Innovations

Link Between Vitamin D and Type 2 Diabetes Mellitus in People of Central Kerala– A Cross Sectional Study

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Abstract **:** *Introduction - Apart from its role in calcium metabolism and bone health,vitamin D has many effects.Due to low sunlight exposure ,there is a high prevalence of vitamin D deficiency in India. Identification of the vitamin D receptor (VDR) in almost all human cells, suggests a role in extra-skeletal diseases like type 2 DM,hypertension. Studies have shown that vitamin D deficiency is an independent risk factor for glycaemic control. Aim – To evaluate association between vitamin D and glycaemic control in people coming for health check up to a tertiary care centre in South India. Materials and Methods - Study was carried out as a cross sectional study in a tertiary care centre in South India for a period of 6 months. Participants (520) were both males and females (337 males and 183 females),between the age group of 20-60 years attending health check up clinic of our hospital.Statistical analysis-Statistical analysis was done using IBM SPSS statistics 20.0.Results - Severe vitamin D deficiency was highly prevalent in people with Diabetes Mellitus(DM) than in people without DM(p value- <0.05).Conclusion – Vitamin D deficiency and its complications were uncommon in India till recently, since India is a tropical country with adequate sun exposure. But vitamin D deficiency was highly prevalent in people with DM, emphasizing the need of early vitamin D supplementation. Therefore, to reduce skeletal and extra skeletal complications, vitamin D deficiency should be identified at the earliest and appropriate action to be taken in our population,having high prevalence.*

Keywords:Vitamin D, Diabetes Mellitus, vitamin D receptor,calcium, disease*

Introduction

Vitamin D plays an important role in the normal functioning of human body.Deficiency of Vitamin D is highly prevalent all over the universe Many of the extra skeletal diseases like type 2 Diabetes Mellitus (DM),Cardio vascular disease,auto immune disease as well as cancer are associated with vitamin D deficiency.Creating awareness among the physicians and public is important to prevent the deadly consequences in future. Indian diet does not satisfy the daily needs of vitamin D.Here,comes the need of fortifying foods with vitamin D through various National programs.Vitamin D is also used to treat Osteoporosis[4,5,6]. Vitamin D regulates metabolism of the bone, by physiologic calcium reabsorption in the gut and thus providing necessary calcium for bone mineralization[7]. Vitamin D receptors (VDRs) are present in almost all the human cells[8]. Several studies have shown association of vitamin D deficiency with a variety of chronic extra-skeletal diseases like DM,Hypertension etc[9,10,11,12,13,14,15]. There is a strong association of Vitamin D deficiency with increased risk of type 2 diabetes mellitus, insulin resistance, and decreased insulin production. In this context, aim of our study was to find an association between vitamin D and DM in people coming for health check up to a tertiary centre in Kochi – Central Kerala, since studies from Central part of Kerala were limited.

Physiology of vitamin D:

