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Original Article

Association of serum vitamin D status with development of type 2 diabetes: A retrospective cross-sectional study

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SUMMARY

Background and aims: Vitamin D deficiency (VDD) has become a growing global public health issue, which is placing increasing burdens on healthcare systems worldwide due to its multifactorial clinical manifestations. However, epidemiological findings pertaining to VDD's link with various disease pathologies, especially type 2 diabetes mellitus (T2DM), remain contradictory. Through a retrospective cross-sectional analysis, this study aimed to contribute towards the construction of a novel framework for understanding the relationship between VDD and T2DM to subsequently inform relevant public health policy.

Methods: A retrospective cross-sectional analysis of the prevalence of diabetes in both VDD and non-VDD groups was conducted. Data pertaining to various biochemical parameters was obtained from the Kasturba hospital database for 500 patients tested for both serum 25(OH)D and blood glucose levels [i.e. random, fasting, post-prandial, and/or hemoglobin A1c (HbA1C)] between 1st January and 30th April 2018.

Results: Within the study sample, 117 (41.1%) of patients with VDD had T2DM, whereas 72 (33.5%) of patients without VDD had T2DM. This indicates no association between VDD and T2DM ($\chi 2 = 2.98$; $p = 0.084$). Still, an OR value of 1.4, despite statistical insignificance (95%CI:0.96–2.0, $p = 0.084$) indicates that there is an

Abbreviations: VDD, vitamin D deficiency; T2DM, type 2 diabetes mellitus; HbA1C, hemoglobin A1C; CRP, C-reactive protein; PTH, parathyroid hormone; Ca, calcium; P, phosphorous.

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approximately 40% greater odds of developing T2DM in VDD patients relative to non-VDD patients. Moreover, the likelihood ratio (LR) is 2.99, which indicates an approximately 3-fold chance of having T2DM as a VDD patient, relative to a non-VDD patient.
Conclusions: Despite the lack of statistical significance, the findings of this study make important contributions to the existing literature and must be considered in light of their inherent limitations. Taking these into account, it becomes clear that these results should not be extrapolated nor assumed to entirely invalidate the hypothesized link between VDD and T2DM. Rather, such gaps warrant need for further research and more robust study designs to draw sufficiently significant conclusions to justify reforms in clinical practice.

1. Introduction

Vitamin D deficiency (VDD) has become a growing global public health issue[1]. Although various researchers have considered different cut-off value for vitamin D status, the majority defined VDD as serum 25(OH)D level <20 ng/mL and insufficiency as serum 25(OH)D level 20–30 ng/mL, which affects almost 1 billion people worldwide, representing a staggering 15% of the global population [1]. Vitamin D plays a vital role in many central homeostatic processes such as bone metabolism, cellular growth, neuromuscular and immune function, and inflammation [1]. As a result, low serum vitamin D levels render an individual susceptible to a multiplicity of health complications, such as multiple sclerosis, autoimmune disorders, infectious, respiratory, and cardiometabolic diseases, and cancer, amongst others [1]. Therefore, the high prevalence of VDD places a large burden on healthcare systems worldwide, which must increasingly deal with its multifactorial clinical manifestations.

However, epidemiological findings pertaining to VDD's link with various disease pathologies, especially type 2 diabetes mellitus (T2DM), remain contradictory. Many observational longitudinal studies and its systematic review and metaanalysis support the hypothesis that VDD may increase risk of T2DM [2–5] and related complications [6]. Yet simultaneously, results of many, more robust, randomized controlled trials (RCTs) appear to suggest otherwise [7,8]. A meta-analysis reported no significant effect of vitamin D supplementation on the risk or incidence of T2DM, but found a probable dose-response effect of supplementation on improvement of glycaemia and insulin metabolism among non-T2DM adults, demonstrating a possible advantage of high-dose vitamin D supplementation for T2DM prevention [9]. Similarly, a RCT found that in those with prediabetes as well as VDD, high-dose vitamin D supplementation improves insulin sensitivity and reduces risk of diabetes progression [10]. The discrepancy in the above findings, along with inherent limitations apparent in the design and conduct of the studies have prolonged the scholarly debate concerning the association between VDD and T2DM. Meanwhile, biomolecular evidence suggests that vitamin D may play a protective role against insulin resistance and many diabetes-associated epigenetic alterations, providing a strong biological basis for the hypothesized etiological relationship. However, epidemiological findings must be more conclusive if they are to invalidate or lend further merit to such molecular work. Due to the uncertainties that currently persist in the scientific literature, there is a need for further research to discern the relationship between VDD and T2DM. Through a cross-sectional analysis of the prevalence of T2DM in VDD and non-VDD groups, this study aims to address the need for more robust scientific literature pertaining to this growing yet controversial body of research. Should this study, alongside others, help strengthen support for this association, the clinical significance of VDD can be further established and new policies aimed at improving national vitamin D status implemented.

