

Prevalence of Vitamin D Deficiency in Diabetic Neuropathy Patients in the South Indian Population

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ABSTRACT

OBJECTIVE This research explores the prevalence of vitamin D deficiency among diabetic neuropathy patients in South India, highlighting the significant impact of this deficiency on health outcomes, particularly of the sensory-motor type.

METHODS A cross-sectional study at Koonaseema Institute of Medical Science and Research Foundation (KIMS&RF) found a significant association between vitamin D deficiency and diabetic neuropathy, with factors such as sociodemographic, risk factors, treatment duration, treatment modality, DPN score, hyperalgesia, and neuropathic pain.

RESULTS A study of 480 diabetic neuropathy patients found that 88.54% of them had a deficiency in vitamin D. Of those patients, 51.43% had insufficiency, while 37.11% had a more severe deficiency. The statistical analysis was conducted using Excel and the 16th edition of SPSS (Statistical Package for the Social Sciences). Literate individuals had lower vitamin D levels, while middle-class individuals were more likely. Obesity was linked to vitamin D insufficiency, with 65.60% of obese patients having insufficient vitamin D. Treatment modalities, sensory thresholds, and neuropathic pain symptoms were also associated with vitamin D deficiency.

CONCLUSIONS This study in South India found that 88.54% of individuals, particularly those with diabetic neuropathy, were deficient in vitamin D. This is lower than in Egypt, but higher than in the UAE and China. Factors affecting vitamin D levels affect both males and females, with a higher impact on males. Complications from diabetic neuropathy are linked to vitamin D insufficiency. Further research is needed to validate these findings.

KEYWORDS vitamin D, diabetic neuropathy, hyperalgesia, neuropathic pain

INTRODUCTION

Diabetic neuropathy (DPN) contributes significantly to the morbidity associated with diabetes mellitus as a chronic microvascular consequence (1). DPN causes significant morbidity, elevated

mortality, and a reduced quality of life for those who have it (2). In addition to raising the chance of lower limb amputation, it also imposes additional financial strain on patients and their families since affected individuals must take more pain killers

and see a doctor more frequently, which makes it harder for them to work or carry out everyday tasks (3). Neuropathic pain, a crippling symptom of DPN, adds significantly to the burden of pain management worldwide.

Researchers anticipate an increase in DPN prevalence due to the ageing of the world's population and the growing number of diabetes mellitus patients (4). People with type 2 diabetes (T2DM) experience DPN more commonly than those with type 1 diabetes (T1DM) (5). Previous epidemiological research examined China's DPN prevalence and reported it to be 57.20%. According to estimates from 2020, between 18.80% and 61.90% of Indians have diabetic peripheral neuropathy (DPN), with the sensory-motor type being the most often reported among diabetics (6).

In addition to being essential for metabolic health, researchers have connected vitamin D to a number of physiological mechanisms that affect diabetic complications such as neuropathy. Because poor glycemic control exacerbates neuropathic symptoms, researchers found a substantial interaction between vitamin D levels and insulin sensitivity (7). The way that vitamin D affects insulin resistance and secretion emphasizes how vitamin D levels may impact the severity of DPN as well as diabetes management.

Researchers indicate that vitamin D deficiency widely prevails across the Indian subcontinent, with some groups reporting deficiency rates as high as 100.00% (8). For South Indian patients with diabetic neuropathy, who may experience exacerbated health problems due to their illness, this alarming statistic raises significant concerns. A systematic analysis emphasizes the critical need for public health interventions, reporting a pooled prevalence of 68.00% for vitamin D deficiency in South Asia, including India (9).

A comprehensive analysis has indicated that a significant percentage of Indians, ranging from 40.00% to 99.00%, may have a deficiency in vitamin D (10). Individuals with diabetes are particularly at risk due to their elevated dietary needs. This study underscores the importance of implementing food fortification programmes and conducting public awareness campaigns to mitigate the prevalence of vitamin D deficiency in individuals with diabetic neuropathy.

In addition to neuropathy, vitamin D insufficiency has been associated with other diabetes-related issues such as retinopathy, demonstrating its broader health impacts (11). This connection underscores the importance of comprehensive monitoring and intervention strategies for diabetic patients. Assy et al. suggests that vitamin D levels may influence not just neuropathy but the entire spectrum of diabetic complications.

Moreover, it is important to highlight the role of vitamin D in enhancing insulin sensitivity, underscoring its significance in effective diabetes management. Improving glycemic control is essential for minimizing the likelihood of complications, and increased insulin sensitivity can directly benefit people suffering from DPN (12). This research investigated the frequency of vitamin D deficiency among individuals with DPN in south India. It extends existing studies which emphasize the notable occurrence of this deficiency and its implications for health outcomes. Furthermore, the report identifies areas where information is lacking and provides recommendations for potential future research topics.

