VITAMIN D DEFICIENCY AND ANEMIA IN PREGNANCY:

HOW ARE THEY ASSOCIATED?

DISSERTATION

Submitted to

THE TAMILNADU DR.MGR MEDICAL UNIVERSITY

In partial fulfilment for the degree

DEGREE OF MEDICINE

IN

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DEPARTMENT OF BIOCHEMISTRY

CHRISTIAN MEDICAL COLLEGE

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CERTIFICATE

This is to certify that the study titled **"VITAMIN D DEFICIENCY AND ANEMIA IN PREGNANCY: HOW ARE THEY ASSOCIATED"** is the bonafide work of Dr. R. S. Logapriya, who conducted it under the guidance and supervision of Dr. Molly Jacob, Professor of Biochemistry, Christian Medical College, Vellore. The work in this dissertation has not been submitted to any other university for the award of a degree.

Dr. Molly Jacob,

Professor,

Department of Biochemistry,

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Vellore.

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Professor and Head of the Department,

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Principal,

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DECLARATION

I hereby declare that the investigations, which form the subject matter of this study, were conducted by me under the supervision of Dr. Molly Jacob, Professor of Biochemistry, Christian Medical College, Vellore.

Dr. R.S. Logapriya,

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ABSTRACT

Background to the study

Iron-deficiency anemia is common in pregnancy, affecting up to 50% and 12-25% of pregnant women in developing and developed countries, respectively. Vitamin D deficiency is also common in pregnancy. There is accumulating evidence in recent years that suggests a link between vitamin D deficiency (VDD) and risk of anemia in pregnancy. It is not clear what underlies this association.

Aim

The aim of the present study was to estimate vitamin D levels and iron-related parameters in blood from pregnant women and determine how these correlate with one another.

Methods:

Subjects of the study were primigravidae, who attended the antenatal clinic of Unit 4, Department of Obstetrics and Gynecology, at Christian Medical College (CMC), Vellore, India. Primigravidae with a singleton pregnancy and no complications of pregnancy were recruited, after obtaining informed consent. Their body mass index (BMI) was determined. A blood sample was collected from each subject and used for estimating vitamin D levels, haematological parameters (haemoglobin and mean corpuscular volume), iron-related parameters (serum iron, ferritin, total iron-binding capacity (TIBC) and transferrin saturation), creatinine (as a marker for renal function) and C-reactive protein (CRP) (a marker

of systemic inflammation). Subjects were categorized into those with and without vitamin D deficiency, based on a cut-off value of 20 ng/mL of serum 25 (OH) vitamin D. Correlation analyses was carried out for the parameters of interest.

Results:

Thirty subjects were recruited for the study. Most of them were vitamin D-deficient (86.66%). There were no significant differences between vitamin D-deficient and sufficient subjects, with regard to hematological and iron-related parameters. Correlations were seen between hematological and iron-related parameters in the subjects, and were in keeping with known biological relationships amongst the parameters of interest. However, there were no correlations found between any of these parameters and vitamin D levels. Serum vitamin D levels correlated positively with the gestational age at recruitment and serum CRP levels correlated positively with TIBC.

Conclusion:

There were no significant differences between vitamin D-deficient and sufficient subjects in this study, with regard to hematological and iron-related parameters. No correlation was found between vitamin D levels and iron-related parameters. Since the sample size in this study was small, an adequate number of subjects would need to be studied to confirm these findings.

REVIEW OF LITERATURE

OVERVIEW OF VITAMIN D METABOLISM

Vitamin D is a fat-soluble vitamin. It exists as ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). Ergocalciferol is produced from ergosterol, found in plants and fungi, by the effect of UVB irradiation (Bikle, 2014). Foods such as fish liver oils, mushrooms, egg yolks, fatty fish and liver are rich sources of vitamin D (Holick and Chen, 2008).

Cutaneous synthesis: 7-dehydrocholesterol (an intermediate of cholesterol synthesis) is present in the specialized skin cells called, called keratinocytes which is the inner layer of epidermis (Khurana et al., 2019). On exposure to ultraviolet rays 7-dehydrocholesterol is converted into pre-vitamin D_3. This conversion is a non-enzymatic reaction. Pre-vitamin D_3 undergoes thermal isomerization over a period of a few hours to form cholecalciferol.

STRUCTURE OF ERGOCALCIFEROL AND CHOLECALCIFEROL

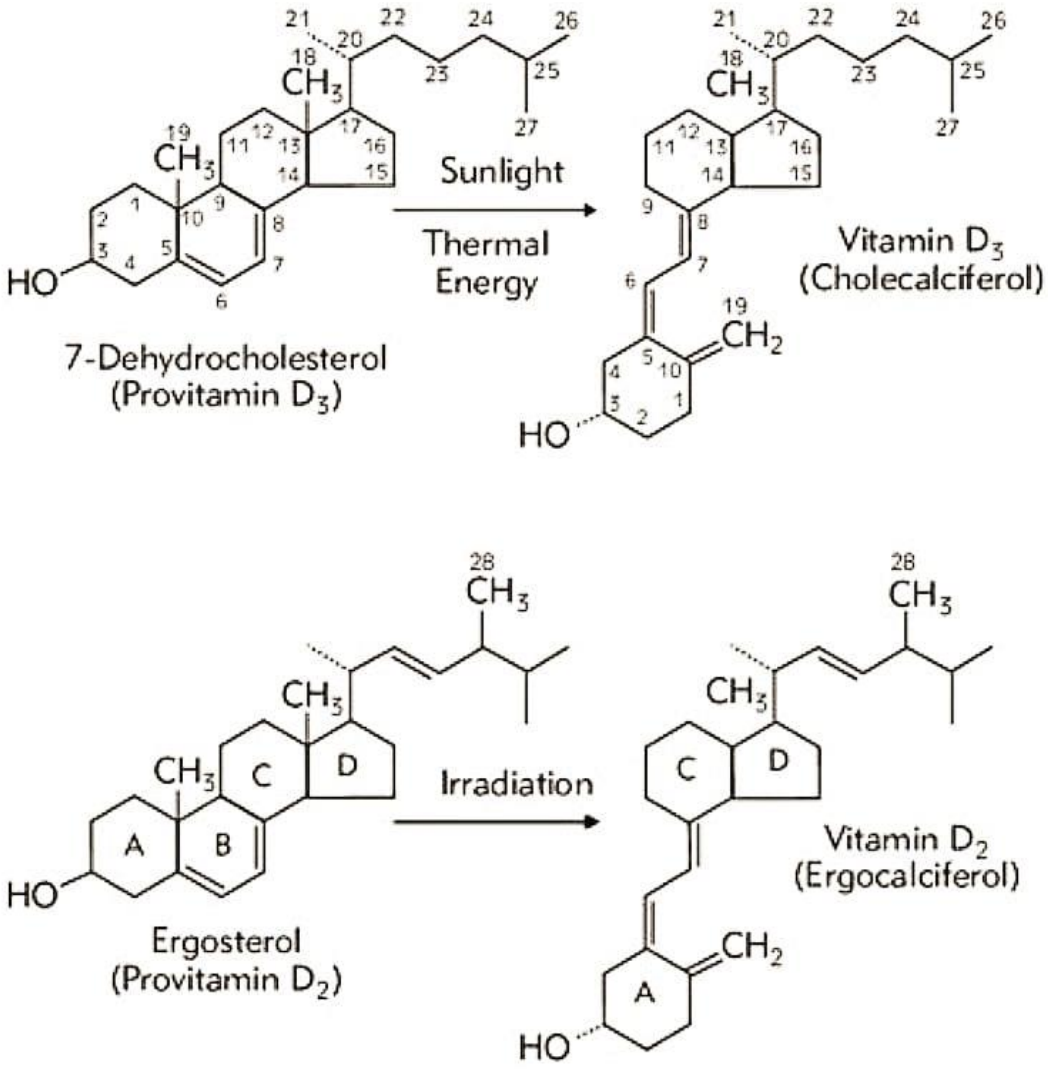


Figure 1: Structure of ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3).

Source: Jovicic, S., Ignjatovic, S., Majkić-Singh, N., 2012. Biochemistry and metabolism of vitamin D / Biohemija i metabolizam vitamina D. Journal of Medical Biochemistry 31. https://doi.org/10.2478/v10011-012-0028-8

In Liver: Once formed in the skin, cholecalciferol enters the circulation and is hydroxylated by 25-hydroxylase in the liver to produce 25-hydroxy vitamin D (calcidiol).

In Kidney: 25-hydroxy vitamin D is further acted upon by 1α-hydroxylase (CYP27B1) in the kidney to produce 1, 25-dihydoxy-vitamin D [$1,25(OH)_2D$] or calcitriol, which is the active form of vitamin D. This conversion takes place in the cells of proximal convoluted tubules and is the rate-limiting step in vitamin D synthesis (Khurana et al., 2019). An inactive metabolite, 24-hydroxycalcidiol is also produced in the kidney when 25-hydroxy vitamin D undergoes 24-hydroxylation (Rodwell et al., 2018).

The major storage and circulatory form of vitamin D is 25-hydroxycholecalciferol. In the plasma, it is bound to vitamin-D binding protein (VDBP). The half-life of VDBP is 3 days. VDBP has greater affinity for 25-hydroxy vitamin D_3 than for calcitriol (Khurana et al., 2019).

Calcitriol, being lipophilic, spontaneously dissociates from the carrier protein (Vitamin D binding globulin) and diffuses across plasma membrane of target cells (Oren et al., 2004). It produces its actions via the vitamin D receptor (VDR). In its free state, VDR is mainly present in the cytoplasm and widely expressed in the intestinal epithelium, osteoblasts, chondrocytes, monocytes, macrophages, T-lymphocytes, etc (Wang et al., 2012).

(Nagpal, 2005). When calcitriol binds to VDR, this complex translocates to the nucleus and dimerizes with retinoid X receptor (RXR). Here, it binds to vitamin D response elements (VDRE) which are present in the enhancer regions of genes that are responsive to Vitamin D-mediated actions (Nagpal et al, 2005; Pike et al, 2012; Ferrier, 2017).

VITAMIN D METABOLISM

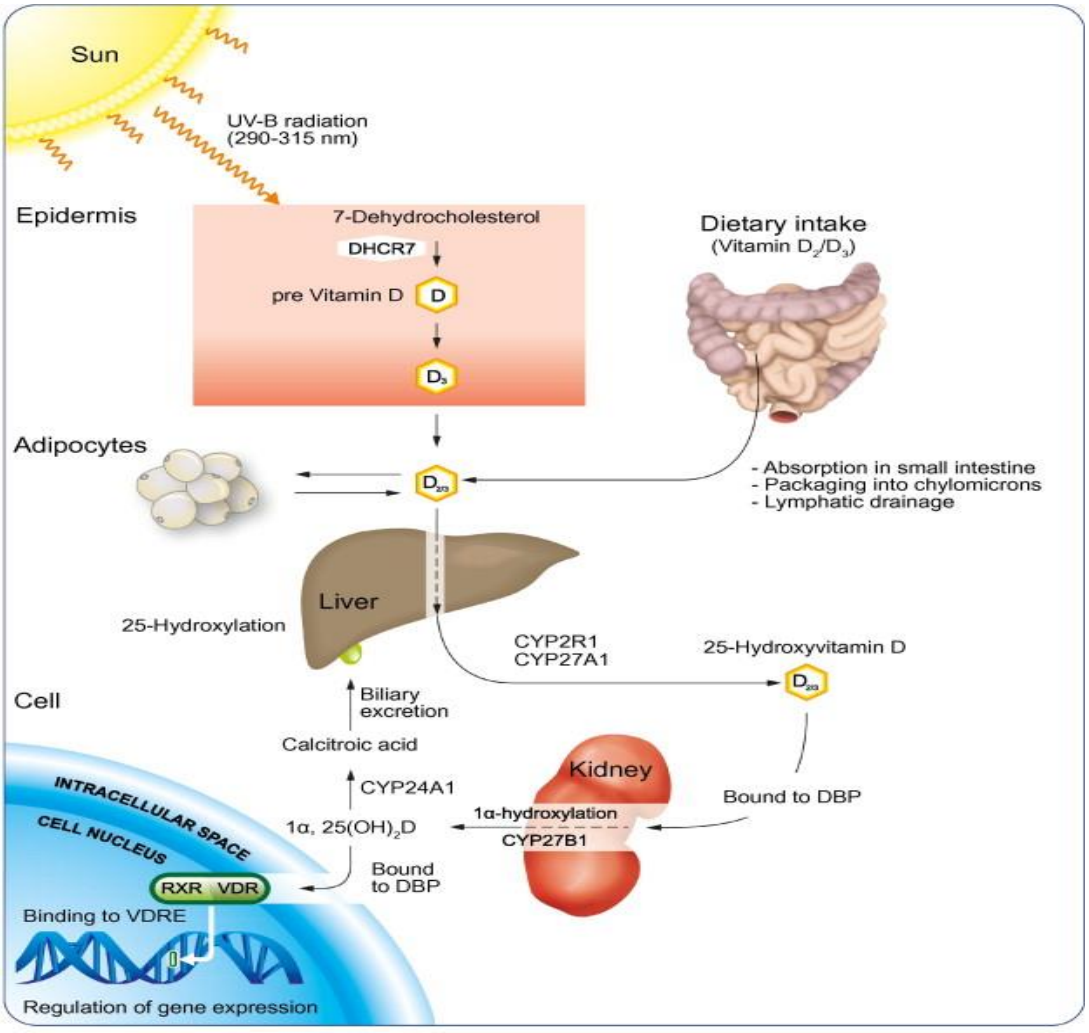


Figure 2: Overview of vitamin D metabolism

Source: Kitson, M.T., Roberts, S.K., 2012. D-livering the message: The importance of vitamin D status in chronic liver disease. Journal of Hepatology 57, 897–909. https://doi.org/10.1016/j.jhep.2012.04.033

FUNCTIONS OF VITAMIN D

Vitamin D has two discrete functions. One, as a circulating hormone and the other as a locally acting cytokine (Adams and Hewison, 2010).

Intestinal calcium absorption

Calcium entry into enterocytes: In the intestinal epithelial cells, vitamin D increases the formation of a calcium binding protein called calbindin. In the brush border of the intestinal epithelial cells, calbindin helps to transport calcium into the cytoplasm of these cells. Two calcium channels exist, namely, TRPV5 and TRPV6. They contain six membrane-spanning domains and are members of the transient receptor potential vanilloid receptor subfamily. These channels are expressed in the duodenum, kidney, jejunum and in various other tissues. Calcitriol increases the expression of TRPV6 which plays a critical role in the absorption of calcium (Melmed et al, 2016). Intestinal calcium absorption is directly proportional to the amount of calbindin present in the cells. Since, calbindin remains for several weeks in the cells even after the removal of calcitriol, the calbindin effect on calcium absorption is prolonged (Hall and Hall, 2020). Vitamin D also increases calcium absorption by other mechanisms. This effect is rapid and is independent of the calbindin synthesis (Rodwell et al., 2018).

Transcellular transport of calcium: Two calcium ions bind to each calbindin molecule on its EF hand structure. Calbindin's affinity for calcium is four times greater when compared to other intracellular calcium-binding proteins. During calcium absorption, calbindin buffers the intracellular free calcium concentration. In addition, calbindin associates with microtubules and helps in the transport of calcium from the apical to the basolateral side of the enterocyte (Melmed et al, 2016).

Exit of calcium from the enterocytes: This is the last step in the intestinal calcium absorption and is also dependent on calcitriol. Calcium extrusion depends mainly on the mechanism of calcitriol inducible ATP-dependent calcium pump (PMCA1b). This pump has approximately 2.5 times greater affinity for calcium when compared to calbindin. This pump helps to transport calcium across the enterocyte's anti-luminal (basolateral) surface. Transport of calcium across the basolateral membrane is also helped by Na^{+}/Ca^{2+} exchanger (Melmed et al, 2016).

