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RECENT DIAGNOSTICS METHODS FOR VITAMIN-D DEFICIENCY IN HUMAN IMMUNE SYSTEM

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ABSTRACT

Vitamin D has attracted increased clinical and educational interest over the last twenty years. Serious cases of vitamin D have shown a rise in the number of young people displaying signs of vitamin D deficiency, i.e., rickets and hypocalcaemia. The vitamin D deficiency of two forms is typically measured in the blood. Serum 25(OH)D is an indication of skin and nutritional availability of vitamin D synthesis. This is the greatest indicator of the vitamin D intake, which shows our food, sunlight and other nutrients for the amount of vitamin D. The existence or absence of vitamin D can be established by only blood tests, while vitamin D deficiency can be established by diagnostic methods like Dia Sorin (LIASION), IDS I-SYS, Roche assay, LC-MS/ MS, QCIA (CLIA), Siemens ADVIA Centaur (Abbott, ARCHITECT), HPC, RFI, etc. In developing countries including India, the national surveys of serum 25(OH)D levels in demographic groups of babies and young children below the age of 4 should concentrate on assessing vitamin D deficiency with the developed countries. Breast-fed child-bearing girls, pregnant mothers, breastfeeding mother and ethnic minorities are more vulnerable to vitamin D deficiency and thus of considerable significance. In the present situation where the lethal virus is circulating across the continents and the lack of a special cure for the new coronavirus, it is desperately important to look for alternate ways to deter and maintain rapid viral replication. Supplementing with vitamin D could minimise the frequency, severity, and probability of pneumonic death in the current COVID pandemic (consequential to a cytokine storm). Tocopherol could reduce the respiratory tract infections caused by viruses by inducing the adaptive immunity. It also decreases the viral infection risk by producing antimicrobial peptides like cathelicidin and defensin. In India, it is recommended that COVID-19 people to consume more vitamin D supplementations to enhance their immunity.

KEYWORDS

Vitamin D, 25-Hydroxy vitamin-D, 25(OH)D, Sunlight, Diagnosis and Calcium.

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INTRODUCTION

For our bodies to naturally grow and produce vitamins are substances required. It has to be supplied with a diet or an enhancement. These are important for human wellbeing and defects can have health implications and diseases. Vitamin D (calciferol) is a fat-soluble vitamin that consists of two forms: 1. D2 (ergocalciferol) 2 Vitamin. And D3

(Cholecalciferol) vitamin. Vitamin D is not structurally similar in all forms[1]. Foods dependent on fortification, plant food, and additives are used to make ergocalciferol available. Cholecalciferol comes from fortified foodstuffs and animal feed supplements that are internally made with ultraviolet (UV) radiation (fatty fish, cod liver oils, eggs and liver). Vitamin D helps people consume calcium as the primary bone-building blocks[2]. Vitamin D deficit induced bone disease or bone disease (Wallace). Vitamin D plays a part in human neurological, muscle stimulation and immune systems. Since exposure to sunlight and UV rays, our body spontaneously shapes vitamin D. Vitamin D has a postulated role in changing the risk of developing different diseases and the function of the diseases in bone metabolism and calcium homeostasis. A lack of diagnostics of vitamin D deficiency in the medical practice, research, and rehabilitation therapy, results in neglect in the evaluation of children who suffer from vitamin D deficiency. Therapy in untreatable people with a high vitamin D deficiency incidence contributes to positive health outcomes[3].

The census rate of vitamin D deficiency

Recommendations for vitamin D supplements as required for high-risk populations by the Health Department[4].

In 2001–2006 there was ample vitamin D, a serum 25-hydroxyvitamin D (25OHD) of 50-125nmol / L in the two-thirds population. Around 1-4 per cent (serum 25OHD 30-49nmol / L) is susceptible to deficiency of vitamin D and 8 per cent (serum 25OHD lower than 30nmol /L), respective to vitamin D deficiency.

Ethnicity and Age of women vary in the risk of vitamin D deficiency. In people who were younger, male or non-Hispanic women, who were pregnant or breastfeeding, the incidence was lower[5].

Vitamin D risk improved by 85% from 1988 to 1994 and by 90% in 2001 to 2002. The prophylaxis programme curtailed development in vitamin D deficiency for the two sexes because of lack of understanding, but did not improve in 2005-2006, as seen in Figure No.1.

The latest dietary reference intakes of calcium and vitamin D have recently been published by the Institute of Medicine (IOM). The IOM identified four vitamin D status groups based on 25-Hydroxyvitamin D serum: (i) risk of deficiency, (ii) risk of inadequacy, (iii) sufficiency, and (iv) above which there may be the reason for Table No.1 shows Different levels of Vitamin-D deficiency[6].