METHODS

Subjects

The study recruited 480 adult subjects aged 20-60 from the General Medicine OPD, Kona-seema Institute of Medical Science and Research Foundation, based on criteria such as diagnosis of T2DM mellitus, stable antidiabetic treatment, painful distal symmetrical and sensorimotor polyneuropathy, diabetic neuropathy, DPN characterized by a Neuropathy Disability Score (NDS) of 6 or higher, a reported Visual Analogue Scale (VAS) score of at least 4 for pain, and a Neuropathy Symptom Score (NSS) of 5 or above. Participants with T1DM, who were pregnant or lactating, and those with related endocrine disorders were excluded from the study. Other exclusion criteria included concurrent trials, inability to provide informed consent, malignancies, chronic liver disease, anemia, renal failure, obsessive-compulsive disorder, chronic inflammatory conditions, hypersensitivity to medication, and non-compliance with study medication.

Study design

A cross-sectional study was conducted in the Konaseema area of Andhra Pradesh. The study

focused on individuals with DPN who were receiving treatment at the General Medicine Out-patient Department of the Konaseema Institute of Medical Sciences and Research Foundation (KIMS&RF).

The study was conducted from September 2023 through August, 2024. The required approval from the Institutional Ethics Committee was obtained before starting the study (reference number IEC/PR/2023: 21/04.03.2023). After being evaluated for study eligibility, individuals found to meet the inclusion criteria a baseline measure of diabetic polyneuropathy was assessed using the NDS score, the NSS, and the VAS (visual analogue scale, to measure painful symptoms). According to the authors, Akter et al., a mean score of more than $NDS \geq 6$, $NSS \geq 5$, and $VAS \geq 4$ indicates diabetic polyneuropathy (13). Vitamin D levels were categorised into three groups: vitamin D deficiency (< 15 ng/mL), vitamin D insufficiency (15-29 ng/mL), and vitamin D sufficiency (≥ 30 ng/mL) based on the national population represented in the NHANES 2001-2004 study (National Health and Nutrition Examination Survey) (14).

Ethics

The study protocol was approved by the Institution Committee of Ethics in Human Research, a division of the Konaseema Institute of Medical Sciences & Research Foundation, in accordance with Indian Council of Medical Research regulations (Ref. No. IEC/PR/2021:114). Each patient who wished to participate in the research signed a written informed permission form after being informed about the study.

Data collection

Upon obtaining written informed consent, sociodemographic information, the duration of diabetes, and the type of therapy received were collected. Common evaluation techniques, e.g., the VAS, NSS and NDS were utilised to assess DPN (15). The Toronto Clinical Neuropathy Scoring System was employed to quantify the severity of neuropathy, using the average DPN score ($NSS + NDS + VAS$) to categorise patients into mild, moderate, and severe groups (16). Blood samples were drawn from the antecubital vein and were then centrifuged at 4,000 rpm, with the resulting serum stored at -20°C for further examination. The COBAS 602 automated platform, which utilises

Chemiluminescent Immunoassay (CMIA) technology, was employed to analyse 25-OH vitamin D levels. Patients with diabetic polyneuropathy were categorised into three groups based on their vitamin D levels: deficient (< 15 ng/mL), insufficient (20-30 ng/mL), and sufficient (> 30 ng/mL).

Quantitative sensory testing

Quantitative sensory testing (QST) was used to assess hyperalgesia, which is an increased sensitivity to pain (17). QST measures changes in sensitivity to different sensations like temperature, touch, and pressure. It helps evaluate the excitability of different pain pathways and provides insights into pain mechanisms. The CASE IV system is a QST that has been specifically designed to evaluate sensory thresholds and pain perception in individuals. This system utilises two different stimulators to measure the sensory thresholds for various stimuli, e.g., pressure, vibration, heat, and cold. The supplier of the CASE IV equipment was WR Medical Electronics Co., located in Maplewood, MN, USA. For temperature tests, the dorsal side of the hand was chosen, while the third finger was used for vibratory testing. (Supplement Table 1) The tests were conducted on the upper extremities of individuals with DPN and a control group without diabetic neuropathy, and the results were carefully recorded. After converting the raw QST findings to Z-transformation values, the criteria for hyperalgesia in the patients were established. It was observed that a difference of 1 SD was present between heat pain threshold (HPT) values of 0.5 and 5, while a difference of 2 SD was noted between the control group and the illness group.

Neuropathic pain screening

Ten "yes/no" questions about neuropathic pain make up the douleur neuropathique (DN)4 Questionnaire (18). The score is determined by adding up the "yes" responses, and ranges from "0" (no neuropathic pain) to "10" (the most severe neuropathic pain).