ACTION OF VITAMIN D ON INTESTINAL ABSORPTION OF CALCIUM

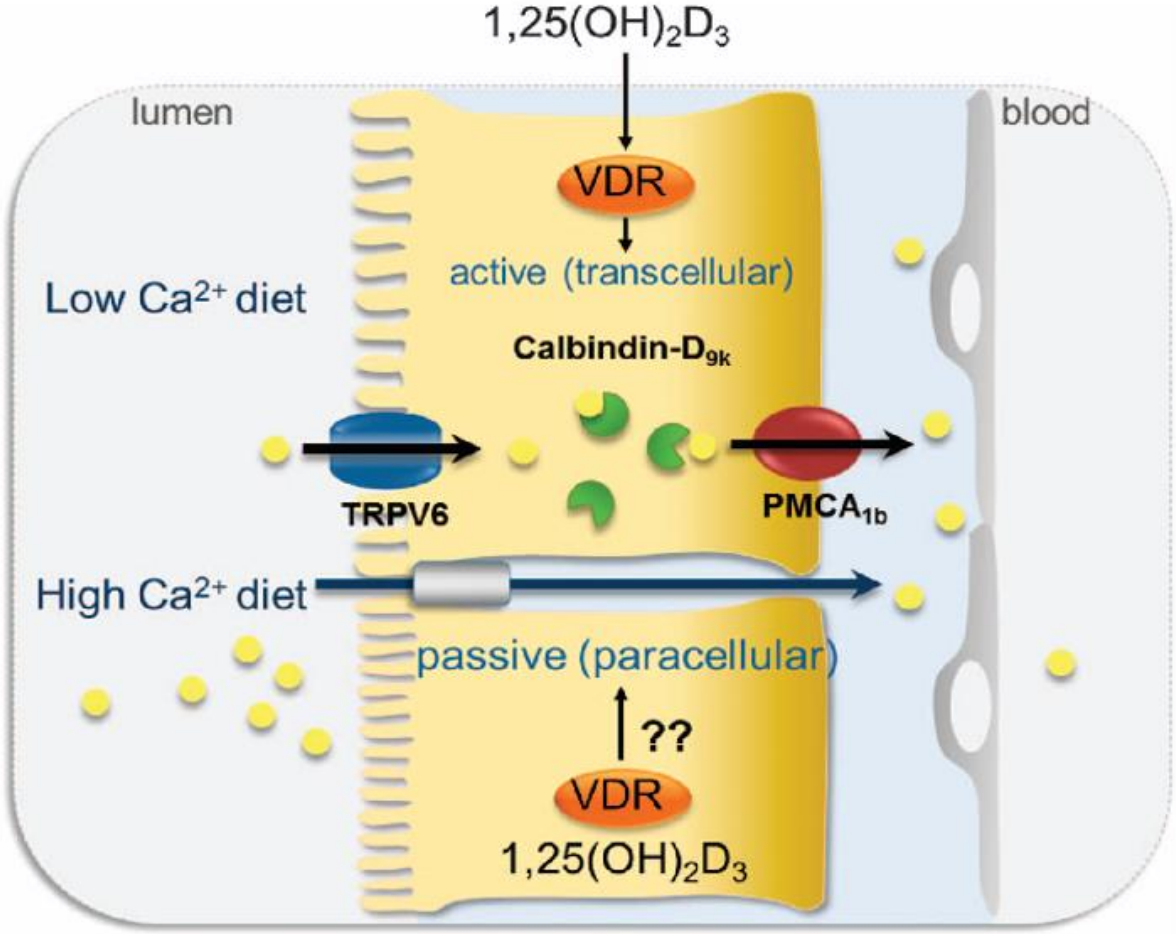


Figure 3: Mechanism of intestinal calcium absorption
When dietary calcium is low or normal, the transcellular active mechanism helps in intestinal calcium absorption via TRPV6, Calbindin-D9k and PMCA1b. During high calcium intake, a passive paracellular pathway also plays an important role.

Source: Christakos, S., Lieben, L., Masuyama, R., Carmeliet, G., 2014. Vitamin D endocrine system and the intestine. BoneKEy reports. https://doi.org/10.1038/bonekey.2013.230

Effect of vitamin D on phosphate absorption in the intestine:

Enhanced phosphate absorption from the jejunum and ileum occurs due to the hormonal action of vitamin D (Burtis et al., 2015). It occurs due to the direct effect of calcitriol on phosphate transporters. Vitamin D also increases calbindin synthesis which increases the intracellular calcium levels. Calcium in-turn acts as a transport mediator for phosphate (Hall and Hall, 2020).

Effect of vitamin D on calcium and phosphate reabsorption in the kidney:

Calcitriol induces the expression of fibroblast growth factor 23 (FGF23). FGF23 is a hormone that plays a key role in phosphate homeostasis by regulating renal phosphate excretion as shown in Figure 4 (Melmed et al, 2016). In the proximal tubule, FGF23 decreases the reabsorption of phosphate and 1α-hydroxylation of 25(OH)D.

FGF23 binds to FGF receptor/ Klotho in the proximal tubule causing phosphorylation of extracellular signal-related kinases (ERK) and activation of glucocorticoid-regulated kinase 1 (SGK1). SGK1 in turn phosphorylates Na^+/H^+ exchanger regulatory factor 1 (NHERF1) resulting in the degradation of the luminal phosphate transporter, NaPi-IIa (Sodium dependent phosphate cotransporter). The expression of NaPi-IIa leads to renal phosphate loss (Melmed et al, 2016).

FGF23 increases the calcium and sodium reabsorption in the distal tubule by increasing the expression of TRPV5 calcium channels and Na^+/Cl^- cotransporter. This process also requires the activation of the FGF receptor/Klotho complex, followed by the activation of ERK and SGK1 kinase as in the proximal tubule (Melmed et al, 2016).

FGF23 ON PHOSPHATE HOMEOSTASIS

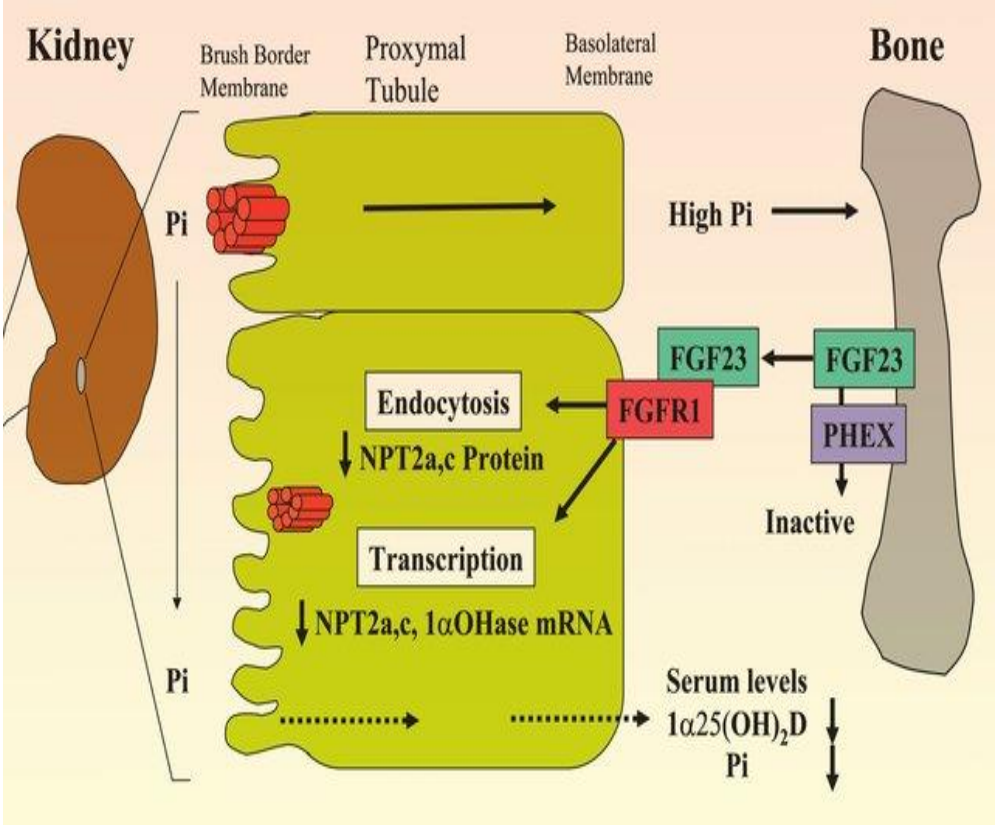


Figure 4: Phosphate homeostasis by FGF23. FHF23 causes phosphate reabsorption in the proximal tubule by degrading the sodium dependent phosphate cotransporter. FGF23 also decreases the 1-α hydroxylase activity.

Source: Takeda, E., Yamamoto, H., Nashiki, K., Sato, T., Arai, H., Taketani, Y., 2004. Inorganic phosphate homeostasis and the role of dietary phosphorus. Journal of cellular and molecular medicine 8, 191–200. https://doi.org/10.1111/j.1582-4934.2004.tb00274.x

Action of vitamin D on the bone:

Calcitriol has numerous effects on bone. These are summarized below:

Bone resorption: Osteoclasts, which are bone-resorbing cells, express calcitriol receptors. Calcitriol binds to these receptors to activate a complex intracellular signaling cascade which results in differentiation, recruitment and fusion of precursor cells into mature osteoclasts. These osteoclasts cause bone resorption with the help of calcitriol, a process in which parathyroid hormone is also required (Khurana et al., 2019). In addition, calcitriol promotes monocyte-macrophage stem cells in bone marrow to differentiate into osteoclasts (Hall and Hall, 2020). Calcitriol, when administered in high doses, will cause resorption of bone by stimulating the RANKL (also known as osteoclast differentiating factor) production from the osteoblast (Melmed et al, 2016) (Hall and Hall, 2020).

Bone mineralization: One of the major roles of calcitriol is to promote mineralization of bone by increasing calcium and phosphorus absorption from the intestine. Type 1 collagen and osteocalcin are the two most abundant matrix proteins present in the bone. Expression of both these proteins is transcriptionally regulated by calcitriol. Calcitriol represses the type I collagen synthesis and induces the osteocalcin synthesis (Melmed et al, 2016). Calcitriol increases the synthesis alkaline phosphatase and osteoblastic proliferation, thus contributing its direct effect on bone formation (Khurana et al., 2019).

OVERVIEW OF NORMAL BONE METABOLISM

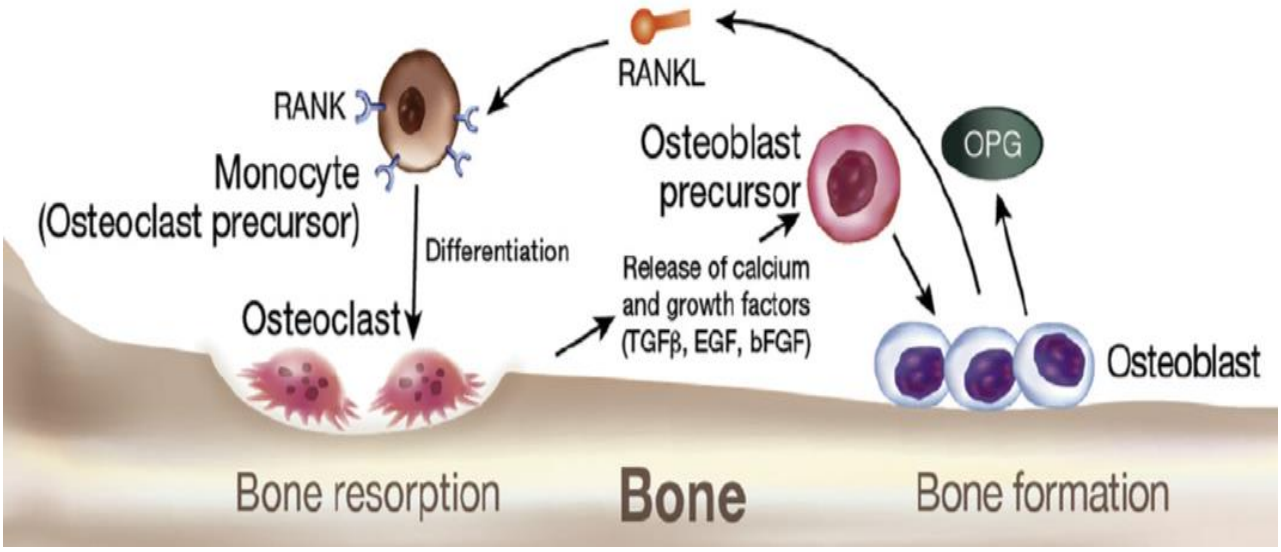


Figure 5: Overview of normal bone metabolism

Showing bone resorption via osteoclast and bone formation via osteoblast. RANKL binds to RANK on osteoclast precursors which differentiation into mature osteoclasts. Resorption of bone matrix is caused by the mature osteoclast releasing calcium and various growth factors like transforming growth factor-β (TGF-β), epidermal growth factor (EGF) and fibroblast growth factor (FGF). These stimulate the precursor osteoblast to differentiate into mature osteoblasts. Negative feedback of this process is taken care of by RANKL which is inhibited by osteopontegerin (OPG)

Source: Modified from Crawford, E., Schally, A., Pinthus, J., Block, N., Rick, F., Garnick, M., Eckel, R., Keane, T., Shore, N., Dahdal, D., Beveridge, T., Marshall, D., 2017. The potential role of follicle-stimulating hormone in the cardiovascular, metabolic, skeletal, and cognitive effects associated with androgen deprivation therapy. Urologic Oncology: Seminars and Original Investigations 35. https://doi.org/10.1016/j.urolonc.2017.01.025

Action of vitamin D on parathyroid gland:

Calcitriol plays a critical role in the growth and development of the parathyroid gland. Both in vitro and in vivo gene expression of parathyroid hormone (PTH) is decreased by calcitriol. This effect of calcitriol is utilized in the treatment of secondary hyperparathyroidism associated with chronic renal failure (Melmed et al, 2016.).

Action of vitamin D on muscle:

Calcitriol has a non-genomic effect on muscle cells which express the vitamin D receptors. Calcitriol causes alteration in phospholipid metabolism and increases amino acid uptake *in vitro* in the muscle cells (Melmed et al, 2016). Calcitriol stimulates the transport of calcium into skeletal and cardiac muscle (Khurana et al., 2019). A calcium binding protein called troponin C increases in concentration upon vitamin D administration. Troponin C is involved in excitation coupling and increases calcium uptake rate by the sarcoplasmic reticulum (Melmed et al, 2016).

Effect of vitamin D on Immunity:

Vitamin D is required for the proper functioning of the immune system and is involved in the process of inflammation (Jameson et al., 2018). Calcitriol stimulates the differentiation and proliferation of immune cells. It has been shown that calcitriol can be synthesized by monocytes, transformed lymphocytes and macrophages. Monocytes,

activated T lymphocytes and promyelocytes express the receptor for calcitriol (Khurana et al., 2019).

Effect of vitamin D on growth:

Vitamin D receptors (VDR) are found in anterior pituitary, ovary, hypothalamus, placenta, fibroblast of skin and endothelium of aorta. Hence, calcitriol is considered to be involved in growth regulation and growth hormone synthesis (Khurana et al., 2019).

Effects of vitamin D on keratinocytes:

Calcitriol has paracrine and autocrine functions by which it stimulates the differentiation of keratinocytes and also causes inhibition of their proliferation. Vitamin D regulates the formation of cornified outer layer of the epidermis with their enzymes and proteins. Due to this action of vitamin D, it is used to treat psoriasis (Khurana et al., 2019).

REGULATION OF VITAMIN D SYNTHESIS:

The conversion of 25-hrdroxy vitamin D into calcitriol by 1-α hydroxylase activity in the liver is strictly regulated when compared to the conversion of vitamin D_3 to 25-hrdroxy vitamin D in the liver. The regulation in the kidney takes place via the following:

1.Plasma calcium: Calcitriol synthesis is regulated by plasma calcium levels by feedback mechanism and indirectly through parathyroid hormone action (PTH). When there is hypocalcemia, parathyroid glad is stimulated to produce PTH. PTH induces 1-alpha hydroxylase in the kidney which increases the formation of calcitriol. When there is hypercalcemia, parathyroid hormone release is inhibited resulting in decreased calcitriol. Adaptive mechanism for the calcium absorption by vitamin D in the intestine depends on these effects. (Khurana et al., 2019).

REGULATION OF CALCITRIOL BY PLASMA CALCIUM

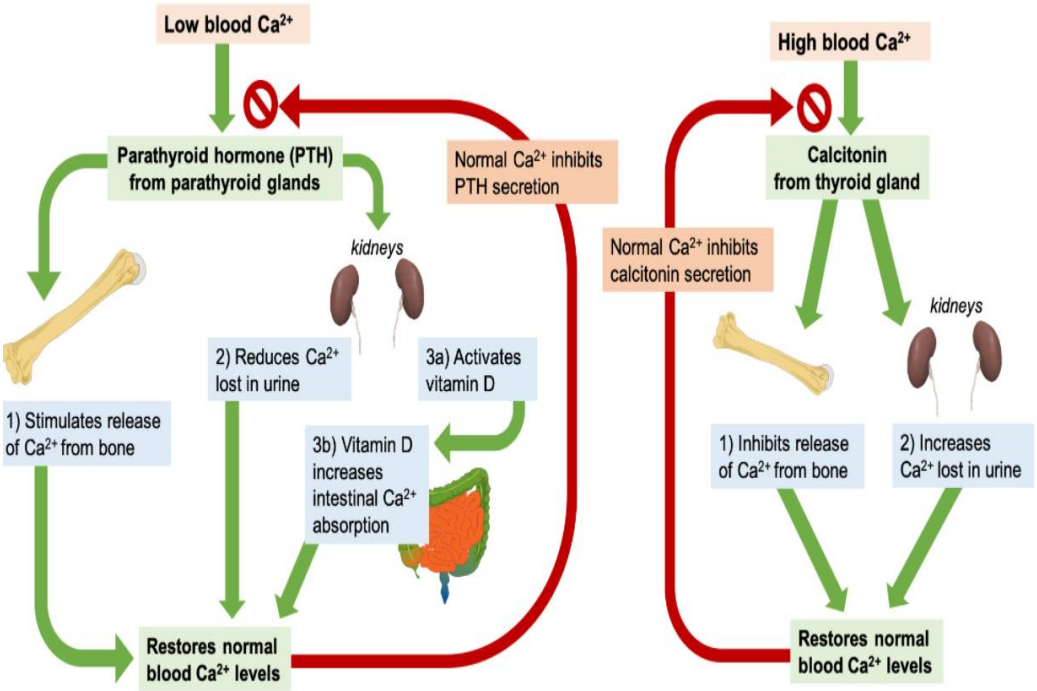


Figure 6: Plasma calcium levels regulate calcitriol synthesis

Source: Alice Callahan, P., Heather Leonard, Me., Tamberly Powell, M.S., 2020. Calcium: Critical forBones and Throughout the Body.

2.Plasma phosphate: Hyperphosphatemia inhibits 1-α hydroxylase enzyme activity, thus causing a decrease in calcitriol synthesis (Burtis et al., 2015). Hypophosphatemia induces 1-α hydroxylase, thereby increasing the synthesis of calcitriol (Khurana et al., 2019).