Effect of Vitamin D Deficiency

Older persons with dark skin (high melanin production), obese, or with kidney or liver conditions, and those with inadequate direct Sun exposure are among the most susceptible people with Vitamin D deficiency danger. Vitamin D human toxicity is very rare and does not cause vitamin D toxicity overexposure to sunlight[7]. Vitamin D toxicity in some rare cases causes Fatigue, constipation, fatigue and damage to the kidneys. NIH's latest guidance notes vitamin – D does not exceed 4,000 I.U (under the age of nine), in addition to the diet or medicines per day. Deficiency of vitamin D is normal in winter and fall (November through March, April through October)[8]. The vitamin D deficiency dependent on the month of blood draw also affects those two seasons. The season is essential because vitamin D is provided by sunlight and exposure and varies by season in the skin for two reasons[9].

Vitamin D requirements

Two Institute of Medicine recommends appropriate dietary supplements or nutritional benefits[10]. The RDA is the normal daily consumption of 97 to 98 per cent of safe people to satisfy their nutritional needs. For people at risk of vitamin D loss, the Endocrine Society has set recommendations according to their age groups Table No.2.

Sources and role of Vitamin D

Three big vitamin D sources are 1. Skin (exposure to UV), 2. Diet and diet and three. Additional add-ons. The strongest forms of vitamin D are egg yolks, saltwater poultry, liver, liquid milk and vitamins D enriched yoghurt, fortified cereals, pantries and orange juices. Vitamin D2 comes from the UV irradiation of yeast sterol which spontaneously occurs in sun-exposed mushrooms, which sunlight hits the skin and humans to synthesise Vitamin D2, D3. Vitamin D is the most normal component[11]. Vitamin D2 is not created by humans. Vitamin D3 is

found in oil-rich fish, including salmon, mackerel and herring. Ingested and integrated into the lymph chain of ingested Chylomicrons into the bloodstream, vitamin D (D representing D2 or D3 or both) reaches into the blood vessels. In humans, UVB-induced conversion of 7-dehydrocholesterol to vitamin D is the primary source of vitamin D on the skin. Vitamin D affects intestines, lungs, lungs, pancreas, bones, nerves, brains, and helps regulate cell cycles[12]. The skin produces Vitamin D stimulated in the body when the human body is exposed to sunlight. Adequate blood amounts of vitamin D are derived from available sources. Extra Vitamins D from strong foods or supplements might come from older adults and people with dark skin. Exposure of the skin to sunlight is important to promote vitamin D regeneration.

Vitamin D occurs in the skin or diet first undergoes vitamin D-25-hydroxylase (25-OHase) to 25(OH) D hydroxylation in the liver (Braegger *et al*, 2013). 25(OH)D requires an additional 25(OH)D-1-OHase (CYP27B1) hydroxylation in the kidneys to shape biologically active forms of vitamin D1, 25(OH) 2D. 1.25(OH),2D stimulates the intestinal uptake of calcium. 10–15 per cent therapeutic calcium and approximately 60 per cent phosphorus are ingested without vitamin D. Sufficiency from vitamin D increase the synthesis of calcium and phosphorus by 30-40% and 80 % respectively[13].

Most tissues and cells of people have vitamin D (VDR) receptors. 1, 25(OH)2D has a wide variety of biological activities, such as cell proliferation inhibition and differentiation, insulin stimulation, inhibition of angiogenesis, renin inhibition and macrophage cathelicidin production stimulation.

Predominance and prevalence of sunshine vitamin deficiency:

Because 25(OH)D in most cases are less than 0.8 IU, in the last few years, the vitamin D deficit was commonly reported and is recorded in Institute for Medicine (IOM). Popular signs and symptoms of vitamin D deficiency in adults, tiresome and infected muscles, depression, weakened injury healing, weakening of bone, hair loss, muscle pain, etc., are defined as 25(OH)D levels of 21-29ng/ml[14]. Incompliance with Vitamin D levels has been defined in the following terms: 25(OH)D levels. The risk of VDD is similarly high for children, young people and middle-aged adults. Women with pregnancy and infant formula take vitamin D supplements with a high risk of VDD[14].

Synthesis of Vitamin D

The active form of vitamin D has a half-life of approximately 15 hr (calcitriol) (1, 25-dihydroxyvitamin D3), and calcitriol (25-hydroxyvitamin D3) has a half-life of approximately 15 days. Vitamin D is binding to body-wide receptors. 25(OH)D is the type of vitamin D that has been converted into 1, 25-dihydroxyvitamin D by renal or extra renal 1α-hydroxylase (i.e., 1, 25(OH)2D) and is circulating at concentrations much lower than 25(OH) D but far more in line with the VDR[15]. Cell types, including the vascular wall, express 1α-hydroxylase, with the subsequent intracellular conversion of 25(OH)D to 1, 25(OH)2D, exercising its impact at cell and tissue level, which have been shown to regulate the expression of 1α-hydroxylases before being catabolized into biologically inactive calcitriol acid factors such as fibroblast growth factor 23 and Klotho Extrarenal 1, 25(OH)2D macrophage productions are improved as a part of an inborn immune reaction to IB (Lips *et al*, 2006) by Toll-like receptors. The growing output of 1.25(OH)2D from keratinocytes in wounds offering a reasonable indication of the vitamin D status of keratinocytes activity should be viewed as an additional example for the extra renal control of 1α-hydroxylase[16]. Vitamin D crosses blood-brain barrier, as seen in Figure No.2.