Statistical analysis

Continuous variables underwent normality testing using the Kolmogorov-Smirnov test to assess their distribution. Variables that exhibited a normal distribution are presented as mean $\pm$ standard deviation; others are reported as median

(interquartile range). Categorical variables are expressed using either percentages or proportions. Statistical Significance was set at $p < 0.05$, and appropriate tests of significance, such as the independent t -test and the chi-square test for categorical data, were employed. Data entry and analysis were facilitated using Excel and the 16th edition of SPSS (Statistical Package for the Social Sciences). Prevalence was determined based on the available data. Associations between vitamin D deficiency and various factors, including duration of diabetic treatment, treatment mode, DPN score, hyperalgesia, and neuropathic pain, were demonstrated using the 95% confidence interval (CI) and odds ratio (OR). The 'yes' response rate in the DN4 questionnaire was compared using McNemar's test.

RESULTS

A total of 424 individuals, 88.54% of the 480 DPN patients who took part in the study, had vitamin D deficiency, according to the national population as reported in the National Health and Nutrition Examination Survey (NHANES 2001-

2004 study). Patients were categorised based on their vitamin D levels, with 180 patients (37.11%) having vitamin D deficiency (< 15 ng/mL) and 240 patients (51.43%) having vitamin D insufficiency (15-29 ng/mL). The mean age of those with vitamin D insufficiency was 38 years with a standard deviation of 12.4 years, and 59.40% of this group were men. Among the female participants, 108 (55.38%) were older compare to males and had vitamin D deficiency, with a statistically significant p -value of less than 0.00004.

Anthropometric, clinical characteristics and vitamin D level in the study population

An assessment of various characteristics in terms of their independence and correlation with vitamin D levels is presented Table 1 presents. Several characteristics, such as education, employment position, socioeconomic level, marital status, and body mass index (BMI) status, exhibited statistically significant associations. The comparison of variables across deficient, insufficient, and sufficient categories is illustrated as row percentages. In the study group, it was

Table 1. Sociodemographic details of Diabetic Neuropathy participants with categorical representation of vitamin D levels

Variable	Total (n = 480)	Vitamin D deficiency (< 15 ng/mL) (n = 180)	Vitamin D insufficiency (15-29 ng/mL) (n = 244)	Vitamin D sufficiency (≥ 30 ng/mL) (n = 56)	p-value significant at $p < 0.05$
Age	37 $\pm$ 11	44 $\pm$ 8.34	48 $\pm$ 12.4	28 $\pm$ 4.55	
Male	285	95	158	32	Chi-square statistic = 16.8596, $p < 0.00004^*$
Female	195	108	79	08	
Education					
Literate	320	108	180	32	Chi-square statistic = 95.4037, $p < 0.00001^*$
Illiterate	160	30	50	80	
Employment status					
Employed	390	190	160	40	Fisher exact $p < 0.0179^*$
Unemployed	90	30	50	10	
Socioeconomic status					
Upper class	170	60	100	10	Chi-square statistic = 80.3553, $p < 0.00001^*$
Middle class	220	80	120	20	
Lower class	90	20	30	40	
Marital status					
Married	396	190	150	56	Chi-square statistic = 26.6185, $p < 0.00001^*$
Unmarried	84	28	24	32	
Hypertension	173	102	63	08	
BMI status					
Obese	320	82	210	28	Chi-square statistic = 33.7539, $p < 0.00001^*$
Non-obese	205	106	88	11	

* $p < 0.05$ were considered statistically significant

BMI, body mass index. In the table, results represent the arithmetic mean $\pm$ predictable error of diabetic neuropathy subjects.

observed that literate individuals ($n = 320$, 66.66%) were more likely to have lower vitamin D levels than non-literate individuals, especially those with insufficiency ($n = 180$, 56.30%); chi-square statistic = 95.4037; $p < 0.00001$. Examination of diabetic neuropathy found it to be relatively independent of socioeconomic position, with middle-class DPN individuals ($n = 220$) showing a higher likelihood of vitamin D deficiency ($n = 120$, 54.54%); chi-square statistic = 80.3553, $p < 0.00001$. Furthermore, it was observed that married individuals ($n = 396$) had a higher prevalence of vitamin D deficit ($n = 190$, 47.97%), while those with DPN tended to experience vitamin D insufficiency (chi-square statistic = 26.6185, $p < 0.00001$). A correlation was found between vitamin D status and BMI, with 65.60% of the 320 DPN individuals who were obese having vitamin D insufficiency. Additionally, 25.60% of the obese DPN patients were compared for vitamin D deficiency.