3.Calcitriol: Calcitriol reduces 1-α hydroxylase activity, thereby decreasing its own production. Calcitriol also induces 24-hydroxylase activity, producing 24,25 dihydroxyvitamin D, which is an inactive form of vitamin D (Burtis et al., 2015). In addition, calcitriol's direct action on parathyroid glad causes inhibition of PTH gene expression (Khurana et al., 2019).

4. Other factors: Calcitriol synthesis is increased by prolactin. Circulating calcitriol levels are increased by estrogen probably due to vitamin D binding protein increase. Metabolic acidosis decreases the synthesis of calcitriol. Low calcitriol in circulation and increased incidence of osteoporosis is seen in hyperthyroidism. Calcitriol formation is stimulated by growth hormone, calcitonin and HCG (Khurana et al., 2019).

VITAMIN D DEFICIENCY

Vitamin D deficiency (VDD) is common worldwide. A blood concentration of <20 ng/mL (50 nmol/L) of 25 hydroxy vitamin D [25(OH)D] is defined as vitamin D deficiency by "The clinical practice guidelines of the Endocrine Society"(Amrein et al.,

2020). Levels between 20–30 ng/mL (50–75 nmol/L) of 25(OH)D are defined as vitamin D insufficiency. A value more than or equal to 30 ng/mL is considered normal (Judistiani et al., 2018a; Richard et al., 2017).

Major causes for impaired vitamin D action are given below (Jameson et al., 2018):

Nutritional deficiency	Deficiency of vitamin D in diet Malabsorption
Decreased cutaneous synthesis	Low exposure to sunlight
Accelerated loss of vitamin D	Due to increased hepatic metabolism of vitamin D induced by certain drugs (e.g., barbiturates, phenytoin and rifampicin) Increased urinary loss of vitamin D (e.g.., in nephrotic syndrome)
Impaired 25-hydroxylation	Due to advanced liver disease Inhibition of 25 hydroxylase by drugs like isoniazid
Impaired 1α- hydroxylation	Chronic kidney disease Hypoparathyroidism 1α- hydroxylase mutation (vitamin D-dependent rickets type 1) Inhibition by drugs like ketoconazole

Major causes for impaired vitamin D action (continued)

Target organ resistance	Vitamin D receptor mutation Drugs like phenytoin
FGF 23 Excess	Oncogenic osteomalacia X-linked hypophosphatemic rickets
Other causes	Obesity

Various causes are responsible for Vitamin D deficiency like impaired cutaneous synthesis of Vitamin D due to reduction in sunlight exposure. Poor dietary intake of vitamin D rich food leads to VDD. Malabsorption of vitamin D occurs in diseases like celiac sprue, cystic fibrosis and small bowel syndrome leading to deficiency.

Vitamin D absorption from the intestine and the skin's efficiency of Vitamin D synthesis markedly reduces with age making the elderly population more prone to develop VDD. Other contributing factors for VDD are fat malabsorption and deep pigmented skin (Jameson et al., 2018).

Vitamin D metabolites are inactivated in an accelerated manner by drugs that induce hepatic cytochrome P450 mixed-function oxidases (barbiturates, phenytoin and rifampin). Impaired enterohepatic circulation in conditions like cirrhosis can lead to loss

of vitamin D. Nephrotic syndrome also leads to accelerated loss of vitamin D (Jameson et al., 2018).

VDD can arise due to impaired 25-hydroxylation seen in liver diseases, 25-hydroxylase mutation and those who are on isoniazid (Jameson et al., 2018).

Impaired 1α-hydroxylation leads to impaired synthesis of the active form of vitamin D, calcitriol. Hypoparathyroidism, 1α-hydroxylase mutation, drugs like ketoconazole and excess FGF23 leads to impaired 1α-hydroxylation. Mutation in 1α-hydroxylase leads to pseudovitamin D-deficiency rickets, an autosomal recessive disorder. It is characterized by growth retardation, rickets, and hypocalcemic seizures.

Vitamin D dependent rickets are of two types, Type-I (VDDR I) and Type-II (VDDR II). Type- I (VDDR I) is due deficiency of the renal 25-hydroxyvitamin D (25(OH)D)-1 alpha-hydroxylase. Prominent features of Type I are muscle weakness and rickets. This condition is reversible with treatment. Type II (VDDR II) II a spectrum that arises due to the intracellular defects in vitamin D receptor (VDR) which arise because of VDR gene mutations. (Paul. V. K, 2019). Insufficient levels of vitamin D increases the risk of developing type 1 diabetes mellitus, insulin resistance, and hypertension. It also leads to cognitive dysfunction resulting in depression. The exact mechanism are not clearly known (Jameson et al., 2018).

Excess circulating FGF23 is the cause for developing oncogenic osteomalacia, and X-linked hypophosphatemic rickets. Obesity causes VDD due to the malabsorption that occurs because of the presence of fat (Jameson et al., 2018).

CLINICAL MANIFESTATIONS OF VITAMIN D DEFICIENCY

Rickets:

The classical clinical presentation of VDD, especially in children before epiphyseal fusion, is rickets. Normal growth plate consists of three layers of growth chondrocytes namely: the reserve zone, the proliferating zone and the hypertrophic zone. Due to impaired vitamin D action, the hypertrophic zone will expand. In the rachitic growth plate, the proliferation and differentiation are normal. During endochondral bone formation the hypertrophic chondrocytes are replaced by osteoblasts. But in rickets, this is preceded by impaired apoptosis of the late hypertrophic chondrocytes resulting in expansion of growth plate (Jameson et al., 2018).

Familial hypophosphatemic rickets is a X-linked dominant condition that is characterized by features of rickets, such as bowing of legs and short stature, with hypophosphatemia in fasting state. In this condition, phosphate reabsorption is impaired in the proximal tubule leading to hypophosphatemia. This results in derangement in the renal 1-α hydroxylase leading to a reduction in calcitriol levels. Limb deformities like genu varum, genu

valgum, coxa vara and short stature occurs in this condition. Deformed skull due to premature fusion of cranial sutures and abnormalities in maxillofacial region is seen. Dental abscess, pulp deformities with intra-globular dentine are frequently encountered. In advanced stages active changes of ricket are rarely seen in spine and pelvis.

HYPOPHOSPHATEMIC RICKET

Figure 7: Lower limb deformities in rickets. From left genu varum, windswept deformity and genu valgum.

Source: Fischer, P., Thacher, T., Pettifor, J., 2008. Pediatric vitamin D and calcium nutrition in developing countries. Reviews in Endocrine and Metabolic Disorders. https://doi.org/10.1007/s11154-008-9085-1

Vitamin D dependent rickets (VDDR) are seen in infants between 3 to 6 months of age and inherited autosomal recessively. In VDDR type I, the clinical features include growth failure, hypotonia, delay in motor functions like standing and walking, and poor control of head movements. Impaired development of tooth enamel and delayed dentition are seen. Thickenings of ankles and wrist, frontal bossing, anterior fontanelle widely opened, positive trousseau and Chvostek sign, rickety rosary and bony deformity are the physical findings seen in this condition. Alopecia, ectodermal defects like milia, oligodontia and epidermal cyst are also seen. Other features seen rickets are metaphyseal hyperplasia resulting in double malleoli, Harrison's groove, recurrent greenstick fractures.

THICKENED WRIST JOINT

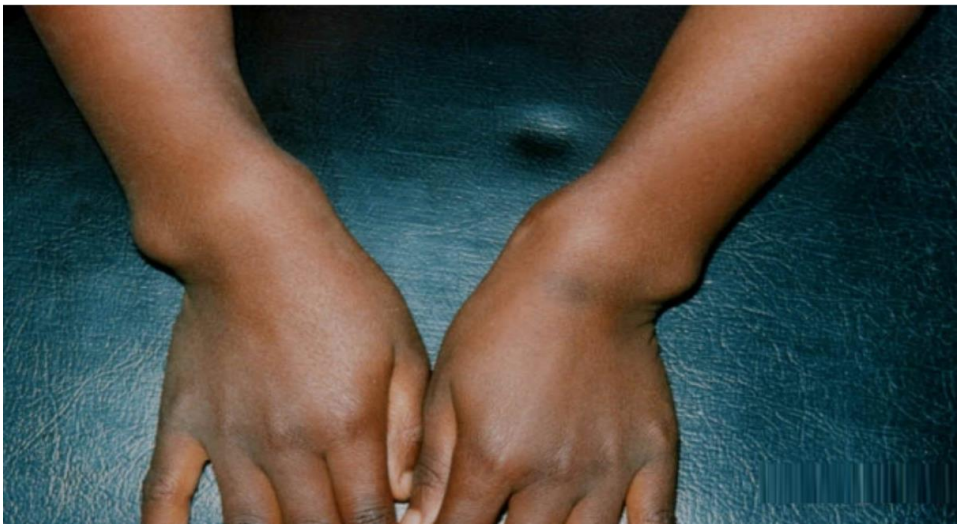
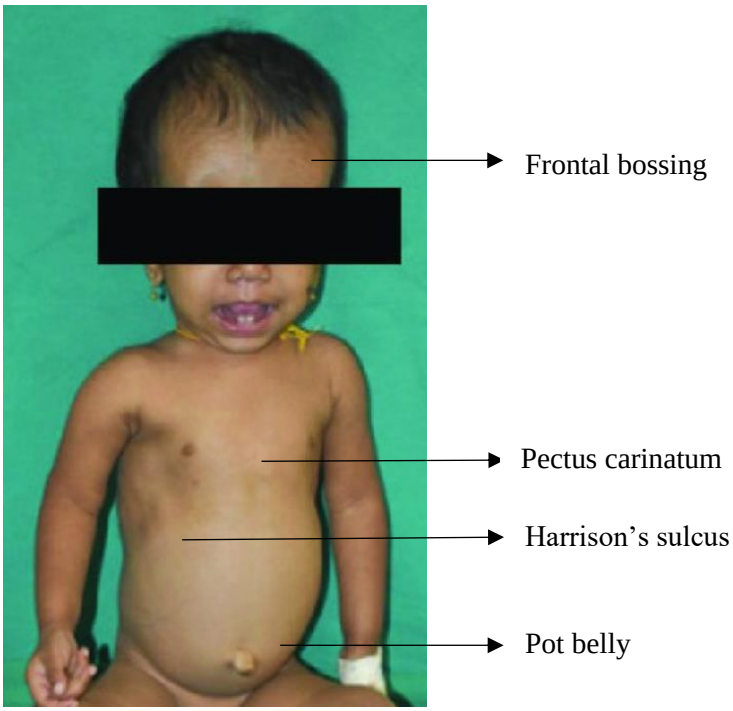


Figure 8: Showing thickened wrist joint

Source: 5 Signs and Symptoms Indicating Lack of Vitamin D | Search Home Remedy [WWW Document], 2013. URL https://www.searchhomeremedy.com/5-symptoms-indicating-lack-of-vitamin-d/ (accessed 1.25.22).

CLINICAL FEATURES OF RICKETS

(A)



(B)

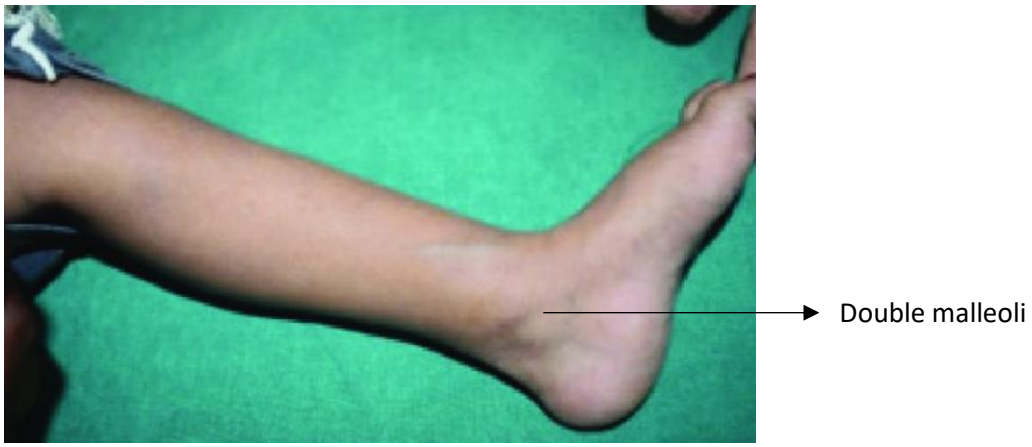


Figure 9: Features of rickets (A) frontal bossing, pectus carinatum, Harrison sulcus, and pot belly appearance. **(**B) Double malleoli

Source: Jeyaraman, M., 2019. Osteopetrorickets - A Paradoxical Association between Osteopetrosis and 6342`Rickets A Rare Case Report. Juniper Online Journal of Case Studies 9. https://doi.org/10.19080/JOJCS.2018.09.555772

Osteomalacia:

VDD is often accompanied by hypocalcemia and hypophosphatemia. This leads to impaired mineralization of the bone matrix, a condition termed as osteomalacia. Phosphate wasting in kidney and usage of drugs like phosphate binding antacids and etidronate results in long standing hypophosphatemia, a feature of osteomalacia. Hence, in VDD hypo-mineralization of the bone matrix is seen. This is inferior to the normal bone resulting in increased risk of fractures (Jameson et al., 2018).

ROLE OF VITAMIN D IN PREGNANCY

To meet the increased maternal and fetal requirement, maternal intestinal calcium absorption is increased during early pregnancy and reaches a maximum during the 3rd trimester (Ritchie et al.,1998). This is mediated mainly by increased formation of calcitriol (Mahadevan et al., 2012). Vitamin D promotes bone growth and maintains skeletal homeostasis in the growing fetus. About 15µg/day (600 IU/day) was established as an adequate intake of vitamin D during pregnancy and lactation by The Food and Nutrition Board of the Institute of Medicine in 2011 (Cunningham et al., 2018).

The metabolism of vitamin D differs between pregnant and non-pregnant states. Although the conversion of vitamin D to 25(OH)D remains the same, the conversion of 25(OH)D to calcitriol undergoes significant changes during pregnancy (Bikle et al.,

1984). There is a 2-3 fold increase in serum calcitriol levels in the first week of pregnancy and this continues to rise through the course of pregnancy (Lund and Selnes, 1979). Renal and placental production of the vitamin D is required for this (Hollis et al., 2011). Prolactin and placental lactogen increases the expression of 1α-hydroxylase in the placenta and fetal kidney. Maternal plasma levels of vitamin D metabolites are greater than fetal plasma levels (Olmos-Ortiz et al., 2015).

VITAMIN D DEFICINCEY DURING PREGANCY AND ITS COMPLICATIONS

Pregnant women in the Middle East and in Asian countries have been reported to have a high prevalence of VDD (De-Regil et al., 2016) (Yuan et al., 2017b). Its prevalence has been reported to be higher in pregnant women from metropolitan areas than in those from medium and small-sized cities in China (Hu et al., 2017). It is also higher among those from low socioeconomic status (Bener et al., 2013). VDD is highly prevalent even in pregnant women from tropical countries where there is adequate exposure to sunlight (Ariyawatkul and Lersbuasin, 2018).

VDD in pregnancy has been reported to be associated with detrimental effects on both mother and fetus. Abnormal angiogenesis that occurs in the presence of VDD is thought to be involved in the development of pre-eclampsia, but the exact mechanisms involved are unclear (Nema et al., 2019).

Children born to HIV- infected mothers, with low levels of vitamin D in blood during pregnancy, were found to have increased risk of stunting and being underweight (Finkelstein et al., 2012a). Inadequate vitamin D during pregnancy has also been reported to be associated with asthma in children (Allan et al., 2015). However, other studies have shown no significant association between vitamin D status and maternal, neonatal and adverse pediatric outcomes such as anemia, iron deficiency, diarrhea and respiratory infections (Lee et al; 2017)(Finkelstein et al., 2012a). Low vitamin D levels were found to be associated with postpartum depression (Pillai et al., 2021) (Abedi et al., 2018).

Vitamin D supplementation in pregnant women has been reported to be beneficial, with better outcomes of pregnancy (Hovdenak and Haran, 2012; Kulia, 2018). Such supplementation was reported to decrease the risk of preeclampsia, and increased length and head circumference at birth (Nema et al., 2020). It has also been reported to prevent gestational hypertension and recurrent preeclampsia in women with previous history of preeclampsia (Behjat Sasan et al., 2017).

Maintaining sufficient maternal blood levels of 25(OH)D by supplementation has been shown to result in down-regulation of anti-angiogenic growth factors, such as soluble FMS-like tyrosine kinase-1(sFH-1) and angiogenic factors, such as vascular endothelial growth factors (VEGF), all of which are thought to contribute to vascular complications in pregnancy, such as pre-eclampsia (Schulz et al., 2017). Vitamin D levels lower than

10.6 ng/ml was reported in Thai women to be associated with a high risk of developing pre-eclampsia. The study concluded that vitamin D deficiency in women require treatment prior to pregnancy (Ariyawatkul and Lersbuasin, 2018). Supplementation of vitamin D during pregnancy has also been reported to reduce the risk for childhood asthma (Allan et al., 2015).

A study conducted among 177 pregnant women in North-East India showed that an alarming 84.18% of the subjects had vitamin D deficiency and 12.44% were found to have vitamin D insufficiency. Only 3.38 % of the subjects were found have sufficient vitamin D levels. In this study, an association was found between vitamin D deficient subjects and adverse pregnancy outcomes like cesarean section, low birth weight, preeclampsia and newborns with lower vitamin D levels (Sharma et al., 2019). Several studies done in India have shown similar findings (Agarwal and Arya, 2011; Arora et al., 2018; Prabha and Kumar, 2020) .