Effects of vitamin D deficiency

Vitamin D helps stabilise the muscles, decreases bone fracture risk and preserves the immune system's work to survive any disease type[17]. Vitamin D deficiency affects limbs and many other body regions. Bones that are too frail and not able to bear their weight (rickets) will become developing children who are not getting adequate vitamin D. Adults that have vitamin D deficiency build weakened bones, known as osteomalacia, due to a reduction in bone density, allowing brittle, breaking bones to form. In winter, vitamin D synthesis tends

to be less than in South India during the winter season, where the sun is gloomy / lower in the sky[18].

Diagnosis

Vitamin D deficiency can be diagnosed by the thin medical record[19]:

A read code related to vitamin D deficiency or rickets.

Prescription of vitamin D (calciferol) at a treatment dose of lesser than 0.8 IU

A serum 25-hydroxy vitamin D (25-OH-D) test result <25nmol/L (<10ng/ml) read code.

Clinical Implications

Tests of vitamin D deficiencies in clinicians using the above-mentioned approaches will detect a short-term vitamin D deficiency[20].

Current Methods for Routine Screening of Vitamin D Levels

The lack of vitamin D contributes to skeletal and non-skeletal diseases, such as malignancies and metabolism. The 25-hydroxyvitamin D level determination is a common test used to determine the condition of vitamin D. Esoteric testing is among clinical chemistry's most important tests[21].

Clinical Significance of vitamin D

The function of vitamin D to protect against malignant neoplasms, coronary disease, and diabetes, as well as osteoporosis and other bone diseases, in calcium and phosphor homeostasis. Individual vitamin D values impair skeletal disease development. Vitamin D in human body conditions. CAP competence tests have been used as medical judgement limit for several levels for total vitamin D[22]. Most clinicians agree that vitamin D interacts with parathyroid hormone (PTH) when making treatment choices. To be more than 50ng/mL of toxicity, appropriate quantities of total vitamin D have been redefined by the IOM. The 25-hydroxy vitamin D sum is the clinician's most important type.

Impact of calciferol on allergic conditions

In the organism, vitamin D performs a variety of essential functions. Machine activation against different infections such as tuberculosis, cancer and reaction diseases such as induration, is an example. Also, evidence has reported that the intervention of allergic diseases may be crucially impacted by calciferol[23]. The vitamin D deficiency (calciferol deficiency) is associated with most allergic disorders of almost all forms and with a vitamin D deficitcoordinate, eczemas and grooming hyposensitivity. The empirical data suggests that in regions less exposed to the sun (northern climates), the hypersensitivity response to different causes such as fruit, medical items, or bug stings is even stronger. Low calciferol levels, especially those with defects in their calciferol receptor genes, contribute to asthma, eczema and allergies. The occurrence of respiratory disorders and allergic disorders of young people have been reduced considerably by the supplements of calciferol given to pregnant ladies. Calciferol stimulates binding mechanism cells that resist chemical release and intensify allergic diseases[24]. Calciferol deficiency may also exacerbate symptoms of allergic reactions or cause allergic irritation. The incidence of illnesses and allergic diseases should not be over-simplified because it is difficult to include the genes and environment of each personality.

Interactions with Medications

Supplements of vitamin D can use several treatments; numerous precautions have been taken every day with patients to take these drugs[25].

Steroids

Corticosteroid medicines, such as Deltasone, are typically administered to avoid inflammation that inhibits metal absorption and affects the metabolism of vitamin D. These results are associated with the reduction of bone mass that leads to complications associated with pathology in long-term use[26].

Other medications

The orlistat drug, which drops the weight (Xenical and Alli TM) and cholesterol-reducing drug, is used to slash the synthesis of vitamin D and alternate fat-soluble vitamins. (Questran, Lo Cholesterol and Prevalite TM). The treatment of epileptic seizures is supported by all of the sodium thiopental and diphenylhydantoin (Dilantin TM); the viscus of vitamin D is reduced to inactive compounds; and the metal is absorbed less[27].

Vitamin D is a steroid hormone which needs several metabolic steps in the human body to be activated. Double vitamin D types i.e., 1. In low-level diets, biological tissue is usable in complements and

multivitamins. 1, 25-dihydroxy vitamin D and 25-hydroxy Vitamin D 2. 25-hydroxy vitamin D is developed in the rentals, and has been converted endogenously and exogenously to the biologically active form[28]. Several substances that can exist in detectable concentrations in tissue are catabolized. Vitamin D metabolism in inactive forms is mainly caused by oxidation reactions in compounds with a medicinal value-focused solely on 25-hydroxy vitamin D and 1, 25-dihydroxy vitamin D and IOM and other organisations, as well as researchers who have the highest significance of the 25-hydroxy vitamin D complete. In infants younger than 1 year and adult plasma tests, 25-Hydroxy Vitamin D3 epimer (C-3 epimer). This vitamin D can be calculated using the latest method of identification of the vitamin D deficit, by LC-MS / MS)[29].