Correlation of vitamin D deficiency with different variables of diabetic neuropathy

The study looked at the relationship between a number of variables and the onset of vitamin D deficiency in diabetic neuropathy. With a non-adjusted odds ratio of 2.93 and a $p < 0.001$, suggesting high statistical significance, it was shown that vitamin D deficiency was significantly more common among patients (52.10%) who were taking long-term diabetes medication. Treatment mo-

dalities, including metformin and sulfonylurea at 49.00% (non-adjusted odds ratio 2.67, $p < 0.001$), moderate DPN score at 50.10% (non-adjusted odds ratio 2.83, $p < 0.001$), and DPN symptoms with hyperalgesia at 69.00% (non-adjusted odds ratio of 3.89, $p = 0.001$), were significantly associated with vitamin D deficiency as shown in Table 2. We studied the relationship between several factors and the onset of vitamin D insufficiency in diabetic neuropathy. It was discovered that of patients receiving long-term diabetic treatment, 52.10% had a significantly high chance of developing vitamin D deficiency, with a non-adjusted OR of 2.93, $p < 0.001$, which is highly significant. As shown in Table 2, along with vitamin D level, treatment modality with Metformin & sulfonylurea 49.00% (non-adjusted OR 2.67, $p < 0.001$), a moderate DPN score of 50.10% (non-adjusted OR 2.83, $p < 0.001$) and DPN symptoms with hyperalgesia 69.00% (non-adjusted OR of 3.89, $p = 0.001$) were also significantly associated with vitamin D deficiency.

Comparison of QST components in the diabetic neuropathy group and the controls without neuropathy

The levels of QST were compared between groups to assess sensory thresholds. In the tests of cold detection threshold (CDT), heat detection threshold (HDT), and vibration detection threshold (VDT), it was found that the group of DPN patients with vitamin D deficiency ($n = 331$) ex-

Table 2. Association between selected variables in diabetic neuropathy and vitamin D deficiency

Variable	n (%)	OR	95%CI	p-value
Duration of diabetic treatment				
0-5 years	150 (31.30)	1.35	1.02-1.82	0.015*
5-10 years	250 (52.10)	2.63	1.70-4.06	< 0.001*
11-20 years	80 (16.70)			
Treatment modality				
Metformin	80 (16.70)	1.40	1.08-1.79	0.010
Metformin with sulfonylurea	235 (49.00)	2.63	1.70-4.06	< 0.001*
Metformin with DPP-4 inhibitors	165 (34.30)	1.93	1.09-3.41	0.009*
DPN Score				
Mild (5-8)	105 (21.90)	1.43	0.95-2.67	0.052
Moderate (9-11)	244 (50.10)	2.93	1.62-4.11	< 0.001*
Severe (12-15)	141 (29.40)	3.05	1.76-4.28	< 0.001*
Hyperalgesia	331 (69.00)	2.89	1.82-4.17	< 0.001*
Neuropathic pain	149 (39.20)	2.03	1.63-3.89	< 0.005*

* $p < 0.05$ were considered statistically significant.

DPN, diabetic neuropathy; CI, confidence interval; OR, odds ratio

Table 3. Comparison of quantitative sensory testing components in the diabetic neuropathy group and the group without neuropathy (control).

Variable	Control: diabetic without neuropathy (N = 56)	Diseases: diabetic neuropathy with vitamin D deficiency (N = 331)	p-value significant at $p < 0.05$
CDT	5.43±1.14	8.33±2.11	0.001
HDT	6.55±1.45	12±2.55	< 0.001
VDT	12.55±2.15	15.2±2.65	0.001
HPT 0.5	18.44±3.15	13.11±2.95	0.005
HPT 5.0	23.85±2.88	20.2±3.05	0.010
HPT 0.5–5.0	5.45±1.32	7.1±1.45	0.005

CDT, cold detection threshold; HDT, heat detection threshold; VDT, vibration detection threshold; HPT, heat pain threshold

Table 4. Comparison of Douleur Neuropathique (DN)4 questionnaire responses of adult patients with painful diabetic neuropathy.