Since all pregnant mothers receive vitamin D supplementation along with calcium from 2nd trimester till delivery, it would be reasonable to expect VDD, if present, to get corrected during the course of pregnancy. But even in studies where vitamin D status was assessed in the 3rd trimester and postnatally, a large proportion of subjects had VDD indicating that the vitamin D supplementation was not adequate or ineffective. The reason for this is not clear. In addition, data regarding pre-conception vitamin D levels

with follow-up data in the 1st, 2nd and 3rd trimesters is not available from the studies done so far in India. Such data is important to understand the cause for VDD in pregnancy despite vitamin D supplementation.

OVERVIEW OF IRON METABOLISM

Iron is an important trace element in the body. It is required for synthesis of haemoglobin, which transports oxygen in blood. It also functions as cofactor for several enzymes in the body (Fu1 et al., 2016). Iron exists in ferric (Fe^{3+}) and ferrous (Fe^{2+}) states which enables it to participate in electron transfer reactions (Gulec et al., 2014).

The average amount of iron present in the body is about 4 to 5 grams. Of this, 65 % is in hemoglobin, 4% in myoglobin, and 1% in cytochrome P450 enzymes and other heme compounds that help in intracellular oxidation. Blood contains 200-400 mg of iron bound to transferrin, the iron-transport plasma protein. About 15 -30% of total body iron is stored in the form of ferritin primarily in the liver and spleen (Hall and Hall, 2020).

OVERVIEW OF IRON METABOLISM

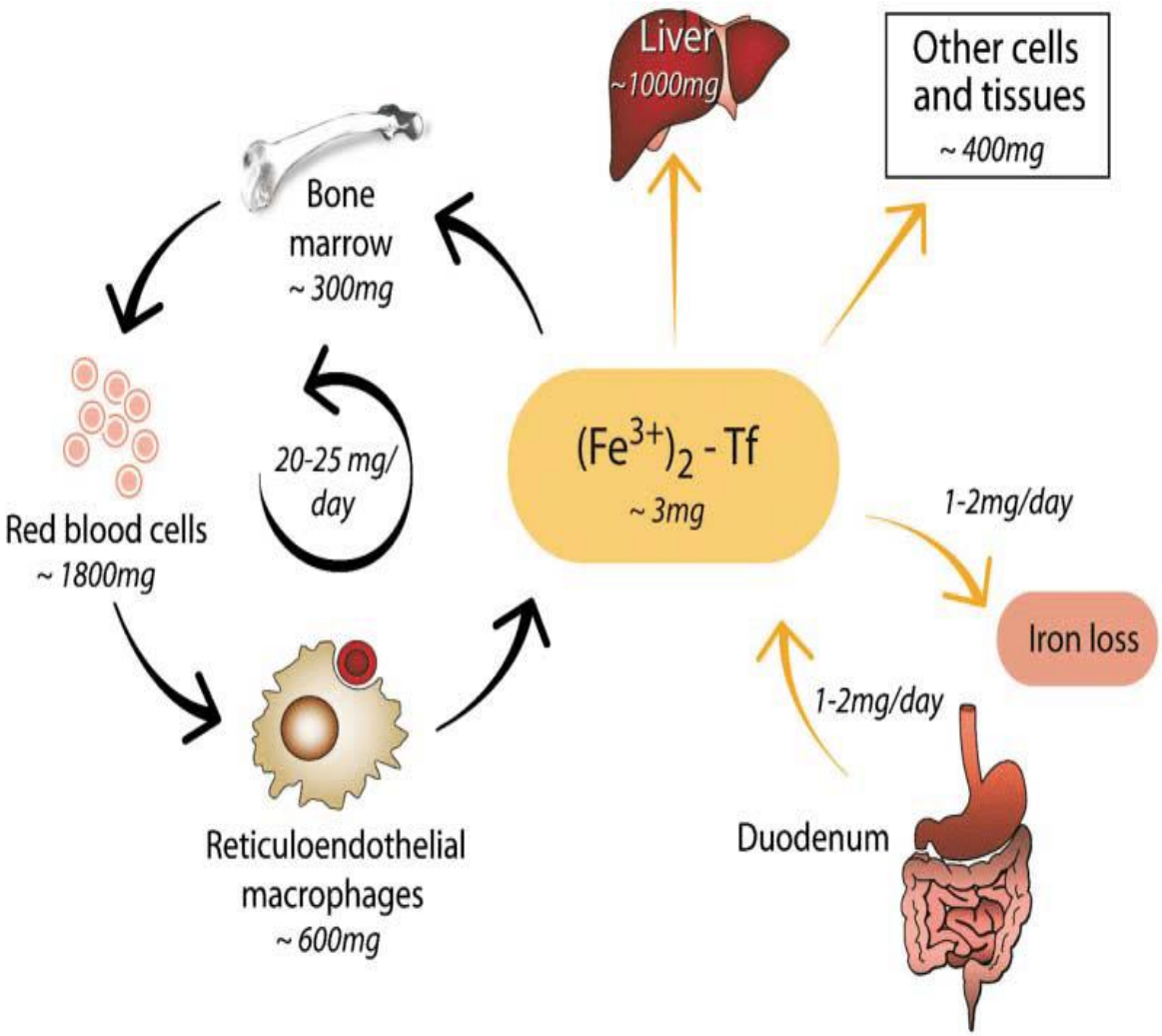


Figure 10: Overview of iron metabolism.

Source: Hentze, Matthias & Muckenthaler, Martina & Andrews, Nancy. (2004). Balancing Acts. Cell. 117. 285-297. 10.1016/S0092-8674(04)00343-5.

Absorption of dietary iron

Since there are no physiologically regulated mechanisms of iron excretion, iron homeostasis is primarily regulated by controlling absorption of dietary iron. Dietary iron exists in two distinct forms: heme iron and non-heme iron. Heme iron is derived from animal products and non-heme iron from plants, animal products, and supplements (Cao et al., 2014).

Iron absorption predominantly occurs in the duodenum and upper jejunum (Edison et al., 2008). Dietary non-heme iron, present in ferric form, is reduced to its ferrous form by a ferrireductase, duodenal cytochrome b reductase (DCYTB), which is present on the brush-border membrane.

Iron can be stored in enterocytes as ferritin or transported into the blood, across the basolateral membrane of enterocytes, by ferroportin (Rodwell et al., 2018). Ferroportin is found on the basolateral membranes of enterocytes in the duodenum, and in hepatocytes, macrophages of the reticuloendothelial system and adipocytes (Donovan et al., 2005).Ferrous iron is then oxidized to its ferric form by a ferroxidase, haephestin.

IRON TRANSPORT ACROSS THE INTESTINAL EPITHELIUM

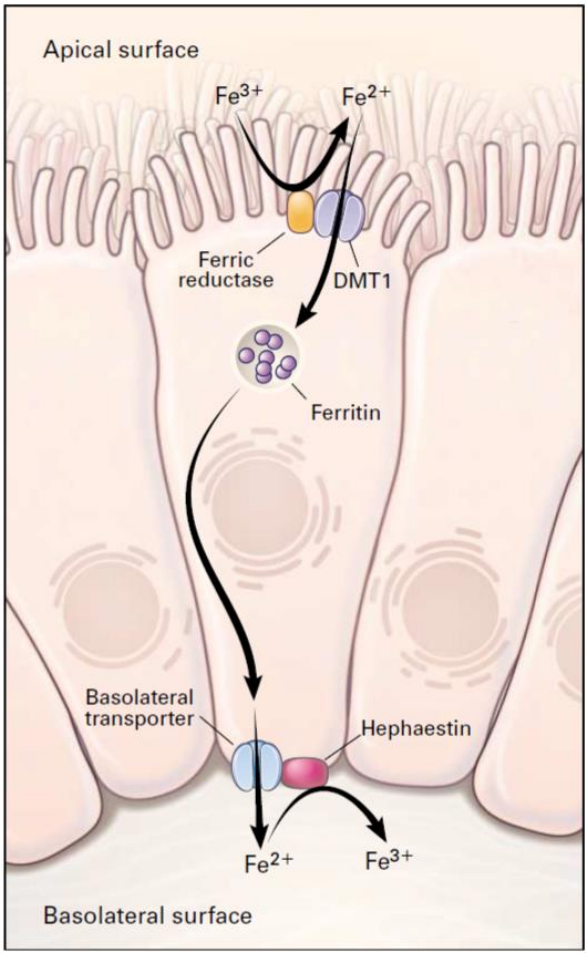


Figure 11: Transport of iron across the intestinal epithelium.
In the apical surface DMT1 tansporter in association with ferric reductase helps in the transport of iron into the intestinal epithelial cells. In the basolateral surface hephaestin is believed to be a form of ferroreductase helping in the transfer of iron into the plasma.

Source: Andrews, N.C., 1999. Disorders of Iron Metabolism. New England Journal of Medicine 341, 1986–1995.
https://doi.org/10.1056/NEJM199912233412607

Transport of iron in blood and uptake by cells

Transferrin helps in the transport of iron in plasma. It is a beta globulin that binds with high affinity to ferric iron (Ferrier, 2014). The apo-transferrin/ferric ion complex is denoted as holo-transferrin (Burtis et al., 2015). Holo-transferrin binds to the transferrin receptors present on the cells forming a complex. The transferrin-receptor complex formed is endocytosed and results in the formation of an endosome. After acidification, the endosome releases iron from the transferrin. This iron is released in its reduced form as ferrous ion (Fe^{2+}).

The divalent metal iron transporter, DMT1, which is expressed on the endosomal membrane, transports ferrous iron into the cytoplasm. The apo-transferrin is cycled back to the plasma membrane where it is released into blood. This entire process is termed as transferrin cycle (Burtis et al., 2015)

TRANSFERRIN CYCLE

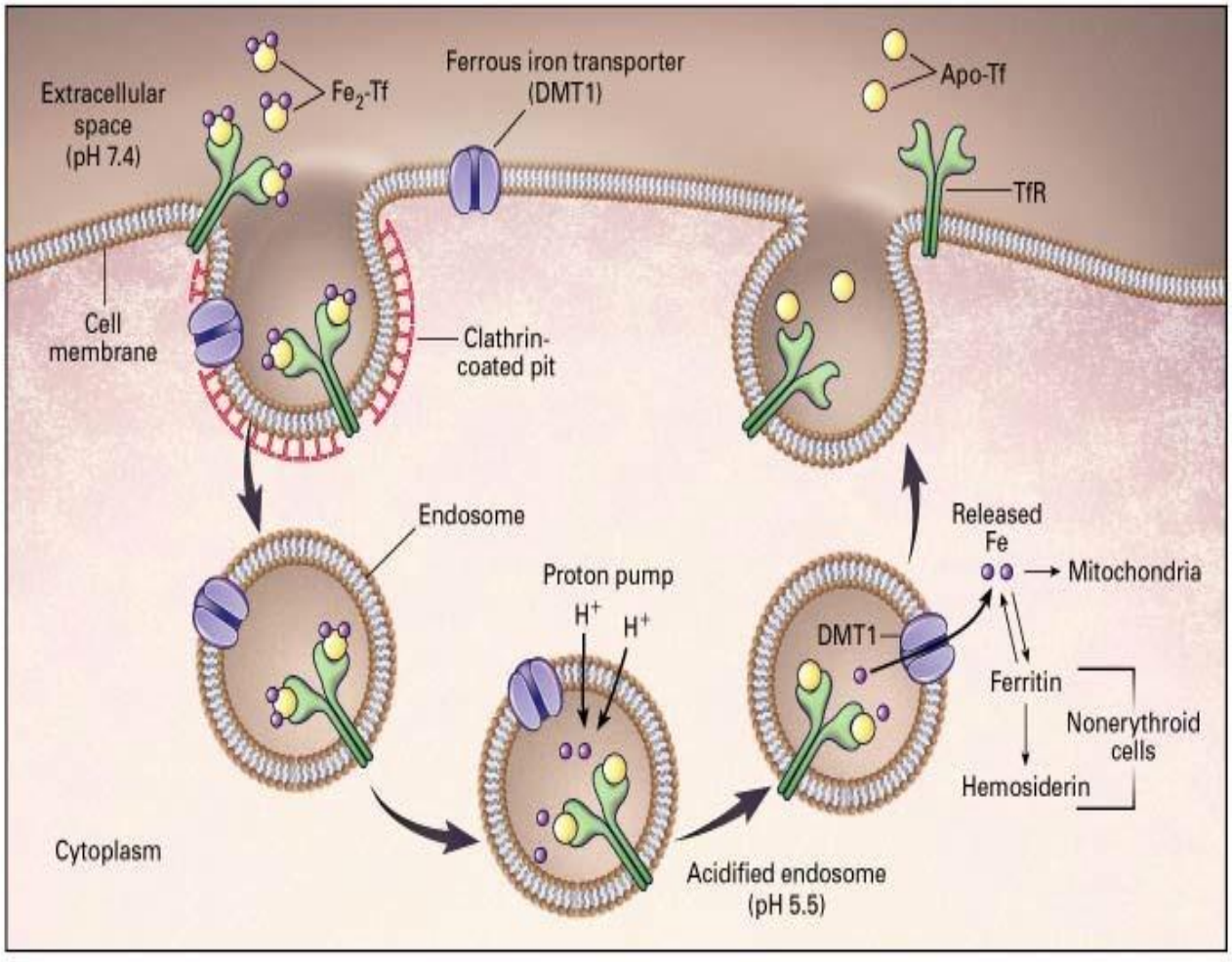


Figure 12: Overview of transferrin cycle

Source: Andrews, N.C., 1999. Disorders of Iron Metabolism. New England Journal of Medicine 341, 1986–1995.
https://doi.org/10.1056/NEJM199912233412607

Storage of iron

Inside cells, iron is stored as ferritin. Ferritin is a globular multi-protein complex made of 24 subunits of 18.5kDa each (Rodwell et al., 2018). There are 2 types of ferritin subunits – L and H. The H subunit has ferroxidase activity and is required for iron loading into the ferritin. The L subunit has a structural role. Together they form globular structure of molecular mass of 440 kDa that can store 3000-4500 atoms in its core.

Iron can be released from ferritin by lysosomal degradation of ferritin. This process is called ferritinophagy and is triggered by conditions where the intracellular iron levels are low. In addition, a small fraction of ferritin is present in blood. Serum ferritin levels are proportionate to intracellular iron stores and is therefore used as a measure of stored iron in the body. Therefore, low serum ferritin indicates iron deficiency and high levels indicate iron overload. However, serum ferritin is also an acute phase reactants and high levels can also be seen in inflammatory conditions and in response to infections.

STRUCTURE OF FERRITIN

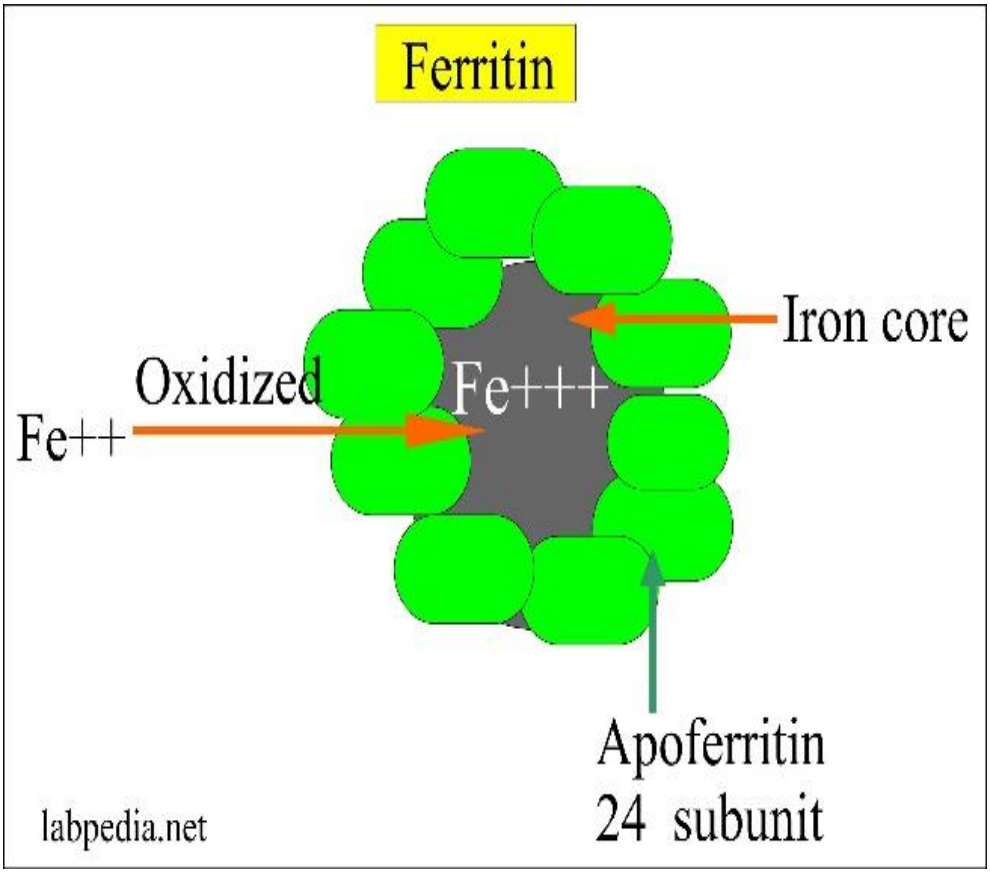


Figure 13: Strucure of ferritin.