METHODS OF VITAMIN D DIAGNOSIS AND MEASUREMENT

VitaminD is calculated using competition-based linking techniques, HPLC and radioimmunoassay (RIA) procedures. A widely used RIA package developed for comparison labs by Día Sorin (Saluggia) is considered to be the best quality. It is used for the determination of diagnostic reference ranges[30]. The Día Sorin 25-hydroxy vitamin D test is a two-stage system involving quick 25-hydroxy-vitamin D extraction and other hydroxylated serum or plasma metabolites, followed by a competitive RIA operation, which uses a 25-hydroxy vitamin D specificity antibody. LC-MS/MS, which can measure vit D2, D3, and D3 epimer have become the sources tool for the study of vitamin D. This approach can be used to separate different types of vitamin D in plasma present in people of various ages and races[31]. 25-hydroxy vitamin D2 and D3 quantitative approaches for HPLC[32]. The kits now available are primarily for the standardisation of test efficiency and the cost-effectiveness and less labour-intensive testing (Tokyo, Japan and Thermos Fisher Science, Sunnyvale, CA). The Hitachi approach uses a reverse phase and diode array detection, which allows simultaneous highly sensitive analysis with maximum wavelengths feasible. This technique is ideal for the study of samples of food and biologics[33].

New methods for chromatography to increase sensitivity and also to test vitamin D have been developed. The system of LC-MS / MS is optimised for simultaneous study of all vitamin D types and metabolites including D2, D3 and serum 25-hydroxy vitamin D. The method is based on an ionisation detector technique known as APPI, which offers enhanced study sensitivity and doesn't require prefabrication measures[34].

The LC-MS approach helps vitamin D2 and vitamin D3 to be isolated and quantified. Some immunoassays measure only one form based on the sensitivity of the anti-method used, whereas others measure all forms similarly (Di Sorin RIA) with varying cross-reactivity tests[35].

Immuno-assay methods

United States Food and Drug Administration (FDA) approved immunoassay methods as well as quantitative chemiluminescent immunoassay (CLIA) methods. DiaSorin, the manufacturer of the commonly used RIA method like CLIA method for its LIAISON platform[36]. This method was developed in year time 2002, to measure total 25-hydroxy vitamin D and other hydroxylated vitamin D metabolites in human serum[37]. The steps used are as follows:

25-hydroxy vitamin D dissociates vitamin D-isoluminol from its binding protein and that binds to the specific firm stage antibody by the addition of tracer along with unbound material is removed[38].

To initiate the chemiluminescent reaction is supplementary the vitamin D-isoluminol tracer. Beam signal formed be alive detected by a photomultiplier as a qualified light unit be used for measurement and inversely proportional to the concentration of 25-hydroxy vitamin D. The Día Sorin LIAISON method used for measuring total vitamin D level is more effective[39].

Abbott Laboratories (Abbott Park, I.L) offers a fully automated immunoassay for 25-hydroxy vitamin D on originator platform. It is a 1-step delayed chemiluminescent microparticle immunoassay (CMIA) with an automated online pre-treatment step designed for vitamin D following routine laboratory

testing. This method received FDA approval in 2011[40].

Immuno Diagnostics Inc. (Woburn, MA) offers an automated CMIA immunoassay technique, (IDS–iSYS) for the quantitative determination of total 25-hydroxyvitamin D moreover other hydroxylated metabolites in human serum or plasma. It reports equal specificity for 25-hydroxy vitamin D_3 also D_2 has kindliness of 5.5ng per mL[41].

The patented Electro Chemiluminescence (ECL) method by F. Hoffman-La Roche AG (Basel, Switzerland) for the raised area within Cobas offers 25-hydroxy vitamin D assay. The test is used on all the Roche Cobas modular experimenter platforms. It received FDA clearance in July 2012[42].

Enzyme immunoassay methods are also available. Diazene Laboratories (Poway, CA) offers a method that uses a homogenous enzyme-coupled vitamin D binding protein to measure total levels of 25-OH vitamin D (i.e., the sum of D_3 and D_2). The vitamin D binding protein recognizes vitamin D_2 in addition to D_3 equally also recognizes the total level of 25-hydroxy vitamin[43].

There are many published results of comparison studies between RIA methods and HPLC and among other immunoassay methods and HPLC; one more recent comparison shows an enhanced understanding of affiliation between immunoassay methods furthermore LC-MS/MS. Vitamin D assay method provides similar absolute values, assay linearity plus assay precision. However, the only analyse that quantitatively perceive total vitamin levels by HPLC, LC-MS and Dia Sorin assay[44].