Vitamin D was discovered by Mc Collum in 1922.It was termed as "D", because it was the fourth vitamin known[16].Chief source of vitamin D is endogenous vitamin D synthesis in the skin[17].To induce the conversion of the precursor 7-dehydrocholesterol to vitamin D, ultraviolet-B spectrum of light is required[1,8]. Approximately 80 to 90% of vitamin D is derived from sunlight, whereas only minor amounts of vitamin D is from dietary sources[18].Fish oil, egg yolk and mushrooms are rich in vitamin D. In countries like United States and Finland, vitamin D fortified milk and orange juice are available. To exert its biological effects, vitamin D has to be converted to its most active form, 1,25-dihydroxyvitamin D(calcitriol)[8]. This involves two hydroxylation steps. First one occurs in the liver, where vitamin D is converted by the enzyme 25-hydroxylase to 25-hydroxyvitamin D(calcidiol). Then, 25(OH)D is transported in the bloodstream to the kidneys by its carrier - vitamin D binding protein (DBP). Proximal convoluted tubule cells of the kidneys express the enzyme 1-alpha-hydroxylase, which catalyzes the conversion of 25(OH)D to $1,25(OH)_2D$. Serum concentrations of $1,25(OH)_2D$ are thus mainly determined by production of $1,25(OH)_2D$ by the kidneys. This is controlled by parameters of mineral metabolism, which help to maintain calcium and phosphorus levels within physiological ranges[1,8]. Parathormone (PTH) increases 1-alpha hydroxylation, whereas fibroblast growth factor-23 (FGF-23) or high phosphate levels, decrease 1-alpha hydroxylation in the kidneys. It was believed that only the kidneys are able to synthesize significant amounts of 1,25$(OH)_2$D[8,19].Now,it was found that many extra-renal tissues are also able to synthesize 1,25$(OH)_2$D on a local and intracellular level[19]. Regulation of extra-renal 1-alpha-hydroxylase is different compared to the kidneys, and is significantly dependent on the availability of 25(OH)D[19]. Based on this, it can be assumed that local tissue levels of 1,25$(OH)_2$D are determined by concentrations of circulating 25(OH)D levels[19]. This is why serum levels of 25(OH)D and not of 1,25$(OH)_2$D are measured to assess and classify vitamin D status. Serum levels of 25(OH)D are up to 1000 fold higher compared to 1,25$(OH)_2$D. Affinity to the VDR is much higher for 1,25$(OH)_2$D, which is thus often called the active vitamin D. On a molecular level, 1,25$(OH)_2$D binds to intracellular VDR, forming a heterodimer with the retinoid X receptor (RXR) and the complex thus formed, translocates to the nucleus[8]. VDR/RXR interacts with several co-regulatory proteins and binds to vitamin D responsive elements. This is followed by either up or downregulation of gene expression, and in this way VDR activation leads to the regulation of approximately three per cent of the human genome, including a variety of genes that are also involved in blood pressure regulation, cerebrovascular and neurological functions[20,21]. 24-hydroxlation initiates degradation of vitamin D, resulting in the production of vitamin D metabolites (*e.g.* 1,24,25$(OH)_3$D or 24,25$(OH)_2$D),which are subsequently converted to calcitroic acid, and is excreted in urine[8].

Association between Vitamin D and DM

Vitamin D receptors are present in almost all the body cells. This has deviated attention to chronic conditions apart from bone diseases. Clinical trials were performed extensively at different levels of diabetes mellitus stages to observe the role of Vitamin D status and to study efficacy of Vitamin D supplementation. Maintaining Vitamin D at adequate levels can be a useful preventive measure, since Vitamin D status in healthy adults was inversely associated with future risk of type 2 DM[22]. Significant negative correlation between 25(OH)D and HbA1c was also observed when compared between diabetic and non diabetic patients[23]. Vitamin D dose in initial years of life is shown to reduce risk of future development of disease modulated by immune protective effects[24]. A meta analysis by Forouhi et al[25] found a strong inverse association between baseline 25(OH)D and incidence of type 2 diabetes. A significant inverse relationship between Vitamin D status and insulin resistance (IR) was also observed, independent of adiposity, in Korean adolescents[26]. A meta-analysis with around 23 studies, found that serum 25(OH)D was significantly lower in patients with type 1 DM than in healthy controls [27,28].

Aim

To evaluate the association between vitamin D and DM in people coming for health check up to a tertiary care centre in Kochi, Central Kerala, since studies from Central Kerala were limited.

Materials and Methods

Study was done as a cross sectional study in a tertiary care centre in Kochi, Central Kerala for a period of 6 months. Participants (520) were both males and females (337 males and 183 females),between the age group of 20-60 years attending the Comprehensive health check up clinic of our hospital.Each subject was interviewed and a standardized questionnaire was computed, containing information on individual characteristics, demographics, anthropometric profile, major risk factors for cardiovascular disease, past medical history, details of sun exposure(type of job and average time of sun exposure in a day) and biochemical parameters.FBS was measured using enzymatic method and the cut off point of 126 mg/dl was considered as diagnostic criterion for the diabetes[29]. PPBS was measured by enzymatic method and cut off point of 140 mg/dl was considered as diagnostic criterion for the diabetes[29]. In order to measure HbA1c, cut off point of 6.5%, based on the Diabetes Control and Complications Trial[30,31] was considered as diagnostic criterion for diabetes. All participants gave their written informed consent to participate in the study, that was approved by the Institutional Ethics Committee

Inclusion criteria: Age group - 20 to 60 years
Both sex

Exclusion criteria:

Age group - below 20 years and above 60 years
People with history of smoking, alcoholism, thyroid disorders, cardiovascular disease, cerebrovascular disease, chronic renal, hepatic and gastro-intestinal disease, skeletal disease, endocrine diseases except DM, acute critical illness, pregnancy, lactation
People on calcium or vitamin D supplementation

Biochemical analysis:

Peripheral venous blood samples (4 ml) were collected from all the participants after an overnight fast of 12-14 hours. Serum was separated by centrifuging at 3000 rpm for 5 minutes. Blood glucose was estimated by hexokinase method on Olympus AU2700 analyser. HbA1c levels were assayed on Bio-Rad D10 system by high performance liquid chromatography-based ion exchange chromatography as per the National Glycohemoglobin Standardization Program standardised to the Diabetes Control and Complications Trial[30,31]. Blood Urea was estimated by enzymatic urease method and serum creatinine by Jaffe's method. Serum calcium was estimated by Arsenazo III method. Liver function was estimated by colorimetry method on Olympus AU2700 analyzer. HDL, LDL, VLDL and triglycerides were estimated by enzymatic method on Olympus AU2700 analyzer.

Vitamin D estimation:

For assessing vitamin D levels, peripheral venous blood samples (2 ml) were collected from all the participants. Serum was separated by centrifuging at 3000 rpm for 5 minutes. Vitamin D remains stable up to 72 hours in room temperature and up to 10 years if stored in – 20 degrees[32] .The minimal detectable limit of vitamin D assay is 3ng /ml. Participants were classified as Vitamin D deficient, insufficient and sufficient on the basis of vitamin D concentration of <20 ng/ml, 20-30 ng/ml and >30 ng/ml respectively according to recent consensus[33,34] . Vitamin D deficiency can be classified as severe (<10 ng/ml) and mild-moderate deficiency (10-20 ng/ml) depending on the vitamin D levels[33] . The quantitative estimation of 25-OHD$_3$ is done using ARCHITECT 25-OH assay, which is a Chemiluminescent Microparticle Immuno Assay (CMIA). The estimated vitamin D is a sum total of both vitamin D$_2$ & vitamin D$_3$.

Biological principles in the procedure:

The ARCHITECT 25-OH is a delayed one step immunoassay.Using CMIA technology, with flexible assay protocols, referred to as Chemiflex, a sample pre treatment was done for the quantitative estimation of vitamin D in human serum. Sample and pretreatment reagents were combined. A reaction mixture was created with an aliquot of sample and pretreated reagent and combined with assay diluents and paramagnetic anti-vitamin D coated micro particles. Vitamin D present in the sample binds to anti-vitamin D coated microparticles. After incubation, a biotinylated vitamin D and anti-biotin acridinium-labelled conjugate complex is added to the reaction mixture and binds to uncoupled binding sites of anti-vitamin D microparticles. After washing, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). An indirect relationship exists between the amount of vitamin D in the sample and the RLUs detected by

ARCHITECT i System optics. The reagents used include microparticles, conjugate, assay diluents, pre-treatment 1, pretreatment 2 , ARCHITECT *i* pre-trigger solution, ARCHITECT i trigger solution and ARCHITECT *i* wash buffer. The measuring interval of this method is from 8 ng/ml (20 nmol/L) to 160 ng/ml (400 nmol/L). This method has high sensitivity and specificity, and the potential interference from hemoglobin, bilirubin, triglycerides, protein, rheumatoid factor and red blood cells is designed to be less than 10%.