Source: Ferritin (serum Ferritin Level) - Labpedia.net, 2020.
URL https://labpedia.net/ferritin-serum-ferritin-level/ (accessed 1.24.22).

REGULATION OF IRON HOMEOSTASIS

Hepcidin is a peptide hormone produced predominantly by hepatocytes. It plays an important role in regulating systemic iron homeostasis (Ganz and Nemeth, 2012; Hernik et al., 2019).Hepcidin has been shown to bind to ferroportin, causing it to be internalized and degraded (Nemeth et al., 2004b). It therefore reduces the absorption of iron from the diet, recycling of iron from macrophages and release of iron from liver stores (Ganz and Nemeth, 2012). Thus, hepcidin decreases the release of cellular iron and causes hypoferremia (Nemeth et al., 2004b).

ACTION OF HEPCIDIN ON FERROPORTIN

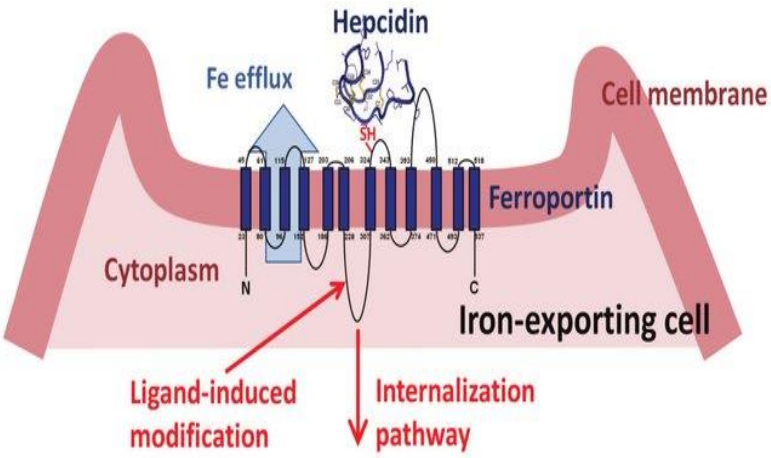


Figure 14: Action of hepcidin on ferroportin. Ferroportin is covalently modified, internalized and degraded once hepcidin binds to it.

Source: Ganz, T., Nemeth, E., 2011. The Hepcidin-Ferroportin System as a Therapeutic Target inAnemias and iron Overload Disorders. Hematology / the Education Program of the American Society of Hematology. American Society of Hematology. Education Program 2011, 538–42. https://doi.org/10.1182/asheducation-2011.1.538

The *HAMP* gene codes for hepcidin. Regulation of hepcidin occurs primarily at the level of transcription. Numerous factors regulate *HAMP* expressions. Increased iron levels in the body, infections and inflammation induce hepcidin synthesis in the liver (Nemeth et al., 2004a, 2003). Physiological processes (such as erythropoiesis) and pathological conditions (such as hypoxia and iron-deficiency anemia) act as negative regulators of hepcidin synthesis (Nicolas et al., 2002; Pasricha et al., 2016; Rishi et al., 2015).

Hepcidin is regulated by serum iron levels and tissue iron stores via the BMP/SMAD signaling pathway (Katsarou and Pantopoulos, 2018). Serum iron levels are sensed by a complex mechanism that involves the HFE protein, transferrin receptors 1 and 2 (TfR1 and TfR2), BMP receptors and the GPI-anchored cell membrane protein, hemojuvelin.

Binding of transferrin to TfR1 on the hepatocyte membrane, induces a chain of intracellular signaling events that eventually results in the binding of HFE to TfR2, which signals via the BMP receptors and HJV to induced hepcidin expression. During condition characterized by low serum iron levels, there is an increase in matriptase – 2 (MT2). Increased MT2 causes cleavage of HJV in the liver. This results in blunting of BMP signaling and decreased hepcidin expression.

Liver iron levels are sensed by the liver sinusoidal endothelial cells which secrete BMP2 and BMP6. BMP2/6 induces hepcidin expression by binding to BMP receptors on hepatocytes and signaling through the BMP/SMAD pathway.

Hepcidin expression is also induced by inflammation. Interleukin-6 is a pro-inflammatory cytokine that plays a key role in increasing hepcidin levels in response to inflammatory stimuli via STAT3/JAK2 pathway. Activin B, another cytokine that increases in inflammatory conditions, activates the transcription of hepcidin via BMP signaling pathway (Zhao et al., 2013).

MAJOR PATHWAYS FOR HEPCIDIN REGULATION

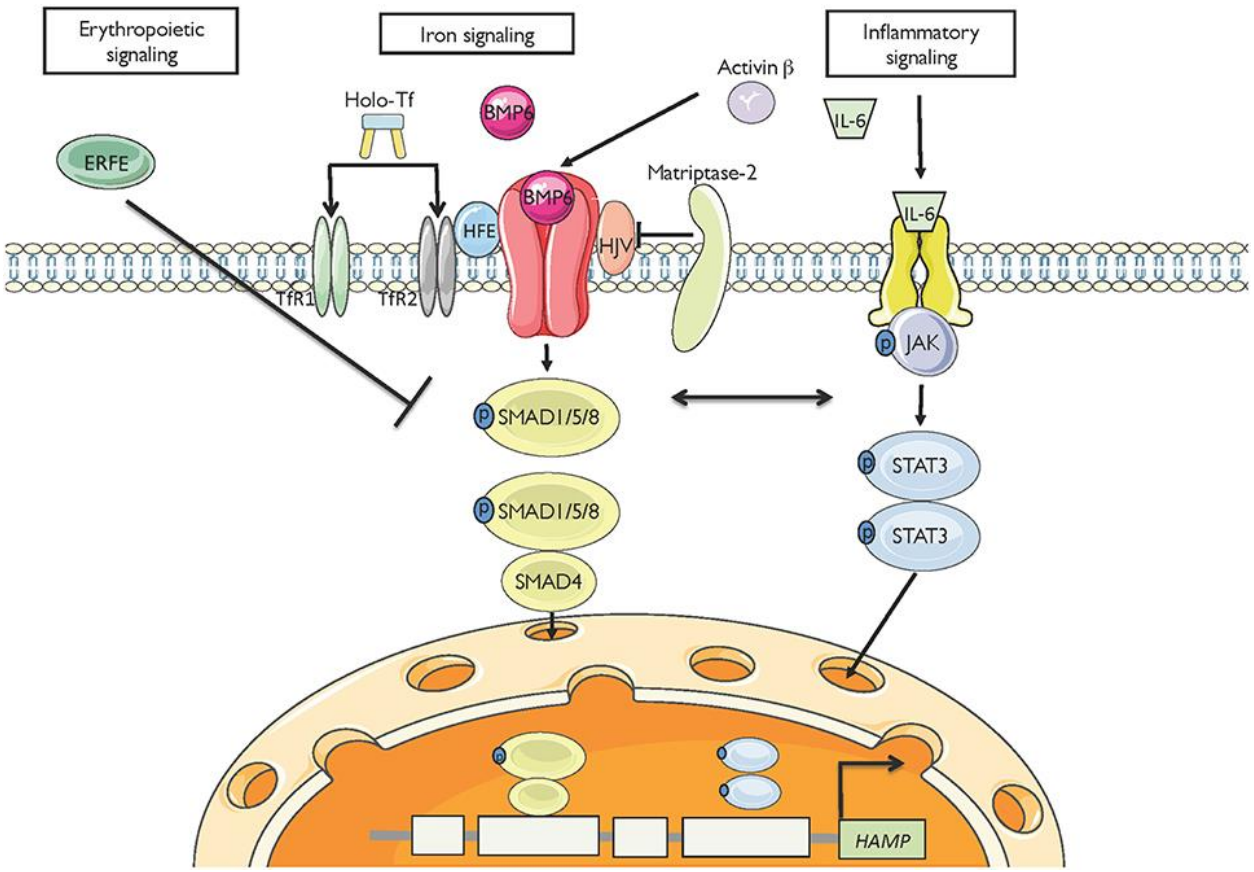


Figure 13: Pathways involved in hepcidin regulation

Hepcidin mRNA transcription via the BMP/SMAD signaling cascade is induced by hepatic iron stores and high serum iron levels. Hepcidin mRNA transcription via JAK/STAT pathway is induced by IL-6. Activin B induces mRNA transcription of hepcidin via the non-canonical BMP/SMAD signaling pathway. High erythropoietic drive (reflected in ERFE) suppresses hepcidin transcription, likely via interferes with BMP/SMAD signaling and causes hepcidin hepcidin suppression.

Source: Sebastiani, G., Wilkinson, N., Pantopoulos, K., 2016. Pharmacological Targeting of the Hepcidin/Ferroportin Axis. Frontiers in Pharmacology 7. https://doi.org/10.3389/fphar.2016.00160

ROLE OF VITAMIN D IN REGULATION OF IRON METABOLISM

Vitamin D has been reported to suppress transcription of hepcidin, as evidenced by a decrease in hepcidin mRNA levels when vitamin D binds to its receptors in monocytes (Bacchetta et al., 2014). Deficiency of vitamin D was found to result in increased synthesis of hepcidin and decreased expression of ferroportin causing an increase in intracellular iron and a decrease in systemic iron (Bacchetta et al., 2014). Thus, it postulated that anemia associated with VDD is mediated by high hepcidin which causes a functional deficiency of iron.

A meta-analysis of studies conducted in the US, France and Asian countries showed that the incidence of anemia was higher in individuals with vitamin D deficiency (Liu et al., 2015). In a cohort study done among healthy African-American adults in U.S, a decrease in serum 25(OH)D < 50 nmol/l was found to be significantly associated with anemia (Smith et al., 2015). Similar finding were reported in elderly as well as in children (Atkinson et al., 2014; Perlstein et al., 2011) . In another study conducted among Korean women, vitamin D deficient women showed an increased risk for developing iron deficiency anemia and anemia of inflammation (Shin and Shim, 2013).

VITAMIND HEPCIDIN-FEROPORTIN IRON REGULATORY AXIS

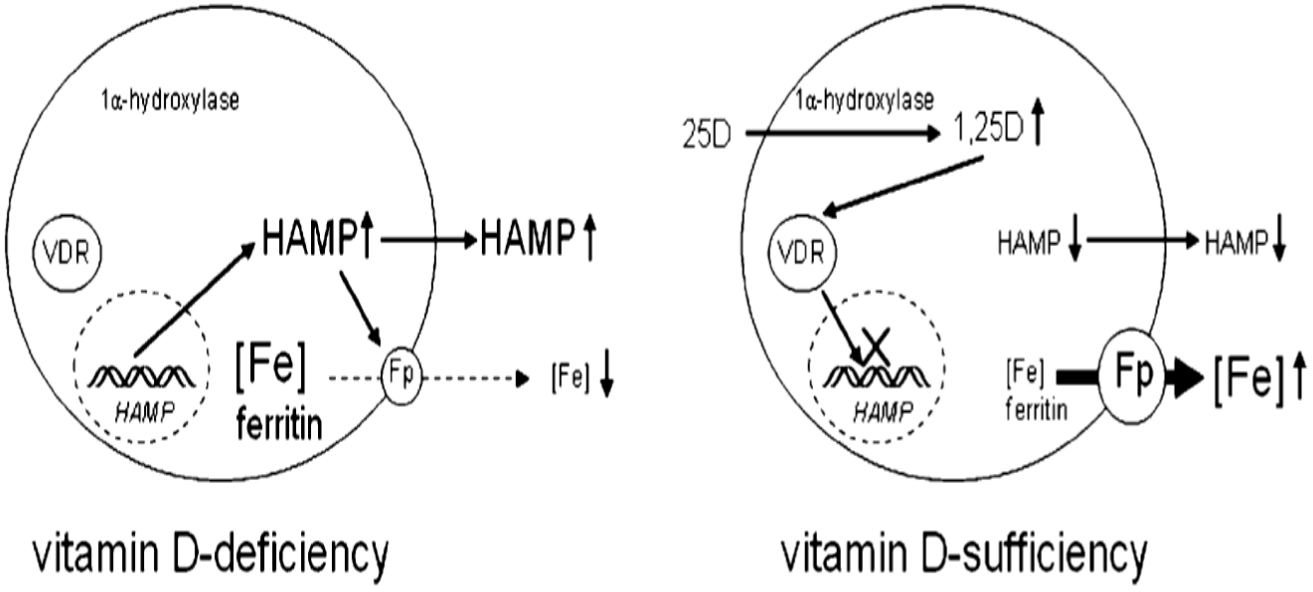


Figure 14: A schematic representation of vitamin D hepcidin-ferroportin iron-regulatory axis. It shows one of the proposed mechanisms to occur inside hepatocytes and monocytes for vitamin D regulation of hepcidin (HAMP)- ferroportin(Fp) interaction. During vitamin D deficient states, there is elevated synthesis of hepcidin causing increase in intracellular and systemic hepcidin levels and a decrease in membrane expression of Ferroportin (Fp) in the hepatocytes and monocytes. This results in suppression iron export leading to iron accumulation and an increase in ferritin intracellularly and decrease in systemic iron. In vitamin D sufficient states, there is decrease in transcription of HAMP. This may lead to a decrease in intracellular and systemic concentration of hepcidin. A subsequent increase is found in the ferroportin (fp) expression in the membranes. This results in enhanced iron export leading to decrease in iron and ferritin intracellularly and an increase in systemic iron levels.

Source: Suppression of Iron-Regulatory Hepcidin by Vitamin D. Bacchetta J et al 2014. https://doi.org/10.1681/ASN.2013040355

ANEMIA IN PREGNANCY

Anemia in pregnancy is defined as hemoglobin level of less than 11 g/dL; a value of less than 10 g/dL is commonly used in developing countries (Cappellini and Motta, 2015). The World Health Organisation (WHO) has estimated that about one-third of women of reproductive age and about 40% of pregnant women worldwide are anemic. It is commonly caused by iron deficiency, which, in turn, is due to blood loss due to hookworm infestation, multiple pregnancies, , episiotomy or as a complication of malaria (Feleke and Feleke, 2020; WHO, Geneva 2014).

Anemia in pregnancy is associated with adverse outcomes in both the mother and child, such as increased maternal and neonatal mortality, preterm delivery, low birth weight and iron deficiency in the new-born (Brabin et al., 2001; Rasmussen, 2001; Cogswell et al., 2003). Hence, screening and treating for anemia is important (Feleke and Feleke, 2020). Prenatal iron supplementation has shown to reduce the risk of low birth weight (Haider et al., 2013). Studies have shown that anemia during pregnancy had a direct impact on neonatal iron stores (Singla et al., 1996) and may also cause cognitive and behavioral problems in children born to severely anemic mothers(McCann and Ames, 2007).

ASSOCIATION OF VITAMIN D DEFICIENCY AND ANEMIA IN PREGNANCY

Deficiency of micronutrients, such as vitamin D and calcium, in pregnancy have been reported to result in health problems, such as anemia and vitamin deficiencies (Dong and Yin, 2018). Observational studies have shown associations between vitamin D deficiency (VDD) and anemia. VDD was found to be associated with increased risk of anemia in women (Shin and Shim, 2013; Suh et al., 2016), children (Atkinson et al., 2014) and infants (Jin et al., 2013). Low serum 25(OH)D levels have been reported to be significantly associated with anemia, particularly anemia of inflammation (Smith et al., 2015). Similar findings have been reported in the elderly (Perlstein et al., 2011) and in pregnancy (Yuan et al, 2017; Thomas et al., 2015).

Vitamin D insufficiency was also reported to be one of the predictors of anemia in pregnancy in HIV-infected pregnant women (Finkelstein et al., 2012b). With increasing gestation, the proportion of women with anemia tended to increase in those who were deficient in cholecalciferol (Judistiani et al., 2018a).VDD in pregnancy and childhood have also been strongly associated with development of iron deficiency and iron-deficiency anemia in children (Cihan, 2018). On the other hand, a study on Indonesian pregnant women, showed no association between vitamin D status and anemia (Judistiani et al., 2018b). Hence, unresolved questions exist.

LACUNA ADDRESSED

Studies have indicated an association between the 2 conditions. However, there is little data on this from India. Therefore, this study was done to see if there is a correlation between iron-related parameters and vitamin D levels in blood in pregnant women receiving antenatal care in CMC, Vellore.

THE STUDY

BACKGROUND OF THE STUDY

Iron-deficiency anemia and vitamin D deficiency (VDD) are common in pregnancy. Accumulating evidence in recent years suggests a link between the 2 conditions. However, it is not clear what underlies this association.

HYPOTHESIS

Vitamin D levels and iron-related parameters in pregnancy correlate with one another, and are linked to development of anemia.

AIM

The aim of this study was to determine if there is a correlation between iron-related parameters and vitamin D levels in blood in pregnant women, receiving antenatal care in CMC, Vellore.

OBJECTIVES

To estimate vitamin D levels and iron-related parameters in blood from recruited pregnant women.

To determine if vitamin D levels and iron-related parameters in these women correlate with one another

MATERIALS

Equipment

1. Table-top refrigerated centrifuge (MPW R 350, MPW Poland)

2. Minus 70ºC freezer (Cryo Scientific, Chennai, India)

Miscellaneous consumables used

1. Vacutainer tubes for blood collection (BD Biosciences, Plymouth, UK).

2. Micro-centrifuge tubes (1.5mL), and micro tips (Tarsons Products Private Limited, Kolkata, India).

METHODS

The study was approved by the Institutional Review Board (IRB) at Christian Medical College (CMC), Vellore, India (IRB Min. No. 13722 dated 06.01.2021) (letter of approval in Appendix I).