Specificity is significant for the use of immunoassay methods in particular through admiration to the proportion of 25-hydroxy vitamin D so as towards is quantified[45]. Chromatography methods are more specific but are less convenient to carry out, due to multiple processing steps. LC-MS/ MS and HPLC are expensive require expertise to operate. Semi-automated or automated HPLC and LC-MS/MS procedures are also life form residential. For most LC-MS/MS also HPLC methods, losses are corrected by the inclusion of internal standard may account for a positive bias in results compared with immunoassays[46].

There appears to be a positive bias in results using the LC-MS/MS methods and a slight negative bias with the Dia Sorin LIASON method in the patients classified as vitamin D inadequate or deficient by the Dia Sorin LIAISON method compared with an LC-MS/MS method[47].

In LC-MS/MS method showed changeability to contrast with routine immunoassay. The RIA defers like grades to individuals as of the LC-MS/MS method through a minor activist prejudice. Every one immunoassay deliberates entire 25-hydroxy vitamin D stage apart from used for the Roche examine which process simply vitamin D_3 rank. Mean positive bias was highest with the Abbott Laboratories method[48].

The results from the Abbot ARCHITECT, Siemens ADVIA Centaur immunoassay methods for 25-hydroxy vitamin D levels in addition to LC-MS/MS were studied, both immunoassays' results show the positive bias[49].

Table No.1: Different levels of vitamin-D deficiency in the blood (serum)

S.No	Levels of Vitamin-D Deficiency	Serum Level
1	At the risk of vitamin D deficiency	Serum 25OHD less than 30nmol/L (12ng/mL).
2	At the risk of vitamin D inadequacy	Serum 25OHD 30-49nmol/L (12-19ng/mL).
3	Sufficient in vitamin D	Serum 25OHD 50-125nmol/L (20-50ng/mL).
4	Possibly harmful vitamin D	Serum 25OHD greater than 125nmol/L (50ng/mL).

Ref[51].

Table No.2: Dietary recommendations of vitamin D based on the age group of incidences

S.No	Recommendations as per Institute/ Organisations	Age Group <18yrs RDA	Age Group 19 - 70yrs RDA	Age Group >70 yrs. RDA
1	Institute of Medicine (IOM)	600 IU/day	600 IU/day	800 IU/day
2	Endocrine Society (EU)	600-1,000 IU/day	1,500-2,000 IU/day	1,500-2,000 IU/day

Ref[52].