1.1. Vitamin D deficiency in India

In spite of India's tropical climate, which should theoretically enable high UV light exposure, the country bears a disproportionately high burden of the global VDD crisis. The estimated prevalence of VDD ranges from 70% to 100% in the Indian population [11]. Interestingly, relatively consistent and equally concerning trends regarding vitamin D status have been noted across the entire Indian subcontinent, with few urban-rural disparities despite varied social factors [11]. Individual rural and urban social determinants which simultaneously contribute to VDD may explain this nation-wide universality in vitamin D health indicators. In rural India, dietary factors including lack of animal product consumption and severe protein malnutrition due to financial constraints contribute to low vitamin D intake. Less access to healthcare for VDD screening and management may further exacerbate the issue [11]. Meanwhile, in urban areas, the uptake of non-laborious occupations and reduced sun exposure resulting from overcrowding and high atmospheric pollution may contribute to the VDD epidemic [11]. Other potential attributable factors are common to the entire Indian population. Overall high rates of vegetarianism further decrease dietary intake of animal sources from which vitamin D is primarily derived [11]. Although degradation of dietary vitamin D during heating depends on the type of food items as well as the heating process, it is proven that cooking may affect retention or lead to detrimental loss vitamin D [12]. Therefore, high-temperature cooking practices due to ancient climactic adaptations coupled with a current lack of government regulation on the hygiene and microbial quality control of fresh produce, leads to degradation of vitamin D and further exacerbates the prevailing issue of low vitamin D content in the Indian diet [11]. Moreover, VDD in individuals with obesity is attributed to volumetric dilution in higher volumes of fat in these subjects [13]. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN), a primary thrifty gene, play a role in various cellular processes involved in energy metabolism, downstream insulin regulation, and mitochondrial function [14]. PTEN underexpression promotes excess storage of fat and reduced ability to burn calories [14]. Remarkably, although Indian new born babies have lower weight on average, they have been found to possess higher mean fat storage which is correlated with a higher risk of metabolic syndromes in adulthood [15,16]. Researchers have found that vitamin B12 deficiency as well as one-carbon chain abnormalities might result in the thrifty gene phenotype implicated in the current diabetes epidemic in India [17]. Altogether, this evidence shows increased risk of VDD in Indian populations owing to thrifty-gene obesity. Finally, socio-cultural practices of modest dress code and efforts to attain a pale skin composition are not conducive to adequate sun exposure [11].

Despite the universality of concerning vitamin D status amongst the Indian subpopulation, there has been a lack of public recognition and consequential political will to implement robust public health policy to mitigate the issue [11]. This indicates a lack of epidemiological research pertaining to the national health impact of VDD. As India is currently the “diabetes capital of the world”, with the number of cases expected to rise to 69.9 million by 2025 and an alarmingly high diabetes prevalence in the South Indian population in particular, there is a highly concentrated research effort to discern the causal factors of diabetes in India [18]. The high economic burden of the diabetes epidemic on the Indian healthcare system has further enhanced government interest in public health efforts to understand the roots of and subsequently address this crisis. To date, commonly proposed causes include genetic factors which give rise to an “Asian Indian phenotype” of increased diabetes susceptibility and urbanization-associated increases in sedentary lifestyles and fast food consumption resulting in an “epidemiological transition” towards a “first world disease profile” [18]. While these factors have been long recognized for their contribution to the national diabetes epidemic, resultant policy reform has been slow due to the multifaceted approach needed to address such large-scale social forces [19].

To encourage hastened implementation of new public health policy and political reform to address diabetes in India, there is a need for scholars to identify more direct causes of the epidemic for which sufficiently concrete targets can be designed. The identification of VDD as a risk factor for T2DM in India may precisely serve this need, for studies have shown that relatively simple public health reforms such as vitamin D fortification and supplementation programs are highly effective at improving population vitamin D status [1]. Therefore, the implications of this work are far-reaching; an understanding of the etiological role of VDD in T2DM development may help to inform important public health efforts to

reduce diabetes prevalence whilst simultaneously increasing political will to address the VDD crisis, with its multidimensional health consequences.