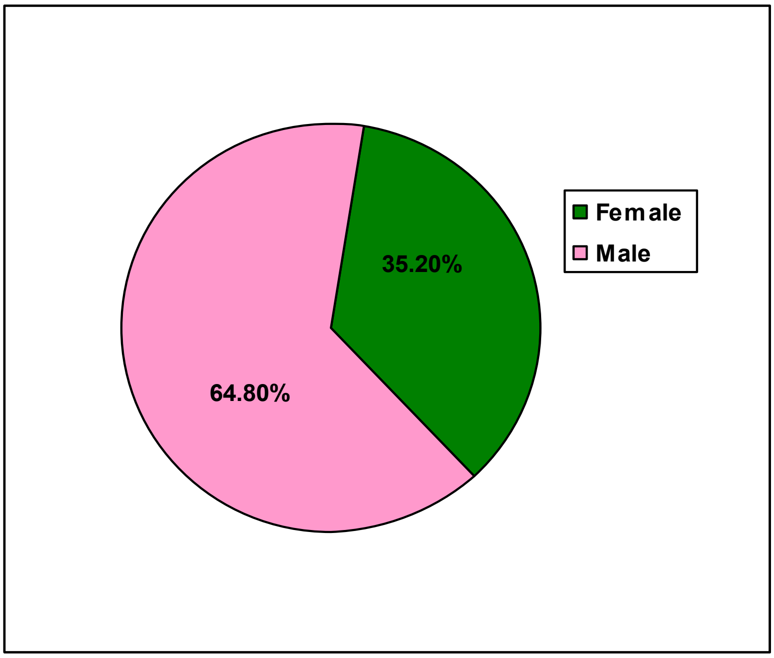
Statistical analysis

Statistical Analysis was done using IBM SPSS Statistics 20.For all the categorical variables, the results were given as %.Pearson's chi - square test was applied to test the relationship between two categorical variables. P - value of < 0.05 were considered as statistically significant.

Results

520 subjects participated in the study.Among 520 participants ,there were 337 (64.8%) males and 183(35.2%) females (Figure 1). 31.54% of the participants were below the age of 40 years and 68.46% were above the age of 40 years(Graph1).45.92±9.77 was the average age of the participants.Out of 520 participants,68 had Diabetes Mellitus(Table 1).Among diabetics,54 were males,14 were females(Table 2).Among diabetics ,89.7% were above 40 years and 10.3% were below 40 years(Table 3).Among 520 participants ,78.85 % were Vitamin D deficient,8.08% had insufficient levels and 13.07 % had sufficient levels of Vit D(Table 4). Among diabetics,83.82% had severe Vitamin D deficiency,4.42% mild moderate vitamin D deficiency and 2.94% had Vitamin D insufficiency and 8.82% had sufficient levels of vit D(Table 5). Non diabetics showed Vit D status of normal in 13.72%,insufficiency in 8.85 % ,Mild moderate Vit D deficiency in 10.17% and severe deficiency in 67.26%(Table 6).Severe vitamin D deficiency was more prevalent in Diabetics (Table 5 and Table 7)(p value <0.05)

Figure



Graph 1: Age distribution of Participants

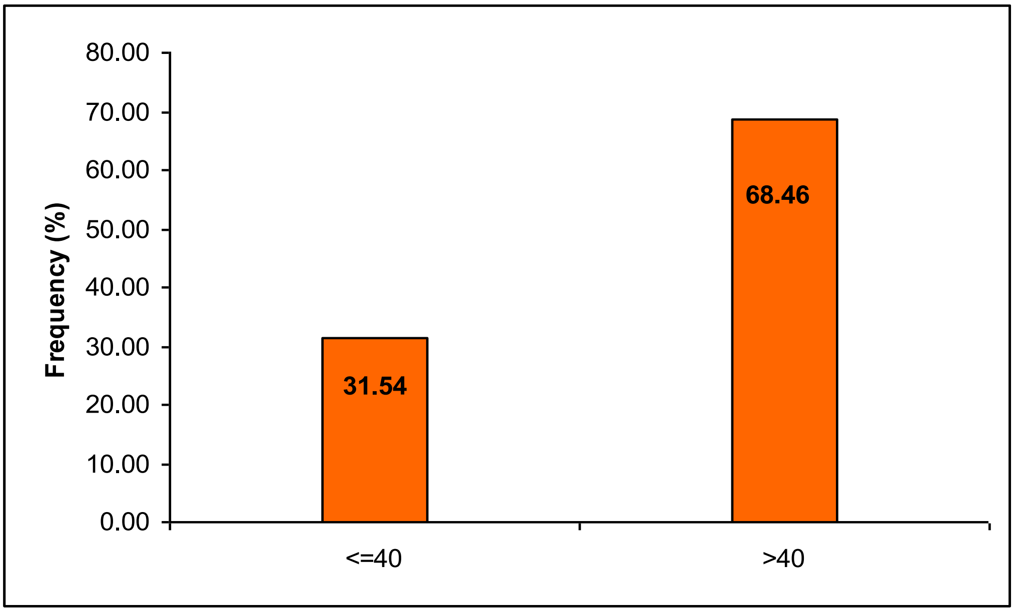


Table 1 : Prevalence of Diabetes Mellitus

DM	Frequency	Percent
Yes	68	13.1
No	452	86.9

Table 2: Distribution of Males and Females among Diabetics

Gender	Frequency	Percent
Male	54	79.4
Female	14	20.6

Table 3: Distribution of Age category among Diabetics

Age	Frequency	Percent
<=40 years	7	10.3
>40 years	61	89.7

Table 4: Prevalence of vitamin D Deficiency, Insufficiency and Sufficiency

Vitamin D status	Frequency	Percent
Vitamin D deficiency	410	78.85
Vitamin D Insufficiency	42	8.08
Vitamin D Sufficiency	68	13.07