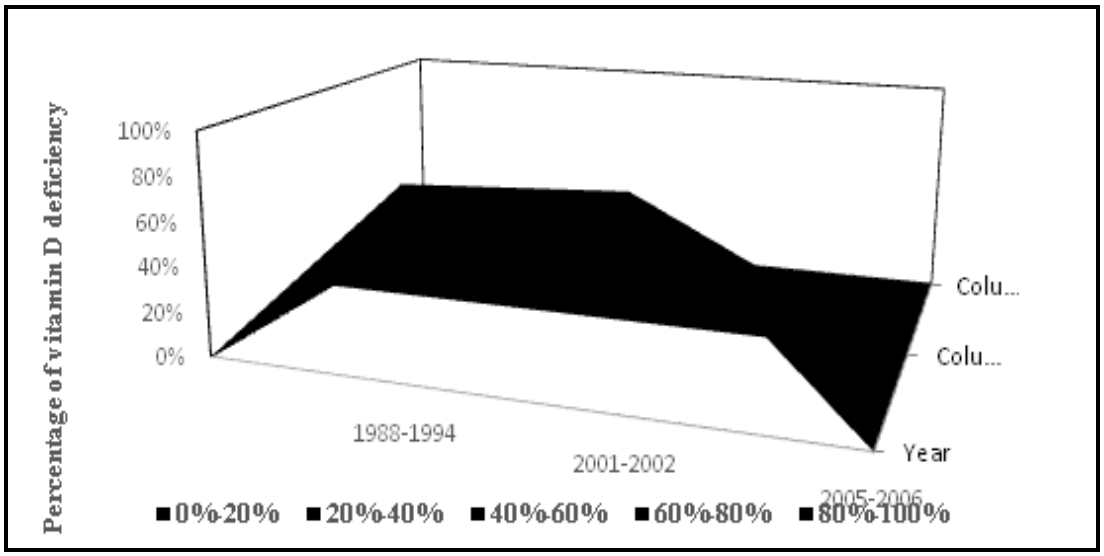

Figure No.1: Percentage of Vitamin -D deficiency during the period 1988-2006

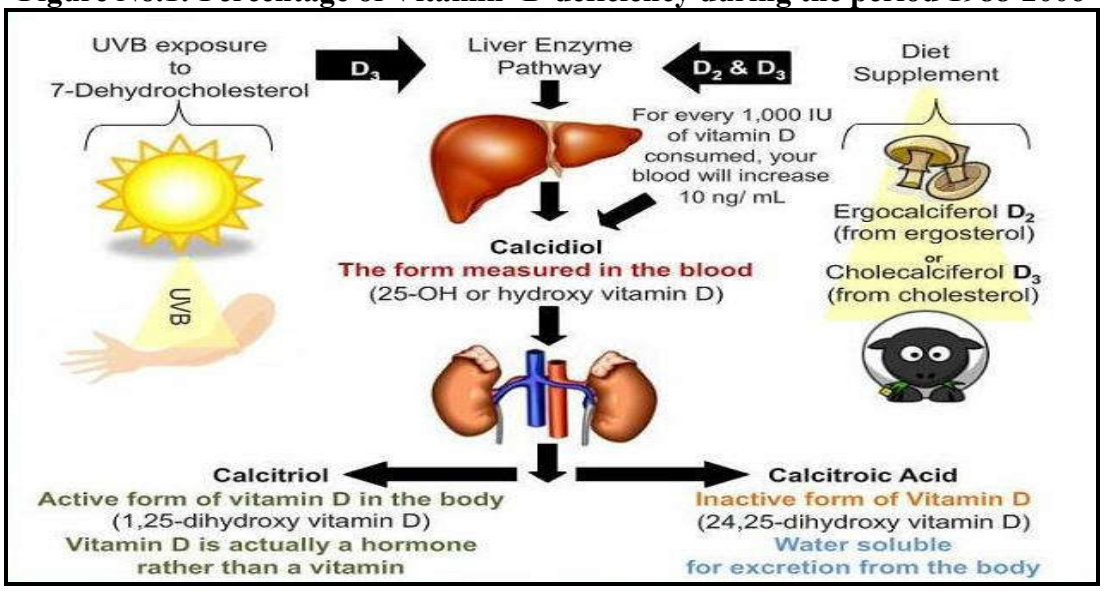

Figure No.2: Synthesis of Vitamin D in human body

CONCLUSION

There is a marked increase in the testing and diagnosis of vitamin D deficiency of children in India over the long-ago decades. Older age, non-white ethnicity as well as social deprivation were associated with higher rates of diagnosis[50]. Automated immunoassays are available for total vitamin D levels and have improved in precision and accuracy. The Dia Sorin LIAISON and Abbott ARCHITECT methods are performed with accuracy. The IDS I-SYS method provides superior accuracy. A rising number of patients are life form counsel to obtain vitamin D complement or smooth to be given upper prescribed amount of vitamin D from end-to-end curative action to lessen their risk for skeletal and non-skeletal illness such as cardiovascular disease or malignant neoplasms. IOM2010 recommend daily allowances of 600 IU/day for most patients established toxicity may be reached at lower circulating levels of vitamin D. Accurate test methods of diagnosis are imperative to differentiate among the inadequate intensity of under treatment and toxic levels of overtreatment.

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AUTHOR CONTRIBUTION STATEMENT

The authors of this research article have contributed sufficiently to the conception and design of this work, as well as the data analysis (where relevant) and text writing. Except as detailed in an attachment, neither the paper nor another with substantially identical content under my authorship has been published or is being considered for publication elsewhere.

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We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Robinson P D, *et al*. The re-emerging burden of rickets: A decade of experience from Sydney, *Arch. Dis. Child,* 91(7), 2006, 564-568.
2. Callaghan A L, Moy R J D, Booth I W, Debelle G, Shaw N J. Incidence of symptomatic vitamin D deficiency, *Arch. Dis. Child,* 91(7), 2006, 606-607.
3. Ferrari R and Prosser C. Testing Vitamin D levels and choosing wisely, *JAMA Internal Medicine,* 176(7), 2016, 1019-1020.
4. Chuanyi M L. Pathology consultation on vitamin D testing: Clinical indications for 25(OH) vitamin D measurement, *American Jour of Clin Patho,* 137(5), 2012, 831-833.
5. Absoud M, Cummins C, Lim M J, Wassmer E, Shaw N. Prevalence and predictors of vitamin D insufficiency in children: A great britain population based study, *PLoS One,* 6(7), 2011, e22179.
6. Munns C F *et al*. Global consensus recommendations on prevention and management of nutritional rickets, *Horm. Res. Paediatr,* 85(2), 2016, 83-106.
7. Schleicher R L *et al*. The Vitamin D status of the US population from 1988 to 2010 using standardized serum concentrations of 25-hydroxy vitamin D shows recent modest increases, *Am. J. Clin. Nut,* 104(2), 2016, 454-461.
8. Kennel K A, Drake M T. Vitamin D deficiency in adults: When to test and how to treat, *Mayo Clinic Proceedings,* 85(8), 2010, 752-757.
9. Reid I R. What diseases are causally linked to Vitamin D deficiency? *Archives of Disease in Childhood,* 101(2), 2016, 185-189.