1.2. VDD and T2DM-review of the literature

An in-depth analysis of the current literature reveals a wide array of evidence which suggests that VDD may be associated with an increased risk of diabetes. Such evidence comes from both epidemiological and biomolecular studies which have respectively identified clinical associations and potential biological mechanisms which may mediate them. Nonetheless, contradictions remain in the current literature, as there exist discrepancies in the findings across different study designs.

1.2.1. Epidemiological research

1.2.1.1. Observational longitudinal studies. In a systematic review, eight different longitudinal cohort studies were identified for having reported an association between vitamin D status and risk of developing T2DM [20]. In a Women's Health Study, an intake of >511 international units (IU)/day of vitamin D was associated with a 27% lower risk of developing T2DM compared with an intake of <159 IU/day [21]. However, this analysis was limited by the lack of adjustment for potentially confounding risk factors for T2DM. In a Nurses' health study which adjusted for multiple covariates, women who reported consumption of >800 IU/day of vitamin D had a 23% lower risk of developing incident T2DM, by validated self-report, compared with women who reported consumption of <200 IU/day [22]. This association, however, was later invalidated after further adjustment for dietary factors. Other nested case-control studies have compared measured blood 25(OH)D concentration with T2DM incidence. After multivariate adjustment in a nested case-control study which pulled data from two cohorts in Finland, participants who were in the highest quartile of serum 25(OH)D levels at baseline (mean 25[OH]D 27.6 ng/mL), compared with those in the lowest quartile (mean 25[OH]D 8.9 ng/mL), had a 40% lower risk of developing incident T2DM [23].

1.2.1.2. Randomized controlled trials. A systematic review identified 11 RCTs which reported the effects of vitamin D supplementation on measures of glycemia or incident diabetes by self-report [20]. There was no effect of vitamin D supplementation on measures of glycemia including fasting plasma glucose or hemoglobin A1c (HbA1C) and insulin resistance measured by homeostatic model assessment (HOMA) in participants with normal glucose tolerance at baseline in seven trials [24–31]. Similar results were obtained from three small underpowered RCTs in which participants with established T2DM were randomized to receive vitamin D supplementation [32–34]. Of the 11 identified trials, only a select few published results of statistically significant improvements in insulin resistance in response to vitamin D supplementation. Furthermore, methodological and other flaws warrant need for further validation of the significance of the findings across most trials. For example, all trials with the exception of the Women's Health Initiative Trial lacked sufficient statistical power for analysis of glycemic outcomes. Additionally, adherence with supplementation was suboptimal in several trials, which may have further weakened any subsequent conclusions drawn. This suggests that RCTs assessing the effect of vitamin D supplementation on T2DM risk should rely on 25 (OH)D concentrations as the exposure variable rather than vitamin D supplementation dose, a conclusion supported by recent research [35].

1.2.2. Biomolecular research

Numerous cellular and molecular biological studies have elucidated potential etiological mechanisms to explain the association between VDD and T2DM [36]. Such mechanisms are multifactorial and primarily center around vitamin D's apparent protective role against insulin resistance, diabetes-associated epigenetic alterations, mitochondrial dysfunction, and pancreatic β-cell apoptosis.

1.2.2.1. Insulin resistance. Evidence pertaining to several cellular pathways supports the notion that vitamin D may provide protection against insulin resistance. It is now understood that insulin

resistance in obesity results largely from the high presence of serum fatty acids and associated metabolites which have been shown to reduce insulin signalling by phosphorylating the insulin receptor substrate [37]. Vitamin D helps to mitigate this effect by helping to maintain the insulin signalling pathway through increasing cell-surface expression of the insulin receptor [38]. Consequently, VDD would render decreased insulin receptor expression, contributing to insulin resistance. Another mechanism by which vitamin D might play a protective role against insulin resistance is by decreasing intracellular calcium concentrations, which in turn increases activity of the glucose transporter-4 (GLUT4) involved in cellular glucose uptake in response to insulin signalling [39]. Furthermore, vitamin D plays an important role in regulating the secretion of various adipokines such as leptin, adiponectin, and resistin [36]. In doing so, it helps to maintain narrow homeostatic ranges of such hormones to prevent the development of insulin resistance which often occurs in the presence of high resistin and/or low leptin and adiponectin [40]. There is also increasing evidence which suggests that Vitamin D acts to reduce the release of chemokines and cytokines that drive inflammation, a major factor implicated in the development of insulin resistance [41]. Finally, by helping to increase the expression of many cellular antioxidants, vitamin D helps to control intracellular reactive oxygen species (ROS) levels [42]. As increased ROS has been shown to reduce the activity of various components of the insulin signalling pathway, low ROS levels play an important preventative role against insulin resistance [37].