Table 5: Prevalence of Severe, Mild moderate vitamin D deficiency, vitamin D insuffiency and vitamin D sufficiency among Diabetics

Vitamin D status	Frequency	Percent
Severe deficiency	57	83.82
Mild Moderate deficiency	3	4.42
Vit D Insufficiency	2	2.94
Vit D Sufficiency	6	8.82

Table 6: Comparison of vitamin D status among Diabetics and non Diabetics

VITAMIND D status	Diabetes Mellitus(DM)		P value
	DM (-) n (%)	DM(+) n (%)	
Severe deficiency	304	57	< 0.05
	67.26%	83.82%	
Mild moderate deficiency	46	3	
	10.17%	4.42%	
Vit D Insufficiency	40	2	
	8.85%	2.94%	
Vit DSufficiency	62	6	
	13.72%	8.82%	

Table 7: Comparison of severe vitamin D deficiency among Diabetics and non Diabetics

DM	N	Mean	Std. Deviation	P value
DM(+)	57	4.9123	1.69327	<0.05
DM(-)	304	4.4622	1.55357	

Conclusion

There is a widespread understanding about the role of vitamin D in the maintenance of normal healthy lifestyle. Vitamin D deficiency is widespread all over the universe. Because of the huge cost in vitamin D testing, it is usually not diagnosed early and ends up in skeletal as well as extra skeletal manifestations. Early diagnosis of vitamin D deficiency can reduce this burden. Our study showed a strong inverse association of 25(OH)D levels and DM, suggesting the role of vitamin D as an independent risk factor for DM. Vitamin D deficiency was associated with an increased risk of having DM in our demographic group.Vitamin D testing and Vitamin D supplementation ,especially in the high risk group,can improve health outcomes in the long run.

Discussion

Diabetes Mellitus(DM) is one of the most common life style diseases encountered at primary care level.Non Pharmacological measures like weight management,low calorie diet,physical activity,cessation of smoking,diabetes self management education and support and psychosocial care are very essential for achieving the ultimate goal and for the improvement of quality of life among the patients with diabetes.By achieving a good glycaemic control,complications associated with DM can be controlled.Vitamin D has several extra skeletal functions including its role in type 2 DM. Hypovitaminosis D is considered as a potential risk factor for the development of type 2 DM.The goal of Vitamin D level screening would be to identify and treat ,before any adverse clinical outcome. In the present study, serum Vitamin D levels were measured in 520 subjects. It was found that 78.85 % of the patients were having Vitamin D deficiency. Hypovitaminosis D at varying prevalence rates has been reported in earlier studies in diabetic patients[35,36,37,38].The main source of Vitamin D is through the exposure to sunlight and from foods that are good sources of Vitamin D such as eggs, sea fish and foods fortified with Vitamin D such as milk and other dairy foods.

Although the exact cause for Vitamin D deficiency in DM is not known, various factors including lack of exposure to sun,skin complexion, dietary habits such as poor intake of Vitamin D rich foods, volumetric dilution and sequestration of Vitamin D in fat depots in obese individuals may increase the susceptibility of an individual to deficiency of Vitamin D[35].Studies have reported that Vitamin D showed an inverse association with HbA1c and PPBS[39]. Vitamin D was shown to be associated with beta cell function and insulin sensitivity in individuals at risk for DM and thus might play a role in the pathogenesis of type 2 DM [40]. Since the physiological role of Vitamin D in pancreatic beta cell function and insulin sensitivity is well appreciated, and considering that most of the diabetic patients in the present study have severe Vitamin D deficiency, it is suggested that Vitamin D levels are measured periodically to identify hypovitaminosis D early.