Subjects

Subjects of the study were primigravidae who attended the antenatal clinic of Unit 4, Department of Obstetrics and Gynecology at Christian Medical College (CMC), Vellore, India.

The following inclusion and exclusion criteria were employed to recruit patients:

Inclusion criteria

Primigravidae in their first trimester, with a singleton uncomplicated pregnancy

Those willing to participate in the study

Exclusion criteria

Those with any complication of pregnancy

Not willing to participate in the study

Informed consent

Eligible pregnant women were identified, based on the inclusion and exclusion criteria listed above. They were invited to participate in the study. Details of this were explained to them. They were provided with an information sheet about the study. This was made available in Tamil, English and Hindi. Those who agreed to participate in the study were requested to sign an informed consent form. The information sheet and informed consent form that were used are included in Appendix II.

Recruitment

Primigravidae in their first trimester, with a singleton uncomplicated pregnancy, who gave informed consent were recruited.

Patients' data

A proforma was prepared to collect relevant data on the patients recruited. This is shown in Appendix III. Relevant medical and obstetric history were elicited from each patient.

Sample size

Sample size calculation was done based on the data reported in a publication by Takaoka et al (2020). In their study, the correlation coefficient reported between vitamin D and hemoglobin levels in pregnant women was 0.51. Using this value, with power set at 90% and allowing for an alpha error of 5%, the sample size obtained (using nMaster 2.0) was 30.

Correlation coefficient between Vitamin D and haemoglobin	0.51	0.51
Power (1- beta) %	90	80
Alpha error (%)	5	5
1 or 2 sided	2	2
Required sample size	**30**	**22**

Formula:

$$n = \frac{\left(z_{1-\beta} + z_{1-\frac{\alpha}{2}} \right)^2}{\left(\frac{r^2}{1 - r^2} \right)}$$

Where,

r : Correlation coefficient.

$Z_{1-\alpha/2}$: Desired confidence level

1-β : Power

Reference:

Takaoka, N., Nishida, K., Sairenchi, T., Umesawa, M., Noguchi, R., Someya, K., Kobashi, G., 2020. Changes in vitamin D status considering hemodilution factors in Japanese pregnant women according to trimester: A longitudinal survey. PLoS One 15, e0239954. https://doi.org/10.1371/journal.pone.0239954

Sample collection

After obtaining written informed consent and after confirming singleton pregnancy with a dating scan, a blood sample was collected from each subject. Blood was used for estimation of hemoglobin and mean corpuscular volume (MCV) in the Department of Transfusion Medicine in CMC, Vellore. Serum obtained from the blood samples (the blood samples

were allowed to clot and then centrifuged at 2500 rpm for 10 minutes to obtain serum) was stored at -70ºC till estimation of serum vitamin D, ferritin, iron, CRP, creatinine and TIBC was carried out.

Estimation of serum iron

Estimation of serum iron was carried out in the Department of Clinical Biochemistry, CMC, Vellore.

Analyzer used: Roche Cobas c 702 modular analyzer

Principle of the method: Guanidine/ ferrozine spectrophotometric method

Guanidine released transferrin-bound ferric ions in the sample. It was reduced to ferrous form by hydroxylamine. When ferrous ions reacted with ferrozine, a purple-colored complex was formed. Using spectrophotometry, the absorbance of the sample was measured at 560 nm. The intensity of the color obtained was directly proportional to the concentration of iron in the sample.

Reference interval

Male: 60- 160 μg/dL

Female: 40-145 μg/dL

Estimation of total iron-binding capacity (TIBC)

Estimation of unsaturated iron-binding capacity (UIBC) was carried out in the Department of Clinical Biochemistry, CMC, Vellore

Analyzer use: Roche Cobas 8000 c 702 modular analyzer

Principle of the method:

At an alkaline pH, a known amount of ferrous iron was added to the sample. The available iron binding sites of transferrin were bound by ferrous ions added. Unbound ferrous ions were measured using the ferrozine method (described above under the estimation of serum iron). The difference between the amounts of ferrous ions added and the unbound ions measured was considered as unsaturated iron-binding capacity (UIBC) of the sample.

UIBC = [Amount of ferrous ion added] - [Amount of unbound ferrous ion]

TIBC was calculated as the sum of serum iron concentration and the UIBC

TIBC = Serum iron + UIBC

Reference interval

Male: 300-400 μg/dL

Female: 250-350 μg/dL

Calculation of transferrin saturation (TSAT)

Transferrin saturation (TSAT) was calculated as the ratio of serum iron level and the total iron-binding capacity of each sample, multiplied by 100.

TSAT = (Serum iron / TIBC) x 100

Estimation of serum ferritin

Estimation of serum ferritin was carried out in the Department of Clinical Biochemistry, CMC, Vellore.

Analyzer use: The SIEMENS - Atellica IM

Principle of the method: Two-site sandwich immunoassay using direct chemiluminescence technology

The method uses 2 anti-ferritin antibodies. The first antibody is a goat polyclonal anti-ferritin antibody labelled with an acridinium ester. It is present in the Lite Reagent. The second antibody is a mouse monoclonal anti-ferritin antibody, which is covalently coupled to paramagnetic particles. It is present in the solid phase. A direct relationship exists between the amount of ferritin present in the patient sample and the amount of relative light units (RLUs) detected by the system.

Reference interval

Men and women > 50 years: 20-320 ng/mL

Women < 50 years: 10-290 ng/mL

Estimation of serum vitamin D

Estimation of serum vitamin D was carried out in the Department of Clinical Biochemistry, CMC, Vellore.

Analyzer use: ROCHE COBAS – e602

Principle of the method: Electrochemiluminescence immunosorbent assay- Competitive principle

1st incubation: 15 µL of the sample was incubated with pre-treatment reagent, namely Sodium hydroxide and dithiothreitol. Vitamin D (25-OH) in the sample was released from the vitamin D-binding protein.

2nd incubation: The pre-treated sample was incubated with the ruthenium-labelled vitamin D-binding protein. A complex was formed between the vitamin D (25-OH) in the sample and the binding protein.

3rd incubation: Streptavidin-coated microparticles and biotin-labelled vitamin D (25-OH) was added. Unbound ruthenium-labelled vitamin D-binding proteins become bind to biotin-labelled vitamin D (25-OH). A complex consisting of the ruthenylated vitamin D-binding protein and the biotinylated vitamin D (25-OH) is formed, and binds to the solid phase, via interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell, where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed. Application of a voltage to the electrode then induces a chemiluminescent emission, which is measured by a photomultiplier.

Reference interval:

25(OH)D value more than 30ng/mL is considered as desirable.

Estimation of CRP

Estimation of CRP was carried out in the Department of Clinical Biochemistry, CMC, Vellore, using the assay for CRP.

Analyzer use: IMMULITE 2000 systems analyzer

Principle of the method: Solid-phase chemiluminescent immunometric assay

The solid-phase bead was coated with an anti-ligand. The liquid phase contained an anti-CRP murine monoclonal antibody, attached to the ligand, and alkaline phosphatase conjugated to rabbit polyclonal anti-CRP antibody in buffer. Pre-diluted sample and the reagent were incubated together with the anti-ligand-coated bead, for 30 minutes. The CRP in the sample formed an antibody sandwich complex. The unbound antibodies were removed. Then a chemiluminescent substrate was added to the reaction tube; the intensity of photons released was measured and was directly proportional to the CRP concentration in the sample.

Reference interval: < 3mg/L

Categorization of subjects as vitamin D-deficient or sufficient:

Subjects were categorized into those who were vitamin D-sufficient (> 20 ng/mL of 25 (OH) vitamin D) or deficient (< 20 ng/mL of 25 (OH) vitamin D).

STATISTICAL ANALYSIS

Descriptive statistics were used. Shapiro-Wilk test was used to determine normality of the data. All continuous variables were expressed as means and standard deviations, or medians with inter-quartile range (IQR) (depending on the distribution of the data). Categorical variables were expressed as numbers and percentages. Spearman's correlation coefficients were used to study the associations between the parameters of interest. A p value of < 0.05 was considered to be statistically significant in all cases. All the analyses were carried out using SPSS version 21.0.

RESULTS

Thirty pregnant women were recruited for the study, as per the inclusion and exclusion criteria described in the study protocol. Of these, 26 subjects were vitamin D-deficient and 4 subjects were vitamin D-sufficient. Data for age, height, gestational age at recruitment, hemoglobin, MCV, serum vitamin D, serum ferritin, serum iron and CRP were found to be normally distributed. Those for weight, BMI, serum creatinine, TIBC and transferrin saturation were found to have skewed distributions. Normally distributed data were expressed as means ± SD, while data with a skewed distribution were expressed as medians with inter-quartile ranges.

Table 6.1 Clinical characteristics of patients in the study (n=30)

Characteristics of subjects	
Age (years)	25.76 (±3.5)
Gestational age at recruitment (weeks)	11.46 (±2.02)
Weight (kgs)	60.1 (53.0-69.3)
Height (cm)	155.73 (±5.14)
Body mass index (BMI) (kg/m^2)	24.89 (23.52-27.71)

Table 6.2 Hematological and iron-related parameters (n=30)

Parameters	
Hemoglobin (gm/dL)	11.65 (±1.23)
Mean corpuscular volume (MCV)(fL)	79.90 (±6.38)
Serum ferritin (µg/L)	24.02 (±18.80)
Serum iron (µg/dL)	68.04 (±37.95)
Total iron binding capacity (TIBC) (µg/dL)	396.78 (361.75-425.27)
Transferrin saturation (Tfsat) (%)	14.55 (11.42-22.57)

Table 6.3 Vitamin D, creatinine and CRP in serum (n=30)

Parameters	
Vitamin D (ng/mL)	12.07 (± 5.97).
Serum creatinine (mg/dL)	0.50 (0.44-0.58)
CRP (mg%)	0.87 (± 0.99)

It was found that 26 subjects (86.66%) were vitamin D-deficient.

Table 6.4 Clinical characteristics of patient in vitamin D-sufficient and deficient groups

Characteristics of subjects	Vitamin D-sufficient (n=4)	Vitamin D-deficient (n=26)	P value
Age (years)	25.75 (± 2.75)	25.77 (±3.64)	0.957
Gestational age at recruitment(weeks)	13.26 (±0.56)	11.18(±2.03)	0.136
Weight (kgs)	62.70 (54.32-67.55)	59.80 (53.05-69.90)	0.061
Height (cm)	154.50 (±3.10)	155.92(±5.30)	0.887
Body mass index (BMI) (kg/m^2)	26.64 (22.12-28.78)	24.89 (23.68-27.34)	0.608

Data were compared by either unpaired student's t test or Mann-Whitney U test, as appropriate.

**p< 0.05*

The 2 groups were similar in terms of their age, gestational age at recruitment and anthropometric data obtained.

Table 6.5 Hematological and iron-related parameters in Vitamin D-sufficient and deficient groups

Parameters	Vitamin D-sufficient (n=4)	Vitamin D-deficient (n=26)	P value
Hb (gm/dL)	11.37 (±1.15)	11.69 (±1.26)	0.760
MCV (fL)	80.25(±6.73)	79.85 (±6.46)	0.910
Serum ferritin (µg/L)	21.45 (±24.04)	24.41 (±18.43)	0.441
Serum iron (µg/dL)	61.92 (±51.74)	68.98 (±36.64)	0.440
TIBC (µg/dL)	398.75(375.47-511.80)	394.20 (349.0-424.70)	0.589
Tfsat (%)	10.44 (5.58-31.20)	14.81 (11.98-22.43)	0.550

Data were compared by either unpaired student's t test or Mann-Whitney U test, as appropriate.

**p< 0.05*

The 2 groups were similar in terms of their hemoglobin, serum ferritin, serum iron, TIBC and transferrin saturation.

Table 6.6 Vitamin D levels, creatinine and CRP in serum in Vitamin D-sufficient and deficient groups

Parameters	**Vit D-sufficient (n=4)**	**Vit D-deficient (n=26)**	**P value**
Vitamin D (ng/mL)	24.32 (±1.18)	10.44 (±3.73)	0.039*
Serum creatinine (mg/dL)	0.58 (0.50-0.67)	0.50 (0.42-0.57)	0.121
CRP (mg%)	1.317 (±0.84)	0.80 (±1.01)	0.996

Data were compared by either unpaired student's t test or Mann-Whitney U test, as appropriate.

**p< 0.05*

Vitamin D levels were significantly lower in the deficient group, as expected.

Table 6.7 Correlation analysis of BMI and gestational age at recruitment: (n=30)

		BMI	GA at recruitment
Weight	Correlation	.914**	
	Sig. (2-tailed)	0.000	
Vitamin D	Correlation		.531**
	Sig. (2-tailed)		0.003

*Correlation analysis was done using Spearman rank analysis. **p < 0.01*

BMI correlated positively with the weight of the subjects. Vitamin D correlated positively with the gestational age at recruitment.

Table 6.8 Correlation analysis of iron-related parameters and hsCRP (n=30)

		Hb	**Serum Ferritin**	**Serum iron**	**TIBC**
Serum ferritin	Correlation	.491**		.552**	-.475**
	Sig. (2-t)	0.006		0.002	0.008
Serum iron	Correlation	.627**	.552**		-.389*
	Sig. (2-t)	0.000	0.002		0.034
MCV	Correlation	.549**		.509**	-.694**
	Sig. (2-t)	0.002		0.004	0.000
TIBC	Correlation	-.415*	-.475**	-.389*	
	Sig. (2-t)	0.023	0.008	0.034	
TSAT	Correlation	.638**	.590**	.979**	-.506**
	Sig. (2-t)	0.000	0.001	0.000	0.004
CRP	Correlation				.362*
	Sig. (2-t)				0.05

*Correlation analysis was done using Spearman rank analysis. * $p < 0.05$*

Serum ferritin correlated significantly and positively with hemoglobin and serum iron, and negatively with total iron binding capacity (TIBC). Serum iron correlated significantly and positively with hemoglobin and serum ferritin, and negatively with TIBC. Mean corpuscular volume (MCV) correlated significantly and positively with hemoglobin, serum iron and negatively with TIBC. TIBC correlated significantly and negatively with hemoglobin and serum ferritin and iron. Transferrin saturation correlated significantly and positively with hemoglobin, serum ferritin, serum iron and negatively with TIBC. Serum CRP levels correlated positively with TIBC. Correlations between hematological and iron-related parameters in the subjects were in keeping with known biological relationships amongst the parameters of interest.

SUMMARY OF THE RESULTS

1. Thirty subjects were recruited for the study. They were all in their first trimester.

2. Most of them were vitamin D-deficient (86.66%).

3. There were no significant differences between vitamin D-deficient and sufficient subjects, with regard to the hematological and iron-related parameters measured.

4. BMI correlated positively with the weight of the subjects.

5. Serum vitamin D levels correlated positively with the gestational age at recruitment.

6. Serum CRP levels correlated positively with TIBC.

7. Correlations seen between hematological and iron-related parameters in the subjects, were in keeping with known biological relationships amongst the parameters of interest.

DISCUSSION

In the present study, 30 women with singleton pregnancies, with no complications of pregnancy, were recruited. There was a very high prevalence of vitamin D deficiency (VDD) among the subjects (86.6%). Of the 30 subjects, 23.33% were found to be anemic. Only one subject (3.33%) had both anemia and VDD, as defined by a hemoglobin value of less than 11 g/dL in the first trimester (Seshadri and Arjun, 2021) and a serum vitamin D levels in blood less than 20 ng/mL (Amrein et al., 2020).

Iron-deficiency anemia and vitamin D deficiency (VDD) are common in pregnancy (Takaoka et al., 2020). Accumulating evidence in recent years suggests that these two conditions are associated with each other, and co-exist (Thomas et al., 2015; Basutkar et al., 2018; Judistiani et al., 2018; Yuan et al., 2017). The underlying mechanisms responsible for the associations seen are not clear.

A study by Yuan et al (2017), on primigravidae in China (n = 2335 and in their second or third trimester), reported that 33.19% of the subjects studied had anemia. Among the anemic women, 72.8% had vitamin D deficiency also, while among the control subjects, only 60.8% had vitamin D deficiency. They reported that low serum levels of 25(OH)D concentration were associated with 80% increase in risk of developing anemia (Yuan et

al., 2017). Thomas et al (2015) have also reported links between vitamin D status and anemia, during pregnancy. Theirs was a prospective longitudinal study on pregnant adolescents (<18 years of age), who had uncomplicated singleton pregnancies. Maternal and neonatal vitamin D and iron status were estimated during mid-gestation (~25 weeks) and at time of delivery (~40 weeks). After adjusting for gestational age at recruitment and race, those with sub-optimal vitamin D status (serum 25(OH)D < 50 nmol/L) were found to have 8 times greater risk of developing anemia, than those who were vitamin D-sufficient (serum 25(OH)D ≥50 nmol/L). Judistiani et al., (2018) studied Indonesian women with singleton pregnancies (of varying parity). Subjects were recruited between 10 to 14 weeks of gestation and followed up through pregnancy. They showed that 96.5% of subjects had hypovitaminosis D (below 30 ng/mL) in their first trimester. Of these 75.5% were deficient and 21% were insufficient. Their cholecalciferol status was not found to be associated with anemia or ferritin values. However, it was found that in cholecalciferol-deficient subjects the occurrence of anemia increased as the trimesters advanced (Judistiani et al., 2018).