1.2.2.2. Epigenetic alterations. With regards to the epigenome, evidence suggests that vitamin D may play a role in increasing the expression of certain DNA demethylases [43]. Such factors, along with vitamin D-induced ROS level decrease, serve to prevent hypermethylation of promoter regions of many diabetes-associated genes which are otherwise inactivated through hypermethylation in the diabetic epigenome [44].

1.2.2.3. Mitochondrial dysfunction. Mitochondrial function may be another factor mediating the link between T2DM and VDD. There is increasing evidence that vitamin D helps regulate mitochondrial function, reducing risk of mitochondrial dysfunction which plays a pathophysiological role in T2DM [44]. Vitamin D receptor signalling pathways serve to increase genomic expression of many of the components responsible for mitochondrial function [44]. Declines in mitochondrial respiration during VDD have been attributed to the resultant reduction in nuclear mRNA molecules and proteins involved in this process [45]. In response to mitochondrial dysfunction, electron transport chain activity declines and this results in increased formation of ROS that induce oxidative stress and consequently contribute to T2DM development [46].

1.2.2.4. Pancreatic β-cell apoptosis. Evidence from numerous studies suggests that vitamin D plays an important role in many Ca^{2+} signalling pathways. This is significant in the context of T2DM because abnormal Ca^{2+} signalling is implicated in pancreatic β-Cell dysfunction which plays a role in the pathophysiology of T2DM [47]. Specifically, vitamin D helps to prevent excessive Ca^{2+} signalling by reducing the expression of L-type Ca^{2+} channels and increasing the expression of various components that act to maintain low resting levels of Ca^{2+}, such as the buffers calbindin D-9k, calbindin D-28k and parvalbumin, Ca^{2+} pumps, the sodium/calcium exchanger (NCX) and the plasma membrane Ca^{2+}-ATPase 1b [48,49]. Excessive Ca^{2+} signalling in response to high glucose levels typically induces apoptosis and subsequent cell death of pancreatic β-cell in the normal pathophysiological progression of T2DM [50]. Therefore, by regulating Ca^{2+} signalling pathways through the aforementioned mechanisms, vitamin D helps to preserve pancreatic β-cell function and hence maintain normal insulin secretion. Moreover, by reducing intracellular levels of ROS which have been linked to enhanced Ca^{2+} signalling, vitamin D further protects pancreatic β-cells from Ca^{2+} overstimulation [51].

2. Materials & methods

2.1. Study design

This study was conducted using a retrospective cross-sectional analysis of the prevalence of diabetes in both VDD and non-VDD groups. Retrospective data pertaining to various biochemical parameters was obtained from the hospital database of Kasturba Medical College and Hospital, Manipal, India.

2.2. Study population

The study population included 500 patients registered through the Kasturba hospital database who had both serum 25(OH)D and blood glucose levels (i.e. random, fasting, post-prandial, and/or HbA1C) tested between 1st January and 30th April 2018. All patients were obtained from the departments of medicine, orthopedics, and dermatology. Patients with incomplete medical records were excluded, as information pertaining to various covariates was required for adjustment in the statistical analysis. Additionally, patients with any conditions that could play a confounding role, such as hepatic or renal diseases or metabolic rickets, and/or those receiving current treatment for VDD (i.e. vitamin D supplementation) were excluded and/or adjusted for in the statistical analysis to ensure greater internal validity. Finally, those taking any medications known to interfere with vitamin D metabolism [i.e. Pregnane X Receptor (PXR) ligands, glucocorticoids, bisphosphates, anti-estrogens, cytostatic agents] were excluded to limit the effect of additional external factors on vitamin D status amongst study participants.