An appropriate action is to be taken by family physicians at primary care level, to reduce this burden. Simple measures such as dietary changes and life style modifications along with Vitamin D supplementation may help in achieving normal Vitamin D levels.Vitamin D sufficiency via sun exposure is untenable for most Indians.Vitamin D rich dietary sources are unaffordable and limited especially for vegetarians. Most of the Indians are vegetarians.Vitamin D rich foods are of animal origin. Vitamin D supplements are unaffordable and not feasible as a population based approach. Foods should be fortified with vitamin D.Milk and milk products can be fortified with vitamin D.Rice and rice flour,which is our staple diet should be fortified with vitamin D and made available to public with minimal cost. Vitamin D supplementation should be done at primary care level for the high risk group,which includes children,elderly,pregnant and lactating women. School going children should be educated about the need of vitamin D in normal functioning of the human body.Vitamin D can be fortified in the mid day meals provided by the government in schools.Daily exercise in schools would also ensure adequate sun exposure.Facilities for vitamin D testing should be made affordable and accessible atleast to high risk group, as mass testing will not be feasible. Early identification of vitamin D deficiency and appropriate intervention may be of primary importance in population, especially like ours, having high prevalence.

Limitations

All the measurements in the present study were done at a single time point which forms a limitation for the present study. Considering the beneficial role played by Vitamin D on glucose homeostasis, the relationship between Vitamin D levels and glycaemic control needs to be explored in further large, well-controlled studies

References

1. *Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266–81.*
2. *Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011;96:53–8.*
3. *Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:1911–30.*
4. *Bischoff-Ferrari HA, Willett WC, Orav EJ, Lips P, Meunier PJ, Lyons RA, et al. A pooled analysis of vitamin D dose requirements for fracture prevention. N Engl J Med. 2012;367:40–9.*
5. *Jain V, Gupta N, Kalaivani M, Jain A, Sinha A, Agarwal R. Vitamin D deficiency in healthy breastfed term infants at 3 months & their mothers in India: seasonal variation & determinants. Indian J Med Res. 2011;133:267–73.*

6. *Balasubramanian S. Vitamin D deficiency in breastfed infants & the need for routine vitamin D supplementation. Indian J Med Res. 2011;133:250–2.*
7. *Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev. 2001;22:477–501.*
8. *Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev. 2008;29:726–76.*
9. *Muscogiuri G, Sorice GP, Ajjan R, Mezza T, Pilz S, Prioletta A, et al. Can vitamin D deficiency cause diabetes and cardiovascular diseases? Present evidence and future perspectives. Nutr Metab Cardiovasc Dis. 2012;22:81–7.*
10. *Pilz S, Tomaschitz A, Drechsler C, Dekker JM, März W. Vitamin D deficiency and myocardial diseases. Mol Nutr Food Res. 2010;54:1103–13.*
11. *Souberbielle JC, Body JJ, Lappe JM, Plebani M, Shoenfeld Y, Wang TJ, et al. Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: Recommendations for clinical practice. Autoimmun Rev. 2010;9:709–15.*
12. *Mirković K, van den Born J, Navis G, de Borst MH. Vitamin D in chronic kidney disease: new potential for intervention. Curr Drug Targets. 2011;12:42–53.*
13. *Soni M, Kos K, Lang IA, Jones K, Melzer D, Llewellyn DJ. Vitamin D and cognitive function. Scand J Clin Lab Invest. 2012;243(Suppl):79–82.*
14. *Hewison M. Vitamin D and immune function: autocrine, paracrine or endocrine? Scand J Clin Lab Invest. 2012;243(Suppl):92–102.*
15. *Pilz S, Tomaschitz A, Obermayer-Pietsch B, Dobnig H, Pieber TR. Epidemiology of vitamin D insufficiency and cancer mortality. Anticancer Res. 2009;29:3699–704.*
16. *Holick MF. McCollum Award Lecture, 1994: vitamin D--new horizons for the 21st century. Am J Clin Nutr. 1994;60:619–30.*
17. *Kochupillai N. The physiology of vitamin D : current concepts. Indian J Med Res. 2008;127:256–62.*
18. *Macdonald HM. Contributions of sunlight and diet to vitamin D status. Calcif Tissue Int. 2013;92:163–76.*
19. *Peterlik M, Cross HS. Vitamin D and calcium insufficiency-related chronic diseases: molecular and cellular pathophysiology. Eur J Clin Nutr. 2009;63:1377–86.*
20. *Pilz S, Tomaschitz A, Drechsler C, Zittermann A, Dekker JM, März W. Vitamin D supplementation: a promising approach for the prevention and treatment of strokes. Curr Drug Targets. 2011;12:88–96.*