Variable	Control: diabetic without neuropathy (N = 56)	Diseases: diabetic neuropathy with vitamin D deficiency (N = 331)
Burning	38 (67.90)	140 (94.0)*
Painful cold	39 (69.60)	146 (98.0)*
Electric shock tingling	26 (46.40)	139 (93.0)*
Pins and needles	33 (58.90)	140 (94.0)*
Tingling	41 (73.20)	146 (98.0)*
Numbness	45 (80.40)	144 (96.6)*
Itching	36 (64.30)	145 (97.3)*
Hypoesthesia to touch	40 (71.40)	139 (93.0)*
Hypoesthesia to prick	44 (78.60)	136 (91.3)*
The pain is caused or increased by brushing	43 (76.80)	145 (97.3)*

*Statistically significant differences ($p < 0.05$; McNemar's test)

Data presented as n (%) of positive responses.

hibited higher sensory thresholds than the non-neuropathic controls in both their painful and nonpainful extremities ($p < 0.05$). Furthermore, it was observed that the DPN group's HPT 0.5 and HPT 0.5–5.0 values were lower than those of the healthy controls ($p < 0.05$). However, there was no statistically significant difference in HPT 5 readings between the groups ($p > 0.05$). For a detailed intergroup analysis, refer to Table 3.

Comparison of the (DN)4 questionnaire in the diabetic neuropathy group and the without neuropathy controls

Comparison of the (DN)4 questionnaire was performed between the groups. The group with DPN with vitamin D deficiency ($n = 149$ patients) had higher neuropathic pain symptoms than the non-neuropathic controls. Statistically signifi-

cant reductions in neuropathic pain symptoms were noted by McNemar's test, p value less than 0.05 (Table 4).

DISCUSSION

Vitamin D is a fat-soluble vitamin that plays a crucial role in various physiological processes. Vitamin D deficiency potentially contributes to bone disorders like osteomalacia and osteoporosis, increasing fracture risk, and is associated with muscle weakness and falls. Furthermore, vitamin D deficiency is linked to autoimmune diseases, cardiovascular issues, and possibly certain cancers. Vitamin D deficiency can contribute to neuropathy through several interconnected mechanisms, including impaired nerve growth and repair, increased inflammation, increased oxidative stress and potential effects on blood

sugar control. Specifically, vitamin D, through its receptor (VDR), plays a role in nerve growth factor (NGF) production, which is crucial for nerve health. Furthermore, vitamin D deficiency can exacerbate inflammation, a process implicated in nerve damage, and may also affect glucose metabolism, a key factor in diabetic neuropathy. Supplementing with vitamin D has an impact on both extra skeletal and skeletal health. In addition to supporting bone health, vitamin D can also affect immunological, neuromuscular, and cell growth, while also helping to reduce inflammation. It's important to note that excessive supplementation of vitamin D can potentially lead to harmful effects such as renal damage and hypercalcemia.

This groundbreaking study is the first of its kind to be conducted in southern India with the primary objective of assessing the levels of serum vitamin D in individuals with diabetic neuropathy. In the study, 88.54% of the subjects were found to have decreased vitamin D: 51.43% had an insufficiency, while 37.11% had a deficiency. These findings indicate a remarkably high prevalence of vitamin D deficiency overall in the population studied. A cross-sectional study conducted in Egypt that included both urban and rural adult populations in Zagazig city, El-Sharkia, revealed significant findings. Two-thirds of the population had low vitamin D levels according to the study, and among those with painful diabetic neuropathy, 35.00% had low vitamin D levels, while 30.00% had painless diabetic neuropathy (19). Additionally, the study identified a notable negative correlation between vitamin D levels and neuropathy scores, suggesting that lower vitamin D levels were associated with higher neuropathy scores. In contrast, our study in southern India reported an even higher percentage of participants with serum vitamin D deficiency of 88.54%, surpassing the findings of the Egyptian study (20). Another cross-sectional study in China highlighted a high prevalence of vitamin D deficiency in distal symmetric subclinical DPN of 78.54%, as opposed to mononeuropathy and radiculopathy, diabetic neuropathy, and distal symmetric polyneuropathy (21). Furthermore, the study observed the highest prevalence of vitamin D deficiency among individuals with diabetic foot ulcers (97.44%), followed by those without foot ulcers (59.18%) (22).

A clinic-based cross-sectional survey conducted in two tertiary-level hospitals in Yogyakarta, Indonesia, revealed that 85.00% of patients were found to have vitamin D insufficiency. Moreover, the study reported that among individuals with painful diabetic neuropathy, the prevalence of vitamin D deficiency was 20.80% in men, 79.20% in women, and 98.11% in vitamin D insufficiency group. The average age of the study participants was 57.83 ± 8.79 years (23). The investigators defined vitamin D deficiency as serum vitamin D levels below 30 ng/mL, and observed a high prevalence of low vitamin D levels. Using the same cut-off for vitamin D deficiency, a similar overall prevalence of 98.11% was found, with an 88.54% prevalence among diabetic individuals. Additionally, the study revealed a 79.20% prevalence of vitamin D deficiency among females with a mean age of 57.83 ± 8.79 years, whereas our study had a higher prevalence of vitamin D deficiency among males age 48 ± 12.4 years (24). In India, both men and women are susceptible to vitamin D deficiency, with a slight tendency for males to be more affected. This tendency is likely influenced by a combination of factors, such as dietary habits, lifestyle choices, and societal norms that impact sun exposure (25). Our study delved into the reasons behind the higher prevalence of vitamin D deficiency in males.