2.3. Study procedure

After identifying the vitamin D status of all study participants according to laboratory test results for serum 25(OH)D levels, participants were divided into two groups: VDD [serum 25(OH)D level < 20 ng/mL) and non-VDD (serum 25(OH)D level > 20 ng/mL] (Fig. 1). These criteria were based on the vitamin D status indicated in the hospital database, which categorizes serum 25(OH)D levels of <20 ng/mL, 20–30 ng/mL, and >30 ng/mL as deficient, insufficient, and normal, respectively. Patients who either had previous diagnoses of diabetes and/or blood glucose levels (i.e. random, fasting, post-prandial, or HbA1C) which fell into the medically accepted diabetic range were labelled as diabetic (with labels confirmed through reference to ICD-10 coding of T2DM (E11) given for case files in hospital data management system) and the proportion of diabetic individuals in both VDD and non-VDD groups was

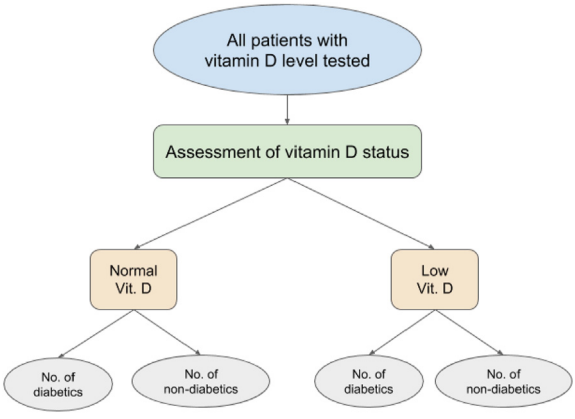

Fig. 1. Study flow chart.

calculated. The relative prevalence of diabetes in VDD and non-VDD groups was then used to determine an odds ratio (OR) which was further analyzed for statistical significance.

2.4. Serum vitamin D test procedure

Laboratory measurements of serum 25(OH)D concentration was determined using the electrochemiluminescence binding assay (Cobas® immunoassay analyzers, Roche Diagnostics). The Cobas® Vitamin D total assay enabled determination of the total 25(OH)D [$25(OH)D_2$ and $25(OH)D_3$]. The stability of 25(OH)D found with the Cobas® Vitamin D total assay is in line with earlier studies using a vitamin D binding protein assay and mass spectrometry. The functional sensitivity was determined to be 4.01 ng/mL (CV 18.5%). The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of $\leq$20%.

2.5. Ethical considerations

The study was conducted after obtaining approval from the Institutional Ethics Committee (IEC) of Kasturba Medical College and Kasturba Hospital, Manipal (Ref. No. IEC 354/2018). The data were collected based on the rules of the Declaration of Helsinki of 1975, revised in 2013.

2.6. Statistical analysis

A standardized frequency analysis was conducted on all quantitative variables, namely serum levels of vitamin D, fasting, post prandial, and random blood glucose, HbA1C, C-reactive protein (CRP), parathyroid hormone (PTH), serum calcium (Ca), and phosphorus (P). For all such measures, respective mean and standard deviation (SD) were also calculated. Pearson Chi-square ($\chi 2$) tests were conducted to determine association of vitamin D status with various factors. Primary outcome measures included $\chi 2$ values for the calculated associations between vitamin D status and diabetes status; and mean serum levels of vitamin D and HbA1C. Furthermore, $\chi 2$ values were determined to analyze the correlations of both vitamin D and diabetes status with various covariates (i.e. CRP, PTH, Ca, and P serum levels).