21. *Pilz S, Tomaschitz A, Ritz E, Pieber TR. Vitamin D status and arterial hypertension: a systematic review. Nat Rev Cardiol. 2009;6:621–30.*
22. *Khan, S. Kunutsor, O. H. Franco, and R. Chowdhury, "Vitamin D, type 2 diabetes and other metabolic outcomes: a systematic review and meta-analysis of prospective studies," Proceedings of the Nutrition Society, vol. 72, no. 1, pp. 89–97, 2013.*
23. *A. Laway, S. K. Kotwal, and Z. A. Shah, "Pattern of 25 hydroxy vitamin D status in North Indian people with newly detected type 2 diabetes: a prospective case control study," Indian Journal of Endocrinology and Metabolism, vol. 18, no. 5, pp. 726–730, 2014.*
24. *C. V. Harinarayan, "Vitamin D and diabetes mellitus," Hormones, vol. 13, no. 2, pp. 163–181, 2014.*
25. *N. G. Forouhi, Z. Ye, A. P. Rickard et al., "Circulating 25-hydroxyvitamin D concentration and the risk of type 2 diabetes: results from the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort and updated meta-analysis of prospective studies," Diabetologia, vol. 55, no. 8, pp. 2173–2182, 2012.*
26. *S. J. Chung, Y. A. Lee, H. Hong et al., "Inverse relationship between vitamin D status and insulin resistance and the risk of impaired fasting glucose in Korean children and adolescents: the Korean National Health and Nutrition Examination Survey (KNHANES) 2009-2010," Public Health Nutrition, vol. 17, no. 4, pp. 795–802, 2014.*
27. *R. Feng, Y. Li, G. Li et al., "Lower serum 25 (OH) D concentrations in type 1 diabetes: a meta-analysis," Diabetes Research and Clinical Practice, vol. 108, no. 3, pp. e71–e75, 2015.*
28. *E. Serra-Planas, E. Aguilera, M. L. Granada et al., "High prevalence of vitamin D deficiency and lack of association with subclinical atherosclerosis in asymptomatic patients with Type 1 diabetes mellitus from a Mediterranean area," Acta Diabetologica, vol. 52, no. 4, pp. 773–779, 2015.*
29. *Reinauer H, Home PD, Kanagasabapathy AS, Heuck C. Laboratory diagnosis and monitoring of diabetes mellitus. World Health organization; 2002.*
30. *The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial. Diabetes 1995;44:968-83.*
31. *Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.*
32. *Hollis BW. Assessment and interpretation of circulating 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in the clinical environment. Endocrinol Metab Clin North Am 2010. 39(2):271-86.*

33. *Harinarayan CV, Ramalakshmi T, Prasad UV, Sudhakar D. Vitamin D status in Andhra Pradesh: a population based study. Indian journal Med Res 127,211(2008).*
34. *Lakshmi R Lakshman, Binu Parameswaran Pillai, Rahul Lakshman, Harish Kumar, Sudha S, Jayakumar RV. Comparison of vitamin D levels in obese and non obese patients with PCOS in a South Indian population. Int J Reprod Contracept Obstet Gynecol. 2013 Sept;2(3):336-343.*
35. *Nwosu BU, Maranda L. The effects of vitamin D supplementation on hepatic dysfunction, Vitamin D status, and glycemic control in children and adolescents with Vitamin D deficiency and either type 1 or type 2 diabetes mellitus. PLoS One 2014;9:e99646.*
36. *Rolim MC, Santos BM, Conceição G, Rocha PN. Relationship between Vitamin D status, glycemic control and cardiovascular risk factors in Brazilians with type 2 diabetes mellitus. Diabetol Metab Syndr 2016;8:77.*
37. *Buhary BM, Almohareb O, Aljohani N, Alrajhi S, Elkaissi S, Sherbeeni S, et al. Association of glycosylated hemoglobin levels with Vitamin D status. J Clin Med Res 2017;9:1013-8.*
38. *Akshay Kumar SV, Kumar S, Bharathy N, Dinakaran A, Ray L. Evaluation of vitamin D status and its correlation with glycated haemoglobin in type 2 diabetes mellitus. Biomed Res 2017;28:66-70.*
39. *Saif-Elnasr M, Ibrahim IM, Alkady MM. Role of Vitamin D on glycemic control and oxidative stress in type 2 diabetes mellitus. J Res Med Sci 2017;22:22*
40. *Kayaniyil S, Vieth R, Retnakaran R, Knight JA, Qi Y, Gerstein HC, et al. Association of Vitamin D with insulin resistance and beta-cell dysfunction in subjects at risk for type 2 diabetes. Diabetes Care 2010;33:1379-81.*