Research studies have extensively documented the frequency of vitamin D insufficiency in healthy individuals and in individuals with diabetes complications such as diabetic retinopathy and diabetic nephropathy in both Indian and Western populations (26). However, there is a lack of statistical documentation regarding the prevalence of vitamin D deficiency in the community of individuals with DPN in south India.

One study uncovered a high rate of vitamin D deficiency among employed individuals, with 39.60% found to be deficient, and 33.30% having insufficiency. These significant findings were obtained from a retrospective observational cross-sectional study conducted in south India which included both urban and rural adult populations in Nalanda District, Kerala, South India (27). The study indicated that 89.20% of employed individuals in the population had low levels of vitamin D. The figures were 27.60% for skilled workers, 28.10% for semiskilled workers, 7.90%

for unskilled workers, 9.20% for professionals, and 8.40% for semi-professionals. This trend is likely influenced by various factors, including dietary habits and employment choices impacting sun exposure, such as skilled and professional workers. The prevalence of indoor work among employees in South India is a significant aspect of the region's workforce, especially with the increased work-from-home practices post-pandemic, particularly in the IT sector (28). The study specifically investigated the reasons behind the greater incidence of vitamin D insufficiency among working people.

In South India, we conducted a comprehensive analysis of various socioeconomic variables among individuals with diabetic neuropathy. Out of the total population studied, 90 individuals (18.80%) were found to be living below or near the poverty level, while 220 individuals (45.70%) belonged to the middle class, and 170 individuals (35.50%) were classified as upper class. Comparing these findings with the national growth benchmark revealed that 31.00% of the population perceived themselves as middle class, aligning closely with national data. A National Institutes of Health (NIH) research study claims that higher socioeconomic classes and urban areas exhibit higher rates of risk factors for cardiovascular diseases (CVDs), including high cholesterol, diabetes, and hypertension (29). Furthermore, in India, it has been observed that families in the upper and middle classes are more inclined to use statins compared to those in lower socioeconomic levels (30). Our study corroborated this trend, revealing a greater incidence of vitamin D deficiency in middle-class households.

A study indicated that 31.30% of married people were insufficient in vitamin D, and 39.60% of them were deficient. Those results were obtained from a community-based cross-sectional study conducted in the West Bengali village of Singur, which revealed that 70.60% of the populace was deficient in vitamin D (31). The study highlighted that vitamin D deficiency is more prevalent among married individuals, particularly women, due to various factors including lifestyle, social roles, and biological differences. Reasons for this higher prevalence include consuming more calorie-dense foods and receiving less sun exposure as a result of domestic responsibilities. Further-

more, differences in fat distribution and hormonal fluctuations may contribute to lower vitamin D levels in women compared to men (32, 33).

According to one study, overall, 210 DPN individuals are obese, with 25.60% of obese individuals found to be deficient in vitamin D and 65.60% insufficient. These significant findings were gleaned from a case-control study conducted in the Endocrinology-Diabetology and Nutrition Department of a tertiary center in Oujda, Morocco. In that study, in the obese population 32.80% had vitamin D deficiency and 65.70% had vitamin D insufficiency (34). Individuals who are obese have a much higher likelihood of experiencing vitamin D insufficiency compared to those of normal body weight. A meta-analysis revealed that obese individuals have a 3.70 OR for vitamin D insufficiency, significantly higher than that of healthy study participants (35). This suggests that vitamin D deficiency is more prevalent among individuals who are obese. Studies have indicated that vitamin D insufficiency is widespread among obese individuals, with rates reaching as high as 78.30% of the population (36). The lower levels of vitamin D in obese individuals are associated with an increased risk of neuropathy.

Association between selected variables in diabetic neuropathy and vitamin D deficiency

Numerous pieces of evidence have established a clear link between vitamin D insufficiency and the consequences of diabetic neuropathy. The current study revealed that vitamin D deficiency is significantly more prevalent in DPN patients experiencing difficulties compared to diabetic patients without complications, with percentages of 88.54% and 11.46%, respectively. The current study also identified a robust correlation between vitamin D insufficiency and the severity of diabetic polyneuropathy, its symptoms, treatment techniques, and the duration of treatment. This study highlighted the high prevalence of vitamin D deficiency among diabetic patients with diabetic polyneuropathy who had been undergoing treatment for 5-10 years (52.10%) compared to those with below 5 years of treatment and above 10 years of treatment. The observed trend could potentially be attributed to the decreased metabolism of vitamin D in prolonged uncontrolled diabetes (37). Additionally, findings from previous

studies by Hassan et al. (2010) suggest that the duration of diabetes not only impacts glucose control but also influences other health outcomes, including vitamin D levels (38). This relationship underscores the importance of healthcare providers monitoring the vitamin D status as part of routine care for patients with prolonged diabetes.