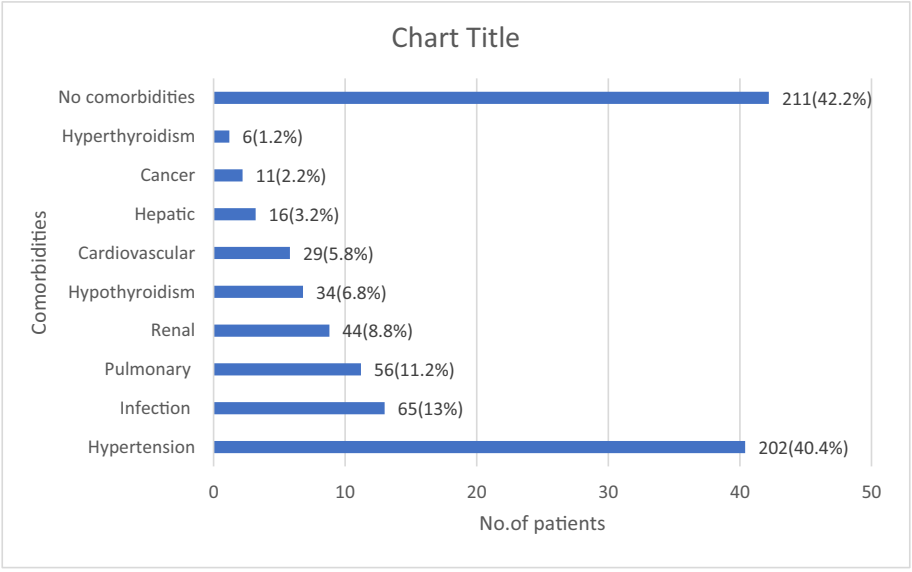

Fig. 2. Major comorbidities among the study population.

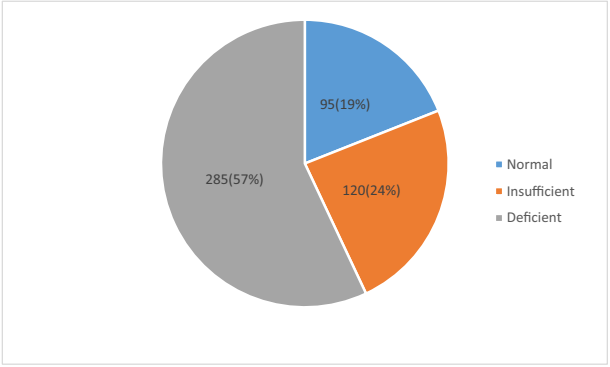

Fig. 3. Vitamin D status among study population (N = 500).

3. Results

3.1. Characteristics of study population

A total of 500 subjects were enrolled in the study, of which 220 (44%) were males and 280 (56%) were females. A total of 213 participants (42.6%) were administered on vitamin D supplementation as of their most recent visit to the hospital. For each of the following comorbidities (Fig. 2), listed in order of decreasing prevalence, the number of recorded participants and corresponding percentage of the study population is outlined as follows: hypertension (202, 40.4%), infection and/or infectious disease (65, 13%), pulmonary disorder (56, 11.2%), renal complication (44, 8.8%), hypothyroidism (34, 6.8%), cardiovascular disease (29, 5.8%), hepatic complication (16, 3.2%), cancer (11, 2.2%), hyperthyroidism (6, 1.2%). Whereas 211 participants (42.2%) had no significant comorbidities.

3.2. Prevalence of VDD and T2DM

Only 95 participants (19%) had normal serum vitamin D levels (i.e. 25(OH)D conc. > 30 ng/mL), with the remaining 405 participants (81%) having sub-normal levels (Fig. 3). Of these, 285 (57%) could be categorized as deficient in vitamin D (i.e. 25(OH)D conc. < 20 ng/mL) and 120 (24%) as insufficient (i.e. 25(OH)D conc. of 20–30 ng/mL). Vitamin D serum levels amongst study participants exhibited a positively skewed distribution with a respective mean and SD of 20.4 and 11.9 ng/mL.

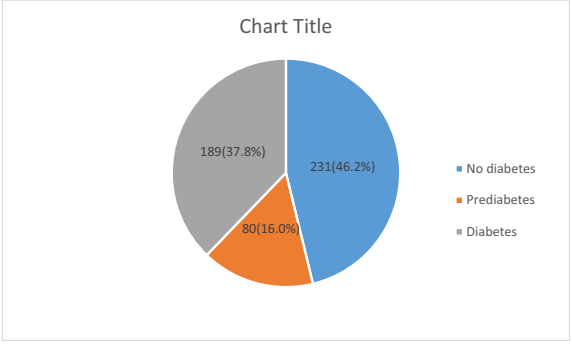

Fig. 4. Diabetes status among study population (N = 500).

With regards to diabetes status, a total of 189 participants (37.8%) could be diagnosed with diabetes according to the aforementioned diagnostic criteria used in the study (Fig. 4). An additional 80 participants (16%) could likewise be diagnosed with pre-diabetes, leaving 231 participants (46.2%) with normal blood glucose levels according to all four indicators used (i.e. fasting, post prandial, and random blood glucose, and HbA1C). The distribution of serum concentrations for HbA1C, the most clinically reliable indicator of diabetes status, was also slightly positively skewed, with a respective mean and standard deviation of 6.5 and 1.8%.