10. Holick M F. Vitamin D status: Measurement, interpretation and clinical application, *Annals of Epidemiology,* 19(2), 2009, 73-78.
11. Terry A H, Sandrock T, Meikle A W, Measurement of 25-hydroxy vitamin D by the nichols advantage, Dia Sorin liaison, Dia Sorin RIA and liquid chromatography-tandem mass spectrometry, *Clinical Chemistry,* 51(8), 2005, 1565-1566.
12. Harvey N C and Cooper C. Vitamin D: some perspective please, Health claims are ahead of the evidence, *Br. Med. J,* 345, 2012, e4695.
13. Atkins D *et al*. Grading quality of evidence and strength of recommendations, *BMJ,* 328(7454), 2004, 1490.
14. Ganji V, Zhang X, Tangpricha V. Serum 25-Hydroxy vitamin D Concentrations and prevalence estimates of hypovitaminosis D in the U.S. population based on assay-adjusted data, *J. Nutr,* 142(3), 2012, 498-507.
15. Schwartz J B *et al*. A comparison of measured and calculated free 25(OH) vitamin D levels in clinical populations, *J. Clin. Endocrinol. Metab,* 99(5), 2014, 1631-1637.
16. Bilinski K, Boyages J. Association between 25-hydroxyvitamin D concentration and breast cancer risk in an Australian population: An observational case-control study, *Breast Cancer Res. Treat,* 137(2), 2013, 599-607.
17. Need A G, Horowitz M, Morris H A, Nordin B E C. Vitamin D status: Effects on parathyroid hormone and 1, 25-dihydroxyvitamin D in postmenopausal women, *Am. J. Clin. Nutr,* 71(6), 2000, 1577-1581.
18. Horsfall L, Walters K, Petersen I. Identifying periods of acceptable computer usage in primary care research databases, *Pharmacoepidemiol. Drug Saf,* 22(1), 2013, 64-69.
19. Dave S, Petersen I. Creating medical and drug code lists to identify cases in primary care databases, *Pharmacoepidemiol. Drug Saf,* 18(8), 2009, 704-707.
20. Bailey R L *et al*. Estimation of Total usual calcium and Vitamin D intakes in the United States, *J. Nutr,* 140(4), 2010, 817-822.
21. Holick M F *et al*. Evaluation, treatment, and prevention of vitamin D deficiency: An endocrine society clinical practice guideline, *Journal of Clinical Endocrinology and Metabolism,* 96(7), 2011, 1911-1930.
22. Chen H, McCoy L F, Schleicher R L, Pfeiffer C M. Measurement of 25-hydroxyvitamin D3 (25OHD3) and 25-hydroxyvitamin D2 (25OHD2) in human serum using liquid chromatography-tandem mass spectrometry and its comparison to a radioimmunoassay method, *Cli. Chi. Acta,* 391(1-2), 2008, 6-12.
23. Maeda S S, Kunii I S, Hayashi L, M. Lazaretti-Castro. The effect of sun exposure on 25-hydroxyvitamin D concentrations in young healthy subjects living in the city of Sao Paulo, Brazil, *Brazilian Journal of Medical and Biological Research,* 40(12), 2007, 1653-1659.
24. Krasowski M D. Pathology consultation on vitamin D testing, *Am. J. Clin. Pathol,* 136(4), 2011, 507-514.
25. Tolppanen A M, Fraser A, Fraser W D, Lawlor D A. Risk factors for variation in 25-hydroxyvitamin D 3and D 2 concentrations and vitamin D deficiency in children, *J. Clin. Endocrinol. Metab,* 97(4), 2012, 1202-1210.
26. Boyages S, Bilinski K. The vitamin D paradox: Bone density testing in females aged 45 to 74 did not increase over a ten-year period despite a marked increase in testing for vitamin D, *J. Endocrinol. Invest,* 36(11), 2013, 914-922.
27. Thacher T D *et al*. Increasing incidence of nutritional rickets: A population-based study in olmsted county, minnesota, *Mayo Clin. Proc,* 88(2), 2013, 176-183.
28. Hollis B W. Assessment and interpretation of circulating 25-hydroxyvitamin D and 1, 25-dihydroxyvitamin D in the clinical environment, *Endocrinology and Metabolism Clinics of North America,* 39(2), 2010, 271-286.

