational age infants.

In adults, a serum 25(OH) D concentration <50–80 nmol/l is now considered insufficient for optimal bone health. Maternal vitamin D deficiency during pregnancy has been reported and infants born to vitamin D insufficient mothers become vitamin D deficient after 8 weeks if not supplemented with vitamin D. Infants born to vitamin D deficient mothers, if not supplemented will reach a state of deficiency more quickly otherwise. Significance of estimation of vitamin D levels in new borns is that many immune cells express the vitamin D receptor, including T and B cells, dendriticcells as well as macrophages [15]. The biologic effects of 1,25-vit D3 are primarily mediated via the nuclear transcription factor, vitamin D receptor (VDR), which triggers expression of vitamin D-responsive genes.The importance of vitamin D in calcium and phosphate homeostasis is well known, recent studies have demonstrated that vitamin D has "nonclassical" effects, including an important role in down regulating immune responses [16].

There are reports which indicate insufficient supply with vitamin D may lead to the dysregulation of the human immune response and may therefore be an underlying cause of infectious diseases and immune disorders [17]. Vitamin D not only stimulates innate immunity but also modulates adaptive immunity to minimize inflammation and autoimmune diseases. The active vitamin D- 1,25(OH)2D, which is produced in monocytes or macrophages, is released to act locally on activated T lymphocytes, which regulate the synthesis of cytokines, such as interleukin 2 (IL-2), IL-4, IL-10, gamma interferon and activated B lymphocytes, which synthesize immunoglobulin's. 1,25-vit D3 decreases specific inflammatory responses in mature circulating neutrophils and that this is impaired in neonatal cells [18]. Vitamin D can trigger resolution of inflammatory responses. There are reports which suggest decreased expression of 1α-hydroxylase in neonatal neutrophils, when compared to adults. 1α-hydroxylase is required for the generation of 1,25-vit D3, the biologically active form that triggers binding to response elements in the promoter regions of vitamin D responsive genes.Vitamin D signaling has emerged as a key regulator of immunity in humans.

Neonatal sepsis is characterized by signs and symptoms of infection with or without accompanying bacteremia in the first month of life and is an important cause of morbidity and mortality [19]. The incidence of neonatal sepsis varies between 1 to 8 neonates per 1000 live births. Newborns are more susceptible to infections as both innate and adaptive immune systems are not entirely developed. The relationship between vitamin D deficiency and infections, especially lower respiratory tract infections (RTIs), has been demonstrated in children and newborns. Low cord blood 25-hydroxy vitamin D levels in healthy newborns were found to be associated with an increased risk of developing respiratory syncytial virus infections during infancy. Vitamin D was reported to have a complex effect on immune functions as it enhanced innate immunity while it also downregulated the acquired immune response [20]. The mechanical barrier of the skin and other epithelial

surfaces constitute the first barrier to infections and activated vitamin D has an important role in maintaining the integrity of epithelial cells by encoding the proteins needed for several tight junctions. Vitamin D has anti-inflammatory actions on neutrophils. It inhibits B cell proliferation and blocks B cell differentiation and immunoglobulin secretion [21].

Vitamin D appears to play an important role in the integrity of the innate immune response.The significance of maternal deficiency during pregnancy as in the present study is that the fetus is developing in a state of hypovitaminosis D, which likely has significant effects on innate immune function and influencing fetal bone development. With severe maternal vitamin D deficiency, the fetus may rarely develop rickets in utero and manifest this deficiency at birth. Studies examining the vitamin D status of mothers of rachitic children have demonstrated a high prevalence of maternal vitamin D deficiency. Further, there is a higher prevalence of vitamin D deficiency in the mothers of rachitic than non-rachitic children. The connection may be a combined effect of the positive relationship between maternal vitamin D nutritional status and vitamin D status in early infancy. Vitamin D has important immune-modulating properties of vitamin D which may help to establish a proper maternal immune response to the placenta [22]. The shift from vitamin D sufficiency to deficiency has occurred in many populations as a result of insufficient sun exposure and inadequate corrective vitamin D supplementation [1]. Published data indicate that vitamin D deficiency may be an unrecognized public health problem in women, nursing mothers and children in many populations. Strategies to prevent vitamin D deficiency and achieve adequate intake of vitamin D and calcium in women and throughout childhood would not only prevent rickets but may also reduce the risk of osteoporosis as well as other disease processes that have been associated with vitamin D deficient states in adults.

V. Conclusion

Vitamin D is an essential nutrient with well-established roles in calcium metabolism and the prevention of rickets. Vitamin D has an important role in preventing infection during pregnancy or early childhood via its immune modulating effects.

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