3.3. Association between VDD and T2DM

Within the study sample, 117 (41.1%) of patients with VDD had T2DM, whereas 72 (33.5%) of patients without VDD had T2DM (see Table 1). This indicates no association between VDD and T2DM ($\chi 2 = 2.98$; $p = 0.084$). Still, an OR value of 1.4, despite statistical insignificance (95%CI:0.96–2.0, $p = 0.084$) indicates that there is an approximately 40% greater odds of developing T2DM in VDD patients relative to non-VDD patients. Moreover, the likelihood ratio (LR) is 2.99, which indicates an approximately 3-fold chance of having T2DM as a VDD patient, relative to a non-VDD patient. However, there is no statistically significant association between vitamin D status and HbA1C ($p = 0.07$).

Table 1
Association between VDD and T2DM.

VDD status	Diabetes status		Total	Statistical test*
	Yes	No		
Yes	117 (41.1%)	168 (58.9%)	285 (100%)	$\chi 2 = 2.98$
No	72 (33.5%)	143 (66.5%)	215 (100%)	$p = 0.084$
Total	189 (37.8%)	311 (62.2%)	500 (100%)	OR:1.4(CI:0.96–2.0)

* Pearson Chi-square ($\chi 2$); VDD: Vitamin D Deficiency.

3.4. Associations involving other covariates

$\chi 2$ test were conducted to determine the degree of association of both vitamin D and T2DM status with other covariates, including serum levels of CRP, PTH, Ca, and P. However, a statistically significant association was only found between vitamin D status and serum Ca level ($p = 0.01$).

4. Discussion

4.1. Main findings and significance

A few main findings pertaining to vitamin D status and its corresponding associations emerged from this study. Of most significance is the high prevalence of poor vitamin D status found in the study population, with 81% of patients exhibiting serum vitamin D levels below the optimal healthy range, and a positively skewed distribution with a mean almost 10 ng/mL below the minimum healthy vitamin D serum level. This finding is compatible with previous studies reporting a 70–100% prevalence of VDD in India [11], lending further merit to such claims. Yet interestingly, Karnataka, as other South Indian states, ranks on the lower end in terms of state-wide incidence (30–40%) of vegetarianism [52], a contributor to low vitamin D dietary intake [53]. Similarly, the prevalence of consumption of beef, a high source of vitamin D [53,54], is relatively high in Karnataka relative to the Indian average [52]. This raises concerns regarding the high prevalence of VDD observed in this Karnataka study population where risk factors such as vegetarianism may be relatively less present than in populations across other Indian states.

Similarly, the high proportion of patients with serum glucose levels in the diabetic and/or prediabetic range, precisely 53.8% or just over half of the study population, is indicative of the widely reported burden of diabetes in India and the growing public health threat that it poses. Although the

proportion of patients with T2DM was higher amongst those with VDD (41.1%) than amongst those without VDD (33.5%), the association between VDD and T2DM was not statistically significant in this study. In contrary, the results from risk and LR estimation provide justification for further investigation of this association. As the LR denotes how likely a VDD patient is to have T2DM in relation to a non-VDD patient, a value of 2.99 suggests that a statistically insignificant p-value for the association may be insufficient evidence alone to reject the possibility that VDD plays a role in the etiology of T2DM. Furthermore, the statistically significant association of vitamin D status and serum Ca level observed in this study is consistent with the established literature concerning their physiological links in associated homeostatic pathways.

4.2. Analysis of results, strengths, and limitations

The high prevalence of VDD amongst study participants, while compatible with previous epidemiological findings, may be due in part to the higher tendency of those tested to be deficient in vitamin D. This limitation is attributable to the inherently biased selection of participants according to retrospective vitamin D testing. This may have amplified the detected prevalence and hence led to an overestimation of the epidemiological burden of VDD in the mainstream south Indian population. The effect on serum vitamin D of other comorbidities, which may have been present at higher than average rates in this study population due to their selection from a tertiary healthcare setting, can also not be ruled at as a potential contributor to an over- or under-estimated VDD prevalence.

Existing ambiguities in the mainstream literature regarding the epidemiological and clinical distinction between deficiency and insufficiency of vitamin D pose additional challenges to comparing findings concerning the categorical distribution of vitamin D status amongst study participants to that of the greater Indian population. Therefore, while the detected VDD prevalence of 81% is in line with the 70–100% prevalence rates reported across Indian subpopulations, few cross-study comparisons can be drawn regarding the epidemiological distinction between deficiency and insufficiency of vitamin D.