The present study also highlighted the high prevalence of vitamin D deficiency among diabetic patients with diabetic polyneuropathy who had been undergoing treatment with metformin with sulfonylurea $n = 235$ (49.00%), in comparison to those with only metformin and treatment with metformin with DPP-4 inhibitors. Research has shown that combining metformin and sulfonylurea is effective in managing T2DM. It's important to be aware of potential adverse effects, such as vitamin deficiencies, when using these medications. In particular, the use of metformin may lead to reduced absorption of vitamin B12 (39). In long-term patients who continued to use sulfonylurea, studies have indicated that B12 levels were lower in 10 out of 17 observational studies of T2DM patients taking metformin, with reductions ranging from 28.00% to 48.00% (40). A deficiency in B12 can disrupt important metabolic functions, potentially impacting the body's ability to metabolise vitamin D and potentially worsening bone density-related health issues. Furthermore, a cross-sectional study revealed a correlation between communities at risk of vitamin D deficiency and the presence of anaemia due to B12 deficiency (41). Our study compared the frequency of vitamin D deficiency among diabetic patients receiving different treatments. We found a high frequency of vitamin D deficiency among diabetic patients with diabetic polyneuropathy who were treated with metformin and sulfonylurea. This was observed in 49.00% of the patients in the study group of 235 individuals. This discovery emphasises the influence of B12 deficiency on vitamin D levels in diabetic neuropathy.

The study also revealed a significant incidence of vitamin D deficiency among diabetes patients with a moderate polyneuropathy score ($n = 244$) at 50.10%, contrasting with individuals having mild and severe polyneuropathy scores. This retrospective, cross-sectional research involved Emirati patients, including those from the secondary care unit at University Hospital Sharjah, and pro-

duced noteworthy findings. The study indicates that 50.00% of the individuals had painful diabetic neuropathy, and approximately two-thirds of the population exhibited inadequate vitamin D levels (42). Furthermore, a cross-sectional study carried out in Indonesia established a correlation between vitamin D levels and the severity of neuropathy, with moderate neuropathy accounting for 35.80% and severe neuropathy for 43.30% of the cases of vitamin D deficiency (23).

In individuals with T2DM, there is a significant association between DPN and vitamin D deficiency. Studies show that a higher DPN score is linked to lower vitamin D levels, with some reports indicating a higher prevalence of DPN in those with vitamin D deficiency (43). Specifically, patients with painful DPN tend to have lower serum vitamin D2 levels. According to a meta-analysis, diabetic people who have low vitamin D levels are 1.22 times more likely to experience DPN than those with normal vitamin D levels (44).

Diabetic peripheral neuropathy is characterised by two main signs: hyperalgesia and neuropathic pain. These signs result from sensory and motor deficits caused by nerve injury in the extremities. The development and persistence of neuropathy are greatly impacted by hyperalgesia, which involves an increased sensitivity to pain (45).

This study revealed a significant frequency of vitamin D insufficiency among diabetic patients with diabetic polyneuropathy who experienced hyperalgesia, in contrast to individuals with neuropathic pain. The study conducted a case-control comparison of neuropathy with and without pain, and the results were found to be important. According to the findings, individuals with vitamin D deficiency exhibited a notably higher NDS compared to those with painful PDN (46). The study also observed that assessments of significantly high NDS scores, including the vibration perception test, cold sensation test, and warm sensation test, were abnormal in cases of vitamin D deficiency with painful diabetic neuropathy. Elevated NDS scores were found to indicate hyperalgesia in DPN patients, where the body becomes more sensitive to pain and pain signals are heightened. The prevalence of vitamin D insufficiency was associated with hyperalgesia, a condition linked to increased pain sensitivity in various illnesses like DPN and fibromyalgia, as reported in previous studies (47).

CONCLUSIONS

The study revealed a significant difference in serum vitamin D levels between diabetic patients with and without neuropathy. In southern India, a significant 88.54% of individuals were found to be deficient in vitamin D, especially those with diabetic neuropathy. Subnormal vitamin D levels were more common in DPN patients who were obese and had long-term uncontrolled diabetes. This suggests that the occurrence and prognosis of DPN are influenced by multiple factors, with vitamin D levels potentially playing a vital role. The link between vitamin D levels and DPN deserves careful consideration, and further interventional studies involving larger numbers of patients may provide additional evidence.

Limitations of the study

The small number of sites included in this study was one of its main shortcomings. To provide a more accurate representation of the South Indian population, a multicentre study would be appropriate. Furthermore, due to the study being conducted in a hospital setting, the findings may not be applicable to the general public. Additionally, the vitamin D levels of patients without neuropathy were not compared in this study. It is evident that clinical trials and further research are crucially needed.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

AUTHOR CONTRIBUTION

V.M: conceptualization, methodology, investigation, formal analysis, and drafting the manuscript; M.K: investigation, Formal analysis, and writing-original draft, supervision, and project leadership; G.V: writing-original draft and inves-

tigation; M.N: visualization, and writing-review & editing. All authors have reviewed and endorsed the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted following the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of the Faculty of Medicine, Konaseema Institute of Medical Sciences & Research Foundation, in accordance with Indian Council of Medical Research regulations (Ref No. IEC/PR/2021:114).

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study.

SUPPLEMENTARY MATERIALS

The following supporting information can be downloaded at: [Supplementary file](#)

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Supplement Material

Application details for the QST measurement:

The testing was conducted in a space free from interruptions and distractions, with an air conditioner set at 22°C. With the use of an adjustable chair, the patients' positions were brought into harmony with the examination table. Test stimuli were provided to ensure that all patients understood the procedure, along with information regarding the testing's progression. In order to prevent the patients from seeing the level of the stimulus, the computer screen was turned away. The third finger was used for vibratory testing, while the dorsal side of the hand was selected for temperature testing. The patients' and healthy volunteers' upper extremities were examined, and the findings were noted.

Vibratory testing:

A component of the system, in the form of a lump of putty, was placed on the middle finger as the test site. The stimulator was positioned in the region between the fingernail and the distal interphalangeal joint. The system supplied putty and sponges to ensure the patients remained still during testing and to eliminate potential causes of external vibrations.

Thermal testing:

Before the test, the researchers made sure the participants' hands were dry and, if necessary, dried them with a towel. The 3x3 cm thermal stimulator was securely positioned on the dorsal side of the hand using velcro straps to ensure complete contact and participant comfort. Skin temperatures were measured using the QST system, and all stimuli were administered following the measurements and prior to each thermal test. The investigation employed the '4-2-1' method, one of the algorithms available for the Case IV system, to assess sensory thresholds. The researcher begins by testing and establishing the estimated threshold in this algorithm. Based on this predicted threshold, 20 stimuli are administered to the patient at each measurement. The strength of the subsequent stimulus is determined by the participants' answers; if they are unable to experience the stimulus, the subsequent stimulus is often more intense. The gadget randomly presents null stimuli among all these stimuli, and the accuracy of the participants' answers is examined because it is anticipated that they will respond negatively to them. The test is repeated for that component once three positive responses to the null stimuli are received.

A thermal stimulator was utilised to apply increasing thermal stimuli using a non-repeating ascending null stimuli algorithm to measure the heat pain threshold (HPT) stimulus. During the stimulation, participants rated their pain levels on a numerical scale ranging from 0 (indicating

no pain) to 10 (representing the worst conceivable pain). The test concluded when the discomfort reached a level corresponding to five on the numerical scale. Temperature values were recorded in comparison to the numerical scale, where the initial bothersome level corresponded to numerical scale 1 (HPT 0.5) and the pain level corresponded to 5 (HPT 5). Additionally, the difference between HPT 0.5 and HPT 5 (HPT 0.5–5) was calculated to demonstrate the fluctuation of pain scales with temperature.

Neuropathic pain screening:

Subjective Peripheral Neuropathy Screen Questionnaire (SPNSQ)

Neuropathic symptom
Do you ever have legs and/or feet that feel numb?
Do you ever have any burning pain in your legs and/or feet?
Are your feet too sensitive to touch?
Do you get muscle cramps in your legs and/or feet?
Do you ever have any prickling or tingling feelings in your legs or feet?
When you get into the tub or shower, are you unable to tell the hot water from the cold water with your feet?
Do you ever have any sharp, stabbing, shooting pain in your feet or legs?
Do you feel weak when you walk?
Are your symptoms worse at night?
Do your legs and/or feet hurt when you walk?
Are you unable to sense your feet when you walk?
Is the skin on your feet so dry that it cracks open?
Have you ever had electric shock-like pain in your feet or legs?

	Yes	No
Pain		
characteristic		
Burning Painful cold		
Electric shock Tingling		
Pins and needles		
Numbness		
Itching		
Hypoesthesia to touch		
Hypoesthesia to prick		
The pain is caused or increased by brushing		