In a prospective observational study done on pregnant women in South India (n =101) (Basutkar et al., 2018), a positive significant correlation was found between serum vitamin D concentrations and hemoglobin levels ($r_s = 0.49$, $p < 0.001$). They reported that serum vitamin D levels showed a positive correlation with ferritin ($r_s = 0.783$, $p< 0.001$), iron ($r_s = 0.788$, $p< 0.000$), hematocrit ($r_s = 0.729$, $p< 0.001$), and transferrin ($r_s =$

0.740, $p< 0.001$) and a negative correlation with TIBC ($r_s = -0.744$, $p< 0.000$). In the present study, vitamin D concentrations in blood did not show any correlation with hematological (hemoglobin and MCV) or iron-related parameters (iron, ferritin, transferrin saturation and TIBC) studied. Since both studies were done on South Indian subjects, a detailed comparison was done of the study by Basutkar et al (2018) and the present study. This is shown in the tables below.

Comparison of the characteristics of the subjects in the study by Basutkar et al (2018) and in the present study

	Basutkar et al., 2018	***Present study***
Number of subjects	101	30
Gestational age at recruitment (weeks)	Between 26 and 28 weeks	Less than 14 weeks
Gravid status	Both primi- and multigravidae, with singleton pregnancy	Only primigravidae with singleton pregnancy

	Basutkar et al., 2018	*Present study*
Inclusion criteria Hemoglobin (gm/dL) Serum ferritin (µg/L) Vitamin D (ng/mL)	 7 to 10.9 Less than 12 Less than 12	No such inclusion criteria were used
Exclusion criteria	Diseases complicating pregnancy	Diseases complicating pregnancy

It is clear from the above table that there were many major differences in the characteristics of the subjects in the study by Basutkar et al (2018) and the present study. This is likely to underlie the differences in observations of the 2 studies, which are summarized below.

Comparison of parameters studied by Basutkar et al (2018) and in the present study

	Basutkar et al	***Present study***	
	Vit D < 12 ng/mL (n=101)	***Vit > 20 ng/mL (n=4)***	***Vit < 20 ng/mL (n=26)***
Age (years)	23.2 (±2.3)	25.75 (± 2.75)	25.77 (±3.64)
BMI (kg/m^2)	22.6 (± 3.0)	25.85 (±3.58)	24.72 (±4.18)
Hemoglobin (gm/dL)	9.35 (± 0.89)	11.37 (±1.15)	11.69 (±1.26)
RBC (10^6/mm^3)	3.38 (± 0.23)	Not done	
Platelet count (10^3/mm^3)	445.30 (± 38.66)	Not done	
Total count	8.57 (± 1.02)	Not done	
Hematocrit	33.37 (± 2.67)	Not done	
MCV (fL)	74.95 (± 3.93)	80.25(±6.73)	79.85 (±6.46)
MCH	28.04 (±3.08)	Not done	
MCHC	33.11 (±2.37)	Not done	
Serum iron (µg/dL)	73.0 (± 15.3)	61.92 (±51.74)	68.98 (±36.64)
TIBC (µg/dL)	445.2 (± 43.3)	428.67 (±79.60)	393.07 (±59.76)
Transferrin	15.0 (± 4.81)	Not done	

	Basutkar et al	***Present study***	
	Vit D < 12 ng/mL (n=101)	***Vit > 20 ng/mL (n=4)***	***Vit < 20 ng/mL (n=26)***
Ferritin (µg/L)	13.0 (± 3.8)	21.45 (±24.04)	24.41 (±18.43)
Tfsat (%)	15.0 (± 4.8)	21.45 (±24.04)	24.41 (±18.43)
Vitamin D (ng/mL)	16.56 (± 5.88)	24.32 (±1.18)	10.44 (±3.73)
CRP (mg%)	Not done	1.317 (±0.84)	0.80 (±1.01)
Creatinine (mg/dL)	Not done	0.58 (±0.09)	0.49 (±0.09)
PTH (pg/mL)	30.70 (± 5.51)	Not done	

From the above table, it is clear that subjects in the study by Basutkar et al (2018) were anemic and iron-deficient, in addition to being vitamin D-deficient. These and the other differences in their characteristics make it difficult to make direct comparisons between their study and the present one, which had only 4 subjects in the vitamin D-deficient group.

VDD during pregnancy has also been reported to be associated with increased risk of gestational diabetes mellitus (Zhang et al., 2008) and as an independent risk factor for bacterial vaginosis (Bodnar et al., 2009). Vitamin D-deficient mothers were also found to have increased incidence of eclampsia and pre-eclampsia (Singla et al., 2015; Ullah et al.,

2013). It has also been reported that VDD during pregnancy causes proximal muscle weakness, contributing to increased indications for caesarean sections, when compared to women with normal vitamin D levels (Bischoff-Ferrari et al., 2006; Merewood et al., 2009).

Braithwaite et al. (2019) have shown, in their randomized control trial in the United Kingdom, that supplementing pregnant women with cholecalciferol (1000 IU/day) did not affect hepcidin, ferritin and CRP levels in serum. Since VDD is highly prevalent in Indian pregnant women, it has been suggested that they be supplemented with vitamin D, without checking vitamin D status. A daily dose of 1000-2000 IU of vitamin D has been suggested (Seshadri and Arjun, 2021). The WHO recommends 200 IU (5µg) per day of vitamin D in pregnant women documented to be vitamin D-deficient (WHO, 2016). However, assessment of vitamin D status is not part of routine antenatal care in India. Empirical clinical practice usually involves use of calcium supplements, which include vitamin D (~200 IU per day). Such doses, however, may not suffice to treat vitamin D deficiency.

In the present study, vitamin D-deficient and sufficient women were found to be similar with regard to the iron-related parameters studied. However, the calculated sample size was not achieved within the time frame of the study. It was possible to recruit only 30 subjects in all, due to the constraints of the pandemic. Of these, most (86.6%) were

vitamin D-deficient. Only 4 of them vitamin D-sufficient. It would be necessary to study an adequate number of patients in this group as well to draw definitive conclusions. It would also be useful to study these parameters over the course of pregnancy.

CONCLUSION

In this study, there were no significant differences between vitamin D-deficient and sufficient subjects, with regard to hematological and iron-related parameters. However, an adequate number of patients need to be studied to confirm these findings.

LIMITATIONS OF THE STUDY

1. Only 30 subjects were studied, of whom 26 subjects were vitamin D-deficient and 4 subjects were vitamin D-sufficient. More patients need to be studied to have an adequate number of control subjects for comparison.

2. This was a cross-sectional study of primigravidae in their first trimester. It would have been good to have followed up the patients over the pregnancy. However, it was not possible to do this due to the circumstances of the pandemic.

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Appendix I

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., Dr. Min (clinical)
Director, Christian Counselling Center
Chairperson, Ethics Committee

Dr. Anna Benjamin Pulimood, MD., Ph.D.,
Chairperson, Research Committee, Principal

Dr. Suceena Alexander, MD., DM., FASN.,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

March 25, 2021

Dr. R.S.Logapriya,
PG Registrar,
Department of Biochemistry,
Christian Medical College,
Vellore – 632 002.

Sub: Fluid Research Grant: New Proposal:
Vitamin D deficiency and anemia in pregnancy: how are they associated?
Dr. R.S.Logapriya, Employment Number: 21595, PG Registrar, Biochemistry, Dr. Molly Jacob, Employment number: 14509, Biochemistry Nikhitha Mariya John, Biochemistry, Dr. Joe Varghese, Employment number: 20405, Biochemistry, Dr. Jagadish R, Employment number: 21102 Biochemistry, Dr. Manisha Beck (employment number: 31924), Obstetrics and Gynecology, Dr. Thenmozhi Mani, Biostatistics.

Ref: IRB Min. No. 13722 [OBSERVE] dated 06.01.2021.

Dear Dr. R.S.Logapriya,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Suceena Alexander, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Suceena Alexander
Secretary (Ethics Committee)
Institutional Review Board

Dr. Suceena Alexander, MD.,DM.,FASN.
Secretary - (Ethics Committee)
Institutional Review Board
Christian Medical College,
Vellore - 632 002, Tamil Nadu, India.

Cc: Dr. Molly Jacob, Biochemistry CMC, Vellore

1 of 5

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., Dr. Min (clinical)
Director, Christian Counselling Center
Chairperson, Ethics Committee

Dr. Anna Benjamin Pulimood, MD., Ph.D.,
Chairperson, Research Committee, Principal

Dr. Suceena Alexander, MD., DM., FASN.,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

March 25, 2021

Dr. R.S.Logapriya,
PG Registrar,
Department of Biochemistry,
Christian Medical College,
Vellore – 632 002.

Sub: Fluid Research Grant: New Proposal:
Vitamin D deficiency and anemia in pregnancy: how are they associated?
Dr. R.S.Logapriya, Employment Number: 21595, PG Registrar, Biochemistry, Dr. Molly Jacob, Employment number: 14509, Biochemistry Nikhitha Mariya John, Biochemistry, Dr. Joe Varghese, Employment number: 20405, Biochemistry, Dr. Jagadish R, Employment number: 21102 Biochemistry, Dr. Manisha Beck (employment number: 31924), Obstetrics and Gynecology, Dr. Thenmozhi Mani, Biostatistics.

Ref: IRB Min. No. 13722 [OBSERVE] dated 06.01.2021.

Dear Dr. R.S.Logapriya,

The Institutional Review Board (**Blue**, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "Vitamin D deficiency and anemia in pregnancy: how are they associated?" on January 06, 2021.

The Committee reviewed the following documents:

1. IRB Application Format
2. Patient information sheet and Consent form (English, Tamil, Hindi)
3. Cvs. Of Drs. Jagadish, Joe, Logapriya, Manisha, Molly J, Nikhitha, Thenmozhi.
4. Proforma
5. No. of documents 1 – 4.

The following Institutional Review Board (**Blue**, Research & Ethics Committee) members were present at the meeting held on January 06 2021 in the New IRB Room, Christian Medical College, Vellore 632 004.

Ethics Committee Blue, Office of Research, I Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284508 Fax: 0416 – 2262788 E-mail: research@cmcvellore.ac.in

Dr. B.J. Prashantham, M.A., Dr. Min (clinical)
Director, Christian Counselling Center
Chairperson, Ethics Committee

Dr. Anna Benjamin Pulimood, MD., Ph.D.,
Chairperson, Research Committee, Principal

Dr. Suceena Alexander, MD., DM., FASN.,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Name	Qualification	Designation	Affiliation
Dr. Anna Benjamin Pulimood	MD, PhD	Principal, Chairperson-Research Committee, IRB, CMC, Vellore	Internal, Clinician
Dr. B. J. Prashantham	MA (Counseling Psychology), MA(Theology), Dr. Min (Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore	External, Social Scientist
Dr. Suceena Alexander	MD., DM., FASN	Secretary – (Ethics Committee), IRB, Addl. Vice Principal (Research), Professor of Nephrology, CMC, Vellore	Internal Clinician
Dr. Shyam Kumar NK	DMRD, DNB, FRCR, FRANZCR	Professor, Radiology, CMC, Vellore	Internal, Clinician
Ms. Grace Rebekha	M.Sc., (Biostatistics)	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Mr. Samuel Abraham	MA, PGDBA, PGDPM, M. Phil, BL.	Sr. Legal Officer, Vellore	External Legal Expert
Rev. Rainard Pearson	BA., B. Th., M. Div.,	Sr. Chaplin, CMC, Vellore.	Internal, Social Scientist
Dr. Jayaprakash Muliyil	MD, MPH, Dr PH (Epid), DMHC	Retired Professor, CMC, Vellore	External, Scientist & Epidemiologist
Dr. Barney Isaac	DNB (Respiratory Diseases)	Associate Professor, Pulmonary Medicine, CMC, Vellore	Internal, Clinician
Mrs. Pattabiraman	BSc, DSSA	Social Worker, Vellore	External, Lay Person
Dr. Ekta Rai	MD, MRCA	Professor, Head of the Unit 5, Department of Anaesthesia, CMC, Vellore	Internal, Clinician
Dr. Balu Krishna	MBBS MD DNB DMRT	Professor, Department of Radiotherapy, CMC Vellore	Internal Clinician
Dr. HS. Asha	MBBS, DNB	Professor, Department of Endocrinology, CMC Vellore	Internal Clinician

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Chairperson, Research Committee, Principal

Dr. Suceena Alexander, MD., DM., FASN.,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Mrs. Jayalinda Christopher	MSc Nursing	Addl. Deputy Nursing Superintendent, College of Nursing, CMC, Vellore	Internal, Nurse
Dr. Winsely Rose	MD (Paed)	Professor, Paediatrics, CMC Vellore	Internal, Clinician
Dr. Sathish Kumar	MD, DCH	Professor, Child Health, CMC, Vellore	Internal, Clinician
Dr. Santosh Varughese	MBBS, MD	Professor, Nephrology, CMC, Vellore	Internal, Clinician
Dr. Anuradha Rose	MBBS, MD, MHSC (Bioethics)	Associate Professor, Community Health, CMC, Vellore	Internal, Clinician
Mrs. Reena George	MSc Nursing	Addl. Deputy DeanCMC, Vellore College of Nursing, CMC, Vellore	Internal, Nurse
Dr. John Jude Prakash	MBBS, MD,	Professor, Clinical Virology, CMC, Vellore	Internal, Clinician
Mrs. Mahasampath Gowri	M.Sc Biostatistics	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Dr. Rekha Pai	MSc, PhD	Associate Professor, Pathology, CMC, Vellore	Internal, Basic Medical Scientist
Dr. Premila Abraham	M.Sc. Ph.D	Professor, Department of Biochemistry, CMC, Vellore	Internal Clinician

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of Withdrawals for the study entitled: "Vitamin D deficiency and anemia in pregnancy: how are they associated?" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

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Chairperson, Research Committee, Principal

Dr. Suceena Alexander, MD., DM., FASN.,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

The Institutional Ethics Committee expects to be informed about the progress of the project, Any **adverse events** occurring in the course of the project, any **amendments in the protocol and the patient information / informed consent**. On completion of the study you are expected to submit a copy of the **final report**. Respective forms can be downloaded from the following link: http://172.16.11.136/Research/IRB_Polices.html in the CMC Intranet and in the CMC website link address: http://www.cmch-vellore.edu/static/research/Index.html.

Fluid Grant Allocation:

A sum of 6,340/- INR (Rupees Six Thousand Three Hundred and Fourty Only) will be granted for 24 Months.

Yours sincerely,

Dr. Suceena Alexander, MD.,DM.,FASN.
Secretary - (Ethics Committee)
Institutional Review Board
Christian Medical College,
Vellore - 632 002, Tamil Nadu, India.

Dr. Suceena Alexander
Secretary (Ethics Committee)
Institutional Review Board

IRB Min. No. 13722 [OBSERVE] dated 06.01.2021

Ethics Committee Blue, Office of Research, I Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284508 Fax: 0416 – 2262788 E-mail: research@cmcvellore.ac.in

Appendix II

INFORMATION SHEET FOR STUDY PARTICIPANTS

Departments of Biochemistry and Obstetrics and Gynecology (Unit 4), CMC, Vellore

Title of project: Vitamin D deficiency and anemia in pregnancy: How are they associated?

Date:

We would like to invite you to participate in a study that we are doing. This study will help doctors understand better if there is a connection between Vitamin D levels and anemia in pregnancy. It study is being carried out by the Departments of Biochemistry and Obstetrics and Gynecology, Unit 4, at CMC, Vellore.

For the purpose of this study, we need 6 ml of blood sample from each subject in each trimester. We would like to know if you are willing to participate in the study and provide a blood sample for it.

The collection of blood will not cause any harm to you or your baby. Blood samples collected will be used only for research purposes. If there is any sample remaining after this study is complete, we would like your permission for the samples to be stored and used for related studies in the future.

All information provided by you will be kept confidential.

You may not benefit directly by participation in this study. Participation in the study does not entitle you to concession or any other special treatment. You have to pay for all the routine tests that the doctor might ask you to do as part of your antenatal health check-up. However, you need not pay for the tests done as part of this study.

Your participation in this study is voluntary and if you are not willing to participate in the study, you are free to do so. It will not, in any way, affect the treatment you will receive in the hospital.

For your further queries, please contact us on the numbers given below:

Dr. R. S. Logapriya, PG Registrar, Department of Biochemistry, CMC, Vellore

Contact number: 9790877161

Dr. Molly Jacob, Professor, Department of Biochemistry, CMC, Vellore,

Contact number: 04162-2284267

Dr. Manisha Beck, Professor, Unit 4, Department of Obstetrics and Gynecology, CMC, Vellore, Contact number: 04162-228-6185.

Dr. Joe Varghese, Professor, Department of Biochemistry, CMC, Vellore, Contact number: 0416-2284267

Informed Consent Form for Subject

Study Title: Vitamin D deficiency and anemia in pregnancy: How are they associated?

Study Number: _____

Subject's Full Name: _____

Date of Birth / Age: _____

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that the Ethics Committee and regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. [ ]

(iv) I understand that my participation in the study, a blood sample of 6 mL will be collected from me during each trimester of pregnancy. [ ]

(v) I understand that giving these blood samples will not affect my health or that of my baby in a foreseeable way. [ ]

(vi) I understand that the blood samples collected will be used only for research purposes. If there is any sample remaining after this study is completed, I give permission for the samples to be stored and used for related studies in the future. [ ]

(vii) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). [ ]

(viii) I understand that my identity will not be revealed in any information published. [ ]

(ix) I agree to take part in the above study. [ ]

Signature (or Thumb impression) of the Subject/Legally Acceptable:

Signatory's Name: _____ Signature: _____

(or)

Date: _____/_____/_____ Thumb impression:

Representative: _____

Date: _____/_____/_____

Signatory's Name: _____

Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

Signature or thumb impression of the Witness: _____(or)

Date: _____/_____/_____

Name & Address of the Witness: _____

_____

_____

பின் இணைப்பு II

ஆராய்ச்சி பங்கேற்பாளர்களுக்கான தகவல் தாள்

உயிர் வேதியியல் மற்றும் மகப்பேறியல் மற்றும் பெண்ணோயியல்

துறை, சி.எம்.சி, வேலூர்.