29. Lensmeyer G, Poquette M, Wiebe D, Binkley N. The C-3 epimer of 25-hydroxyvitamin D 3 is present in adult serum, *J. Clin. Endocrinol. Metab,* 97(1), 2012, 163-168.
30. Skripnikova I A and Sorokin M Y. Diagnosis, treatment and prevention of vitamin D deficiency (discussion based on the clinical recommendations of the endocrine society 2011), *Osteoporos. Bone Dis,* 15(1), 2012, 34-37.
31. Karras S N *et al*. An observational study reveals that neonatal vitamin D is primarily determined by maternal contributions: Implications of a new assay on the roles of vitamin D forms, *Nutr. J,* 2013, 1-8.
32. Wagner C L, Greer F R. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents, *Pediatrics,* 122(5), 2008, 1142-1152.
33. Van Den Broek I, Mastali M, Tolan N, Daniels S, Van Eyk J, Sobhani K. Evaluation of the first FDA-cleared LC-MS/MS assay for quantification of 25-hydroxyvitamin D2/D3 and C3-epimers on the sciex topaz system, *Clin. Chem,* 2018.
34. Herrmann M, Farrell C J L, Pusceddu I, Fabregat-Cabello N, Cavalier E. Assessment of Vitamin D status - A changing landscape, *Clinical Chemistry and Laboratory Medicine,* 55(1), 2017, 3-26.
35. Lee Y W. Analysis of 25-hydroxyvitamin D2 and 25-hydroxy vitamin D3 in serum using ultra-performance liquid chromatography with tandem mass spectrometry, *Life Sci. J,* 2013.
36. Carter G D *et al*. Proficiency testing of 25-Hydroxy vitamin D (25-OHD) assays, *J. Steroid Biochem. Mol. Biol,* 121(1-2), 2010, 176-179.
37. Saraiva G L *et al*. Prevalence of vitamin D deficiency, insufficiency and secondary hyperparathyroidism in the elderly inpatients and living in the community of the city of Sao Paulo, Brazil, *Arq. Bras. Endocrinol. Metabol,* 51(3), 2007, 437-442.
38. Ladhani S, Srinivasan L, Buchanan C, Allgrove J. Presentation of vitamin D deficiency, *Archives of Disease in Childhood,* 89(8), 2004, 781-784.
39. Yu S *et al*. Is it necessary for all samples to quantify 25OHD 2 and 25OHD 3 using LC-MS/MS in clinical practice? *Clin. Chem. Lab. Med,* 56(2), 2018, 273-277.
40. Lips P *et al*. The prevalence of vitamin D inadequacy amongst women with osteoporosis: An international epidemiological investigation, *J. Intern. Med,* 260(3), 2006, 245-254.
41. Singh R J, Taylor R L, Reddy G S, Grebe S K G. C-3 epimers can account for a significant proportion of total circulating 25-hydroxy vitamin D in infants, complicating accurate measurement and interpretation of vitamin D status, *J. Clin. Endocrinol. Metab,* 91(8), 2006, 3055-3061.
42. Moy R J, McGee E, Debelle G D, Mather I. Successful public health action to reduce the incidence of symptomatic vitamin D deficiency, *Arch. Dis. Child,* 97(11), 2012, 952-954.
43. Farrell C J L, Martin S, McWhinney B, Straub I, Williams P, Herrmann M. State-of-the-art vitamin D assays: A comparison of automated immunoassays with liquid chromatography-tandem mass spectrometry methods, *Clin. Chem,* 58(3), 2012, 531-542.
44. Holmes E W, Garbincius J, McKenna K M. Analytical variability among methods for the measurement of 25-hydroxyvitamin D: Still adding to the noise, *Am. J. Clin. Pathol,* 140(4), 2013, 550-560.
45. Theodoratou E, Tzoulaki I, Zgaga L, Ioannidis J P A. Vitamin D and multiple health outcomes: Umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials, *BMJ,* 348, 2014, 2035.
46. Lewis J D, Bilker W B, Weinstein R B. The relationship between time since registration and measured incidence rates in the General Practice Research Database, *Pharma. Drug Saf,* 14(7), 2005, 443-451.

47. Abdel-Khalik J, Crick P J, Carter G D, Makin H L, Wang Y, Griffiths W J. Studies on the analysis of 25-hydroxyvitamin D3 by isotope-dilution liquid chromatography-tandem mass spectrometry using enzyme-assisted derivatisation, *Biochem. Biophys. Res. Commun,* 446(3), 2014, 745-750.
48. Allgrove J, Shaw N J. A Practical approach to vitamin D deficiency and rickets, *Endocr. Dev,* 28, 2015, 119-133.
49. Misra M, Pacaud D, Petryk A, Collett-Solberg P F, Kappy M. Vitamin D deficiency in children and its management: Review of current knowledge and recommendations, *Pediatrics,* 122(2), 2008, 398-417.
50. Shaw N J, Mughal M Z. Vitamin D and child health: Part 2 (extraskeletal and other aspects), *Arch. Dis. Child,* 98(5), 2013, 368-372.
51. Wallace A M, Gibson S, De La Hunty A, Lamberg-Allardt C, Ashwell M. Measurement of 25-hydroxy vitamin D in the clinical laboratory: Current procedures, performance characteristics and limitations, *Steroids,* 75(7), 2010, 477-488.
52. Reid I R. What diseases are causally linked to Vitamin D deficiency? *Arch. Dis. Child,* 101(2), 2016, 185-189.

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