With regards to diabetes prevalence amongst study participants, there are limitations to extrapolating these findings to the Indian population as a whole due to the unique diagnostic criteria used for defining diabetes status in the specific context of this study. While previous studies have identified diabetes as a major public health threat in India, prior estimates of T2DM prevalence are much lower than those ascertained from this study [18]. This is likely due to the fact that clinical T2DM status is dependent on more stringent diagnostic criteria (i.e. consistently high blood glucose levels upon several consecutive measurements), while, for the purposes of our analysis, a single blood glucose test result in the diabetic range was sufficient to meet the requirements for diagnosis of T2DM. The high proportion of hypertensive study participants may also be implicated in the high diabetes prevalence observed due to the established correlation between these interrelated comorbidities. However, misclassification of T2DM status could have also occurred in the other direction wherein cases of T2DM were missed as a result of an abnormally low blood glucose level upon measurement, depending on the time of day at which the measurement was taken. While the inclusion of participants with a medically documented T2D diagnosis aimed to limit this form of misclassification, it may still have been unavoidable in some, very early stage, T2DM cases. The high proportion of participants exhibiting at least one blood glucose measure characteristic of the diabetic phenotype may indicate a high prevalence of individuals on the verge of becoming diabetic, an additional important measure of the potential diabetes burden in India. Furthermore, the convergent trends of high VDD and T2DM prevalence amongst study participants may lend further justification for continued investigation of their association.

The lack of statistical significance in findings pertaining to VDD's association with T2DM, while important to take into account when drawing conclusions, does not entirely invalidate the hypothesized link. Rather, this finding must be viewed in light of the existing literature and potential limitations in the study design and conduct. The effect of overestimating diabetes cases due to clinically inconsistent diagnostic criteria may have been greater in the smaller non-VDD group and hence decreased the measured difference in diabetes prevalence, weakening any observed correlation. Furthermore, the high VDD prevalence amongst study participants led to a significant discrepancy in the sample sizes of VDD and non-VDD groups, making it difficult to draw robust comparisons between them upon statistical analysis. Such limitations likely also played a mediating role in the relationships

observed with other covariates. While retrospective data collection allowed efficient pooling of information pertaining to various parameters and covariates, it also rendered gaps in the data which may have been disproportionally distributed across study groups, introducing further information bias. Additionally, the inability to detect chronology from retrospective data records allowed us to only make cross-sectional measurements without regard to pathways of causality. Inconsistent temporality likely rendered a more heterogenous study population, making it more difficult to pool results and draw comparisons. Nonetheless, the stronger association observed between vitamin D and HbA1C serum levels is important to take into account, particularly due to the increased consistency and reliability of HbA1C as a clinical indicator of diabetes status. The fact that excluding other, less reliable, glucose parameters from the statistical analysis strengthened the measured association is perhaps indicative of the limitations imposed by their inclusion in the diagnostic criteria utilized for determining diabetes status. As HbA1C is a more robust metric, this result, of higher statistical significance, may therefore also hold greater internal validity.

5. Conclusions and future considerations

Although the findings from this study exhibit several limitations which may reduce the validity and overall generalizability, the findings contribute one further piece of evidence to a growing yet controversial body of research. While the results may not independently address many of the existing ambiguities in the corresponding literature, they lend important justification for the need for further research to investigate this association and address such gaps. Other researchers should view these findings in light of the strengths and weaknesses when drawing conclusions and designing future studies to further investigate the links between VDD and T2DM. Specifically, future research should focus on more robust studies such as prospective cohorts and RCTs to overcome some of the limitations imposed by the weaker retrospective cross-sectional study design. Should sufficient quantity and quality of research ultimately be obtained to address the existing literature gaps and contradictions, novel clinical modalities with profound public health consequences may be implemented for addressing VDD and T2DM, both in India and globally.

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Statement of authorship

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Both Ms. Hannah Marcus and Dr. Sonal Sekhar M were involved in the initial study designing and planning stages. Ms. Hannah Marcus was responsible for the data collection and writing components, and Dr. Sonal Sekhar M assisted with data synthesis, analysis, and editing of the final manuscript. Dr. Muralidhar Varma oversaw the research process and provided the necessary consultation and critically reviewed the final draft of the manuscript. All the authors approved the final draft of the manuscript.

Conflict of interest

Authors have no conflict of interest.

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