திட்டத்தின் தலைப்பு: மகப்பேறு காலத்தில் வைட்டமின் டி குறைபாடு மற்றும் இரத்த சோகை: இவை இரண்டும் எவ்வாறு தொடர்புபடுத்தப்பட்டுள்ளன?

தேதி:

நாங்கள் செய்து வரும் இந்த ஆய்வில் பங்கேற்க உங்களை அழைக்க விரும்புகிறோம். இந்த ஆய்வு மகப்பேறு காலத்தில் இரும்பு மற்றும் வைட்டமின் டி அளவுகளுக்கு இடையிலான தொடர்பை மருத்துவர்கள் நன்கு புரிந்துகொள்ள வழி வகுக்கும். வேலூரில் உள்ள சி.எம்.சி.யில் உயிர் வேதியியல் துறைகள் மற்றும் மகப்பேறியல் மற்றும் மகளிர் மருத்துவத் துறை அலகு 4 இல் மேற்கொள்ளப்படும் ஆய்வு இது.

இந்த ஆய்வின் நோக்கத்திற்காக, ஒவ்வொரு கர்ப்பிணித் தாயிடமிருந்து எங்களுக்கு மூன்று மாதத்திற்கு ஒரு முறை 6 மில்லி இரத்த மாதிரி தேவைப்படுகிறது. நீங்கள் இந்த ஆய்வில் பங்கேற்று உங்களது இரத்த மாதிரியை வழங்க ஒப்புக் கொள்கிறீர்களா என்பதை அறிய விரும்புகிறோம்.

உங்களிடமிருந்து எடுக்கப்பட்ட இரத்தம் உங்களுக்கும் உங்கள் குழந்தைக்கும் எந்தத் தீங்கும் ஏற்படுத்தாது. சேகரிக்கப்பட்ட இரத்த மாதிரிகள் ஆராய்ச்சி நோக்கங்களுக்காக மட்டுமே பயன்படுத்தப்படும். இந்த ஆய்வின் முடிவில் உங்களது இரத்த மாதிரி ஏதேனும் மீதமிருந்தால் அதை நாங்கள் பாதுகாக்கவும் பிற்காலத்தில் இது போன்ற ஆய்வுகளுக்கு பயன்படுத்திக் கொள்ளவும் தாங்கள் அனுமதி தரவேண்டும் என்று விரும்புகிறோம்.

நீங்கள் வழங்கிய அனைத்து தகவல்களும் ரகசியமாக வைக்கப்படும்.

இந்த ஆய்வில் பங்கேற்பதன் மூலம் நீங்கள் நேரடியாக பயன் அடைய மாட்டீர்கள். இந்த ஆய்வில் பங்கேற்பது உங்களுக்கு எந்த சலுகையையும்

சிறப்பு சிகிச்சையையும் பெற்றுத் தராது. மகப்பேறு காலத்தில் உங்கள் மருத்துவர்கள் வழக்கமாக பரிந்துரைக்கும் அனைத்து சோதனைகளுக்கும் நீங்கள் கட்டணம் செலுத்த வேண்டி இருக்கும். இருப்பினும், இந்த ஆய்வின் ஒரு பகுதியாக மேற்கொள்ளப்பட இருக்கும் சோதனைகள் அனைத்திற்கும் நீங்கள் பணம் செலுத்த தேவை இல்லை.

இந்த ஆய்வில் உங்கள் பங்கேற்பு தன்னார்வமானது. உங்களுக்கு இந்த ஆய்வில் பங்கேற்க விருப்பம் இல்லை என்றால் நீங்கள் முழு சுதந்திரத்தோடு அந்த முடிவை எடுக்கலாம். இது எந்த வகையிலும், நீங்கள் மருத்துவமனையில் பெறும் சிகிச்சையை பாதிக்காது.

உங்களுக்கு மேலும் ஏதேனும் கேள்விகள் இருந்தால் கீழ்க்காணும் எண்களை பயன்படுத்தி எங்களை தொடர்பு கொள்ளலாம்.

டாக்டர் ஆர்.எஸ்.லோகாப்ரியா, பி.ஜி. பதிவாளர், உயிர் வேதியியல் துறை, சி.எம்.சி, வேலூர், தொடர்பு எண்: 9790877161

டாக்டர் மோலி ஜேக்கப், பேராசிரியர், உயிர் வேதியியல் துறை, சி.எம்.சி, வேலூர், தொடர்பு எண்: 04162-284267

டாக்டர் ஜோ வர்கீஸ், பேராசிரியர், உயிர் வேதியியல் துறை, சி.எம்.சி, வேலூர், தொடர்பு எண்: 0416-2284267

பொருள் குறித்த தகவலறிந்த ஒப்புதல் படிவத்திற்கான வடிவம்

ஆய்வு தலைப்பு: கர்ப்பத்தில் வைட்டமின் டி குறைபாடு மற்றும் இரத்த சோகை: இவை இரண்டும் எவ்வாறு தொடர்புபடுத்தப்படுகின்றன?

ஆய்வு எண்: _____

பொருள் முழு பெயர்: _____

பிறந்த தேதி / வயது: _____

(i) மேற்கொண்ட ஆய்வின் _____ தேதி இடப்பட்ட தகவல் தாளை நான் படித்துப் புரிந்து கொண்டேன் என்பதையும், கேள்விகளை கேட்கும் வாய்ப்பை பெற்றுள்ளேன் என்பதையும் உறுதிப்படுத்துகிறேன். [ ]

(ii) ஆய்வில் நான் பங்கேற்பது தன்னார்வமானது என்பதை அறிவேன். எந்த நேரத்திலும் எந்த காரணமும் தெரிவிக்காமல் இந்த ஆய்விலிருந்து நான் விலக முழு சுதந்திரம் பெற்றுள்ளேன் என்பதையும் நான் புரிந்து கொள்கிறேன். இந்த ஆய்விலிருந்து நான் விலகினாள் எனது மருத்துவ கவனிப்பு அல்லது சட்ட உரிமைகள் பாதிக்கப்படாது என்பதையும் நான் அறிவேன் [ ]

(iii) நான் ஆய்வில் இருந்து விலகினாலும், தற்போதைய ஆய்வு மற்றும் அது தொடர்பாக மேற்கொள்ளப்படக்கூடிய எந்தவொரு ஆராய்ச்சியையும் பொறுத்தவரை எனது சுகாதார பதிவுகளைப் பார்க்க நெறிமுறை குழு மற்றும் ஒழுங்குமுறை அதிகாரிகளுக்கு எனது அனுமதி தேவையில்லை என்பதை நான் புரிந்துகொள்கிறேன். இந்த அணுகலை நான் ஒப்புக்கொள்கிறேன். [ ]

(iv) நான் ஆய்வில் பங்கேற்கும் போது, என்னிடமிருந்து மூன்று மாதத்திற்கு ஒரு முறை 6 மில்லி இரத்த மாதிரி சேகரிக்கப்படும் என்பதை நான் புரிந்து கொள்கிறேன். [ ]

(v) இந்த இரத்த மாதிரியை கொடுப்பதினால் எனது ஆரோக்கியத்திற்கும் எனது குழந்தையின் ஆரோக்கியத்திற்கு எந்த தீங்கும் ஏற்படாது என்பதை நான் புரிந்து கொள்கிறேன். [ ]

(vi) சேகரிக்கப்பட்ட இரத்த மாதிரி ஆராய்ச்சி நோக்கங்களுக்காக மட்டுமே பயன்படுத்தப்படும் என்பதை நான் புரிந்து கொள்கிறேன். இந்த ஆய்வு முடிந்த பின் மாதிரி ஏதேனும் மீதமிருந்தால், அவற்றை சேமித்து எதிர்காலத்தில் இதுபோன்ற ஆய்வுகளுக்கு பயன்படுத்த நான் அனுமதி அளிக்கிறேன். [ ]

(vii) இந்த ஆய்விலிருந்து எழும் எந்த ஒரு தரவையும் அல்லது முடிவுகளையும் அறிவியல் நோக்கத்திற்காக பயன்படுத்துவதை கட்டுப்படுத்த வேண்டாம் என்று நான் தெரிவித்துக் கொள்கிறேன். [ ]

(viii) வெளியிடப்பட இருக்கும் எந்த தகவலிலும் எனது அடையாளம் வெளிப்படுத்தப்படாது என்பதை நான் புரிந்துகொள்கிறேன். [ ]

(ix) மேற்கொண்ட ஆய்வில் பங்கேற்ற ஒப்புக்கொள்கிறேன். [ ]

- பங்கேற்பாளர் கையொப்பம்: _____(அல்லது)கட்டை விரல்ரேகை:

(சட்டப்படி ஏற்றுக் கொள்ளத்தக்கது)

தேதி: _____/_____/_____

கையொப்பம் இட்டவர் பெயர்: _____

- பிரதிநிதியின் கையொப்பம்: _____

தேதி: _____/_____/_____

பிரதிநிதியின் பெயர்: _____

- புலனாய்வாளர்கள் கையொப்பம்: _____

தேதி: _____/_____/_____

புலனாய்வாளரின் பெயர்: _____

- சாட்சியின் கையொப்பம்: _____(அல்லது) கட்டை விரல்ரேகை:

தேதி: _____/_____/_____

சாட்சியின் பெயர் மற்றும் முகவரி: _____

_____

_____

बायोकैमिस्ट्री विभाग और गाईनेकोलॉजी विभाग, सीएमसी, वेल्लोर

परियोजना का शीर्षक: गर्भावस्था में विटामिन डी की कमी और रक्तक्षय (एनीमिया): वे कैसे जुड़े हैं?

दिनांक:

हम आपको एक अध्ययन में भाग लेने के लिए आमंत्रित करना चाहेंगे जो हम कर रहे हैं। यह अध्ययन डॉक्टरों को बेहतर समझने में मदद करेगा कि गर्भावस्था में विटामिन डी के स्तर और एनीमिया के बीच कोई संबंध है या नहीं। यह अध्ययन, सीएमसी, वेल्लोर में जैव रसायन और प्रसूति एवं स्त्री रोग विभाग, यूनिट 4 द्वारा किया जा रहा है।

इस अध्ययन के उद्देश्य के लिए, हमें प्रत्येक तिमाही में प्रत्येक विषय से 6 मिलीलीटर रक्त के नमूने की आवश्यकता है। हम जानना चाहेंगे कि क्या आप अध्ययन में भाग लेने के लिए तैयार हैं और इसके लिए रक्त का नमूना उपलब्ध कराना चाहते हैं।

रक्त के संग्रह से आपको या आपके बच्चे को कोई नुकसान नहीं होगा। एकत्र किए गए रक्त के नमूनों का उपयोग केवल अनुसंधान उद्देश्यों के लिए किया जाएगा। यदि इस अध्ययन के पूरा होने के बाद कोई नमूना शेष है, तो हम चाहेंगे कि आपके नमूने भविष्य में संबंधित अध्ययन के लिए संग्रहीत और उपयोग किए जाएं।

आपके द्वारा दी गई सभी जानकारी को गुप्त रखा जाएगा ।

इस अध्ययन में भाग लेने से आपको सीधे लाभ नहीं हो सकता है। अध्ययन में भागीदारी आपको रियायत या किसी अन्य विशेष उपचार का अधिकार नहीं देती है। आपको उन सभी नियमित परीक्षणों के लिए भुगतान करना होगा जो डॉक्टर आपको अपने प्रसवपूर्व स्वास्थ्य जांच के हिस्से के रूप में करने के लिए कह सकते हैं। हालांकि, आपको इस अध्ययन के हिस्से के रूप में किए गए परीक्षणों के लिए भुगतान करने की आवश्यकता नहीं है।

इस अध्ययन में आपकी भागीदारी स्वैच्छिक है और यदि आप अध्ययन में भाग लेने के इच्छुक नहीं हैं, तो आप ऐसा करने के लिए स्वतंत्र हैं। यह किसी भी तरह से, आपके द्वारा अस्पताल में प्राप्त उपचार को प्रभावित नहीं करेगा।

यदि आपके कोई और प्रश्न हैं, तो नीचे दिए गए नंबरों पर संपर्क करें ।

डॉ। आर.एस.लोगप्रिया, पीजी रजिस्ट्रार, बायोकैमिस्ट्री विभाग, सीएमसी, वेल्लोर, संपर्क नंबर: 9790877161

डॉ। मौली जैकब, प्रोफेसर, बायोकैमिस्ट्री विभाग, सीएमसी, वेल्लोर, संपर्क नंबर: 04162-284267

डॉ। मनीषा बेक, प्रोफेसर, यूनिट 4, प्रसूति विभाग और स्त्री रोग विभाग, सीएमसी, वेल्लोर, संपर्क नंबर: 04162-228-6185

डॉ। जो वर्गीस, प्रोफेसर, बायोकैमिस्ट्री विभाग, सीएमसी, वेल्लोर, संपर्क नंबर: 0416-2284267

प्रतिभागी के लिए सूचित सहमति फॉर्म का प्रारूप

अनुसंधान शीर्षक: विटामिन डी की कमी और गर्भावस्था में एनीमिया: वे कैसे जुड़े रहे हैं?

अनुसंधान संख्या: _____

प्रतिभागी का पूरा नाम: _____

जन्म तिथि/उम्र : _____

(i) मैं पुष्टि करता हूं कि मैंने उपरोक्त अध्ययन के लिए _____ की सूचना पत्र को पढ़ा और समझा है और मुझे प्रश्न पूछने का अवसर मिला है। [ ]

(ii) मैं समझता हूं कि अध्ययन में मेरी भागीदारी स्वैच्छिक है और मैं किसी भी समय, बिना किसी कारण के, बिना मेरी चिकित्सा देखभाल या कानूनी अधिकारों को प्रभावित किए बिना वापस लेने के लिए स्वतंत्र हूं। [ ]

(iii) मैं समझता हूं कि वर्तमान अध्ययन के संबंध में आचार समिति और नियामक अधिकारियों को मेरे स्वास्थ्य रिकॉर्ड को देखने के लिए मेरी अनुमति की आवश्यकता नहीं होगी और इसके संबंध में किए जाने वाले किसी भी अन्य शोध, भले ही मैं परीक्षण से वापस ले लूं। मैं इस पहुंच से सहमत हूं। [ ]

(iv) मैं समझता हूं कि अध्ययन में मेरी भागीदारी, गर्भावस्था के प्रत्येक तिमाही के दौरान मुझसे 6 एमएल का रक्त नमूना एकत्र किया जाएगा। [ ]

(v) मैं समझता हूं कि इन रक्त नमूनों को देने से मेरे स्वास्थ्य या मेरे बच्चे पर कोई असर नहीं पड़ेगा। [ ]

(vi) मैं समझता हूं कि एकत्र किए गए रक्त के नमूनों का उपयोग केवल अनुसंधान उद्देश्यों के लिए किया जाएगा। यदि इस अध्ययन के पूरा होने के बाद कोई नमूना शेष है, तो मैं नमूनों को संग्रहीत करने और भविष्य में संबंधित अध्ययन के लिए उपयोग करने की अनुमति देता हूं। [ ]

(vii) मैं इस अध्ययन से उत्पन्न होने वाले किसी भी डेटा या परिणामों के उपयोग को प्रतिबंधित नहीं करने के लिए सहमत हूं, बशर्ते ऐसा उपयोग केवल वैज्ञानिक उद्देश्य के लिए हो। [ ]

(viii) मैं समझता हूं कि प्रकाशित होने वाली किसी भी जानकारी में मेरी पहचान उजागर नहीं की जाएगी। [ ]

(ix) मैं उपरोक्त अध्ययन में भाग लेने के लिए सहमत हूं। [ ]

प्रतिभागी का हस्ताक्षर (या अंगूठे का निशान) विषय/कानूनी रूप से स्वीकार्य:

हस्ताक्षरकर्ता का नाम: _____ हस्ताक्षर: _____

(या)

दिनांक: ____/____/_____ अंगूठे का निशान:

प्रतिनिधि: _____

दिनांक: ____/____/_____

हस्ताक्षरकर्ता का नाम: _____

अन्वेषक के हस्ताक्षर: _____

दिनांक: ____/____/_____

अध्ययन अन्वेषक का नाम: _____

गवाह के हस्ताक्षर या अंगूठे का निशान: _____ (या)

दिनांक: ____/____/_____

गवाह का नाम और पता: _____

_____

_____

Appendix III

Proforma for patient's details

Departments of Biochemistry and Obstetrics and Gynecology (Unit 4),

Christian Medical College (CMC), Vellore-632 002

Study title: Vitamin D deficiency and anemia in pregnancy: how are they associated?

Information collected by: Dr.R.S.Logapriya **Date:**

Subject's full name:

Date of birth:

Hospital number:

Address with phone number:

Occupation:

Full name of husband:

Occupation:

Gestational age on day of recruitment (as per LMP):

Last menstrual period (LMP):

Expected date of delivery:

History of any previous pregnancy/abortion/miscarriages: Yes/No

If yes, brief details:

Any previous illnesses/current illnesses:

On any medications currently (including traditional medicines): Yes/No

If yes, brief details

On iron and folic acid supplements: Yes/No

How regular in taking these supplements: