Title: Is diabetic neuropathy associated with vitamin D status? A meta-analysis.

Running title: Diabetic neuropathy and vitamin D

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Abbreviations:

25(OH)D: vitamin D

DN: diabetic neuropathy

BMI: body mass index

PRISMA: Preferred Reporting Items for Systematic reviews and Meta-Analyses

MA: meta-analysis

JBI: Joanna Briggs Institute

Abstract

Several studies have been conducted to investigate the relation between 25-hydroxyvitamin D [25(OH)D] level and diabetic neuropathy (DN). However, there is still no clear conclusion due to differences in study design and cut-off values used in the published work, in addition to the absence of a comprehensive meta-analysis on the topic. The present systematic review and meta-analysis therefore aims at clarifying the association between vitamin D level and peripheral DN in patients with type 2 diabetes mellitus.

Primary research studies that explored the association between 25(OH)D level and diabetic peripheral neuropathy in type 2 diabetes were located from Medline, EMBASE, Web of Science, Cochrane Library, CINHAL, and Google Scholar. Twenty-six studies met the inclusion criteria with 6277 participants where 2218 were diabetic with DN, 2959 were diabetic without DN and 406 were healthy.

Diabetic patients with DN showed significantly lower serum 25(OH)D compared to patients without DN (standardized mean difference (SMD) of -0.92 (95% CI = -1.18 to -0.65, I^2 = 93.3%, $p < 0.0001$). The pooled OR value of vitamin D deficiency was higher in patients with DN, 1.84 (95% CI = 1.46 to 2.33, $p < 0.0001$) and 2.87 (95% CI = 1.10 to 7.52, $p = 0.03$) when using fixed-effects and random-effects models, respectively.

Vitamin D deficiency has been found to be highly prevalent among diabetic patients with neuropathy. Since 25(OH)D has been implicated in glucose hemostasis and showed benefit in reducing neuropathy symptoms, its supplementation is warranted for this population of patients.

Keywords: vitamin D; diabetic neuropathy; diabetes; insulin resistance; nutrition

Introduction

Diabetes and its complications represent major and increasing challenges to healthcare systems worldwide. According to the International Diabetes Federation in 2017, 425 million adults have diabetes in the World from whom one in two adults remains undiagnosed. In 2045, this number will rise to 629 million people.

One of the most common consequences of diabetes is damage of the peripheral nervous system.[1,2] It is estimated that 50% of diabetic patients end up having diabetic neuropathy (DN).[1,3] DN is a microvascular complication leading to nerve damage leading to high morbidity and mortality.[2] While the exact mechanism of DN is still unclear; male gender, increasing age, body mass index (BMI), height and disease duration were identified as potential risk factors for DN.[4,5] Further, several studies have suggested that vitamin D deficiency could be an independent risk factor for DN.[2,5-8] Vitamin D is a steroid that functions as a hormone in the human body. It has also a role in glucose homeostasis and sensitivity to insulin.[9] In addition, recent work has shown that patients diagnosed with vitamin D deficiency (serum level of [25(OH)D] below 20 ng/ml) had a higher prevalence of DN than those who were diagnosed with insufficiency (20 – 30 ng/ml) or sufficiency (30 to 60 ng/ml).[8,10,11] Moreover, many studies suggest the use of vitamin D supplementation could improve the symptoms of DN.[2,12-17]

Despite several studies, there appears to be no consensus among researchers with respect to the relation between 25(OH)D and DN. This may be due to differences in sample size, study design, participant ethnicity and cut-off values used in the published work. Moreover, to our knowledge, there are very few published meta-analyses about this relationship which only included between 6 and 13 studies each, that were either all cross-sectional or all case-control.[18-20] The present systematic review and meta-analysis therefore aims at clarifying the association between vitamin D level and diabetic peripheral neuropathy in patients with type 2 diabetes mellitus.

Methods

Search strategy

A specific search strategy has been elaborated to locate the maximum number of relevant studies using the following electronic databases: Medline, EMBASE, Web of Science, Cochrane Library, CINHAL, EBSCO and Google Scholar, from inception to March 01, 2020. The following Boolean terms were used: [diabetic AND neuropathy AND (''vitamin D'' OR 25(OH)D OR ''25(OH) vitamin D'' OR ''25-hydroxy vitamin D'' OR ''25-hydroxyvitamin D'')]. No language or date limitations were imposed.

Criteria for study selection

All study types were accepted for inclusion but two: case reports and reviews. Only patients having type II diabetes were included. Studies reporting comparisons between healthy, diabetic patients without DN or diabetic patients with DN were accepted for inclusion. Whether by clinical exam, validated tool or nerve studies, diabetic neuropathy assessment should be explicitly reported for a study to be included.

Quality study appraisal

The quality of the included English studies were evaluated by the Joanna Briggs Institute critical appraisal tool for cross-sectional and case-control studies.[21] The quality assessment of the included studies written in Chinese language was reported from the study of Qu et al.[19]

Outcome definition

The primary outcome was set to be the serum level of vitamin D. Units were converted and reported in ng/ml. The secondary outcomes were defined as the prevalence of vitamin D insufficiency and the prevalence of vitamin D deficiency.

Data collection and extraction

This review followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines. An excel sheet was used to record all extracted data. Relevant data such

as sample and patient characteristics, diabetes duration, types of comparison, BMI, fasting plasma glucose, HbA1c, tests used to detect and/or assess DN, and laboratory methods for measuring vitamin D was recorded.

Data analysis

Statistical analysis was conducted using the software StatsDirect (Cambridge, UK). Two methods were used for mean comparison. First, an ANOVA was computed to look for significant differences between groups of comparison (diabetic patients with DN, without DN and in healthy subjects) when applicable. Then, an effect size meta-analysis (MA) using standardized mean difference (SMD) was conducted including those studies which reported their standard deviation values along with their mean values. An odds ratio MA was conducted to look for significant proportion differences related to the prevalence of vitamin D insufficiency and/or deficiency. Heterogeneity was assessed by the I^2 inconsistency test; random-effects estimate was reported whenever I^2 value exceeded 50%. A p value <0.05 was considered as significant.

Results

Search results

The electronic search yielded 94 hit records, where 6 were duplicates. Forty-eight abstracts were excluded after initial checking. The remaining 40 articles had their full-manuscripts available for details. Sixteen were excluded for the following reasons: four studies reported outcomes of vitamin D supplementation to treat DN, 6 reviews, 4 lacked primary outcomes, and two were irrelevant. Reference list checking of the 24 studies yielded another 3 relevant studies that fulfilled inclusion eligibility. In total, 27 studies[1-8, 10, 11, 13, 33, 35-48] met the inclusion criteria. Figure 1 shows details of the search.

Study and pooled sample characteristic results

The total pooled sample included 6277 participants. All studies but one[10] reported the subgroup sample number where 2218 patients were diabetic with DN, 2959 were diabetic without DN and 406 healthy subjects. The mean age of the total sample was 53.7 ±9.2 years. The mean diabetes duration was 10.2 ±3.5 years. Table 1 shows the characteristics of the included studies.

Quality appraisal

Scores of the JBI tool for cross-sectional studies ranged between 5 and 8, out of a maximum value of 8 (Table 2).

Scores of the JBI tool for case-control studies ranged between 7 and 10, out of a maximum value of 10 (Table 3).

The quality of the Asian studies written in Chinese were not assessed in this review. However, those 7 seven studies[39-42,45,47,48] were considered as having a moderate quality according to the Newcastle-Ottawa Scale.[19]

Outcomes

Table 4 summarizes the outcomes of each included studies.

Mean serum vitamin D level

Twenty five studies reported this outcome. Serum vitamin D values were 15.1 ±4.2 ng/ml, 20.5 ±6.4 ng/ml and 27.6 ±8 ng/ml for diabetic patients with DN, without DN and in healthy subjects, respectively.

A two-way ANOVA analysis showed high significance between diabetic patients with and without DN ($p < 0.0001$) where those having DN showed significantly lesser values of serum vitamin D. When compared to healthy people, patients with or without DN had significantly lesser values of serum vitamin D ($p < 0.0001$, for both comparisons).

Similarly, when an effect size meta-analysis was conducted including 25 studies, it yielded a standardized mean difference of -0.92 (95% CI = -1.18 to -0.65, $I^2 = 93.3\%$, $p < 0.0001$), significantly lesser serum Vitamin D values in diabetic patients with DN (Figure 2).

Subgroup analysis based on race showed the following values: SMD of 15 Caucasians studies was -0.81 (95% CI = -1.13 to -0.49, $I^2 = 85.4\%$, $p < 0.0001$), and that of 10 Asian studies was -1.04 (95% CI = -1.45 to -0.59, $I^2 = 96.6\%$, $p < 0.0001$). Based on study design, 7 cross-sectional yielded a SMD of -0.48 (95% CI = -0.69 to -0.26, $I^2 = 75.8\%$, $p < 0.0001$), and 18 case-control yielded a SMD of -1.1 (95% CI = -1.47 to -0.72, $I^2 = 93\%$, $p < 0.0001$).

Prevalence of vitamin D insufficiency

Three studies[5,6,8] including 1996 diabetic patients with and without DN yielded a pooled OR of 0.84 (95% CI = 0.69 to 1.04, I^2 = 86.4%, p = 0.1). Non-significance was found for both models.

Prevalence of vitamin D deficiency

Four studies[5,8,37,44] including 1405 diabetic patients with and without DN yielded pooled OR of 1.84 (95% CI = 1.46 to 2.33, p < 0.0001) and 2.9 (95% CI = 1.10 to 7.52, p = 0.03) when using fixed-effects and random-effects models, respectively. For both models, the I^2 = 91.4%.

Prevalence of vitamin D insufficiency *or* deficiency

When combining insufficiency with deficiency with a total of 2076 diabetic patients with and without DN subjects, five studies yielded an OR of 2.8 (95% CI = 1.39 to 5.79, I^2 = 82.9%, p = 0.004).

Gender-based outcomes

Only Jung et al.[10] reported outcomes based on sex. The mean levels of 25(OH)D were significantly lower in patients with DN than in those without DN; (12.7 ng/mL vs 18.7 ng/mL, P = 0.007) and (9.3 ng/mL vs 13 ng/mL, P = 0.002) in men and women, respectively.

Similarly, the prevalence of vitamin D deficiency was significantly higher in men and women with DN than in those without DN.

Discussion

The aim of the present meta-analysis was to explore the association between diabetic neuropathy and vitamin D levels in patients with type 2 diabetes. Diabetic neuropathy is one of the many complications that develop with diabetes, and among the major causes of death in this population.[20] Thus, it is crucial to identify its risk factors. The present meta-analysis included 26 studies with a total sample size of 6277 participants; mean age of 53.7 (SD= 9.2) and mean diabetes duration of 10.2 years (SD = 3.5). The findings indicated a statistical significance between serum levels of vitamin D and diabetic neuropathy.

Main findings

Results showed that patients with neuropathy had significantly lower levels of serum vitamin D compared to those without neuropathy. Moreover, patients with diabetic neuropathy had 1.84 and 2.87 higher odds of being deficient in vitamin D using both fixed and random effect models respectively. Subgroup analyses of vitamin D serum level showed similar significance; results were not affected by race or study design. The prevalence of vitamin D insufficiency among diabetic neuropathy patients was assessed in 1996 patients but with non-significant differences. When combining both outcomes, deficiency and insufficiency, patients with diabetic neuropathy had 2.8 higher odds of being deficient/insufficient in vitamin D.

Interpretation of the results

The findings are in line with previously published meta-analyses that have proven the association between vitamin D deficiency and diabetic neuropathy.[18] However, our meta-analysis differs considerably given that: a) 26 studies were included (vs. 6 to 13)[18-20], b) our sample size was considerably larger (6277 vs. sample sizes below 3000), c) our weighted SMD was the highest, which gives higher evidence towards an association between vitamin D and DN. Though a correlation could not be stated, the association seem to be very high and warrants future large-sampled prospective trials.

Regarding vitamin D insufficiency, it is important to note that there is lack of consensus regarding the threshold in the literature. As such, to address this ambiguity pooled insufficiency data analyses were conducted to examine its association with diabetic neuropathy. The low statistical significance may be due to the lack of consensus for cut-off values for defining vitamin D levels. Indeed, cut-off points to define vitamin D deficiency, insufficiency, sufficiency, and intoxication vary considerably among studies. For example, vitamin D deficiency may be set as levels below 20ng/ml or 10ng/ml (e.g.[10,23]) and insufficiency at 20-29ng/ml or below 24ng/ml depending on the studies.[14,24] Some authors suggested the importance of the Parathyroid Hormone (PTH) as a functional measure for assessing the “adequacy” for serum vitamin D.[49] Taking into consideration that an optimum level of 25(OH) D is required to suppress PTH activity, results from cross-sectional examinations revealed a value of 30 ng/mL vitamin D as adequate. However, this was only a representation of an average value of the

population and did not reflect the variation of vitamin D adequacy for individuals.[49] This suggests that more studies are needed to determine official cut-offs that can be used globally to determine Vitamin D deficiency, insufficiency and sufficiency.

The predominance of vitamin D deficiency *and* insufficiency was assessed among diabetic neuropathy patients using 5 studies with a sample size of 2076. The purpose of assessing both deficiency and insufficiency was to capture the gap between the values of deficiency and insufficiency found among most studies. Again, our results point out to the necessity of more reliable cut-off values to differentiate between deficiency, insufficiency and sufficiency. Two very recent studies were located at the completion of our review; Darraj et al.[26] reported a vitamin D deficit prevalence of 60.8% among patients with diabetes, and Senyigit[27] demonstrated that diabetic patients with DN had significant lower levels than those without the neuropathy.

Vitamin D role in diabetes and diabetic neuropathy

Previous work has shown that diabetic neuropathy is associated with decreased nerve growth factor (NGF) expression in human diabetic nerves and that exogenous NGF can reverse some of the pathological changes in diabetic nerves.[28-30] In parallel, vitamin D is known to induce NGF synthesis in human cell lines.[31] Therefore, Vitamin D may protect diabetic patients from neuropathy through its enhancement of NGF synthesis. In fact, in an experiment on streptozotocin-diabetic rats, a vitamin D3 derivative induced NGF synthesis and prevented neurotrophic deficits.[32]

In addition, vitamin D supplementation was previously shown to result in a significant improvement in neuropathic pain management in diabetic patients[3] as well as a reduction in neuropathy symptom scores.[2] Similarly, a number of clinical trials have linked vitamin D supplementation with the improvement of both pain and neuropathy-specific quality of life in diabetic patients concluding that vitamin D is an independent risk factor for the presence and severity of diabetic neuropathy.[4]

Recent published work has also suggested that vitamin D deficiency may play a role in the pathogenesis of small-fiber neuropathy particularly affecting nociceptor fibres.[33] Other studies proposed that the association between vitamin D deficiency and neuropathy stems from the

pathogenesis of type 2 diabetes through β-cell function, insulin secretion and plasma calcium levels.[34] In that context, vitamin D supplementation was shown to cause an increase in serum calcium, a reduction in serum free fatty acids, higher insulin secretion as well as better glucose tolerance.[2] These findings and mechanisms suggest the importance of vitamin D levels when assessing diabetic neuropathy as well as the effect of vitamin D on the control of insulin in diabetic patients.

Limitations

The present meta-analysis does have some potential limitations. First, severe heterogeneity was observed between included articles, and sensitivity analysis found one article made a great influence on this meta-analysis. Second, some included articles were small-sampled; however, the pooled sample of 6277 participants could be considered as a fair representative of the population. Larger prospective comparative studies are needed to support our findings. Third, the definition of DPN was not uniform due to a variety of different measurements of DPN and that might have impacted our results. Fourth, different methods were used to assess peripheral neuropathy. Fifth, grey literature was not searched with the possibility of missing relevant unpublished articles, conference abstracts or public reports. Sixth, other potential confounding factors which were not investigated and reported in the included studies (season variations at time of measurement, levels of sun exposure and populations of different ethnicities) could have introduced bias to the true association between vitamin D level and DPN. Though we conducted 2 subgroup analyses, one based on race and the other based on study design, heterogeneity was still too high. Selection bias could be one cause of such heterogeneity, despite the fact that quality scores ranged from good to excellent.

Conclusion

In conclusion, the available literature exhibits that vitamin D deficiency could be used as a predictor of diabetic peripheral neuropathy in older adults. However, despite the validation of the studies used in this meta-analysis, more randomized controlled trials and prospective studies as well as studies testing the optimum serum vitamin D for normal functioning should be conducted. These additional studies are necessary to understand the mechanism directly linking vitamin D to diabetic neuropathy and to set recommendations for vitamin D supplementation in order to prevent or slow down the development of DPN in diabetic patients.

Author Contributions

The study was designed by KY. Each author equally contributed to the literature search and data extraction. Data analysis was completed by KY. All authors contributed to the interpretation of the results and the manuscript draft which then was approved by all.

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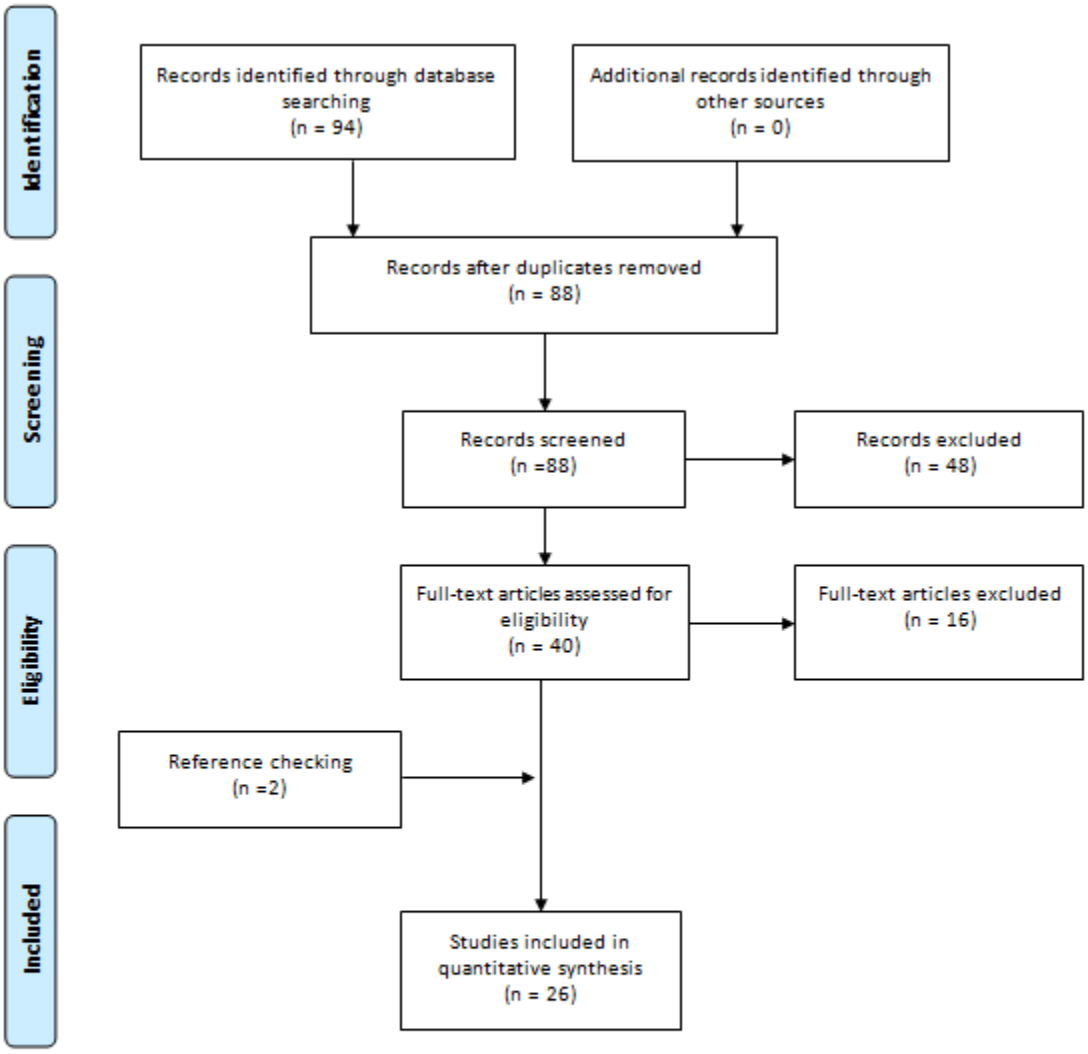
Conflicts of interest: None



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Figure 1. PRISMA Flow Diagram

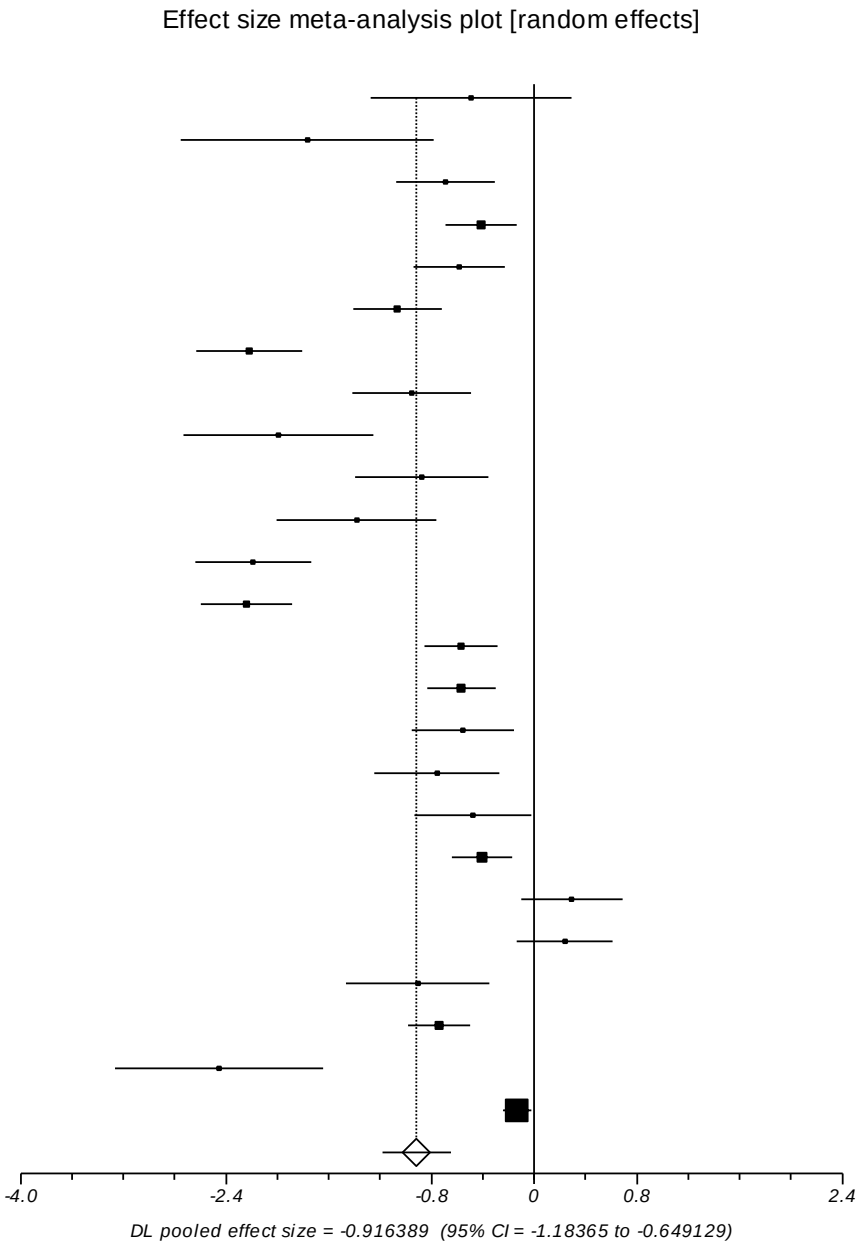


Figure 2. Forest plot of main finding

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Table 1. Characteristics of included studies

Studies	Study type	Total sample	DM with DN sample	DM without DN sample	Healthy sample	Age (years)	Diabetic duration (years)	Tests used for DN	Measurement method of Vitamin D
Yoho et al, 2009	Prospective case-control	41	13	13	15	59 ±9	NR	PE + MFT	NR
Chaychi et al., 2011	Retrospective case-control	22	11	11	NR	58 ±8	NR	MNSI + NCS	LC/MS
Skalli et al., 2011	Cross-sectional	111	62	49	NR	71 ±11	DM with DN: 18 ±10 DM DM with DN: 12 DN: 10 ±7	PE + MFT	RIA
Shehab et al., 2012	Cross-sectional	210	87	123	NR	DM with DN: 58 ±10 DM without DN: 59 ±11	75 subjects >10 135 subjects <10	NSS + NDS + NCS+	RIA
Soderstrom et al., 2012	Cross-sectional	591	287	304	NR	> 40	NR	PE + MFT	ECLIA

Ahmadieh et al., 2013	Cross-sectional	210	86	50	74	59	8.6	UKSS + ISL	RIA
Bajaj et al., 2014	Retrospective case-control	288	56	102	130	53 ±8	5 ±3	PE + NCS	NR
Xiao LF et al., 2014	Case-control	146	76	70	NR	NR	NR	NR	ECLIA
Zhang JP et al., 2014	Cross-sectional	80	37	43	NR	NR	NR	NR	ECLIA
Kheyami et., al 2014	Prospective case-control	81	12	43	26	Healthy Control: 40 ±16 without DN: 43 ±13 With DN: 52 ±16	NR	PE + NCS	ELISA
Alamdari et al., 2015	Prospective case-control	62	33	29	NR	DM with DN: 53.9 DM without DN: 56.6	DM with DN: 7.7 DM without DN 9.2	NCS	RIA
Celikbilek et al., 2015	Prospective case-control	118	24	25	69	Healthy Control: 59 ±11	NR	NCS	ELISA

						DM without DN: 56 ±10			
Jung et al., 2015	Cross-sectional	257	NR	NR	NR	59 ± 2	8 ±8	EP + MNSI + MFT	RIDK
Wang YF et al., 2015	Retrospective case-control	120	60	60	NR	NR	NR	NR	ECLIA
Cui et al., 2015[a]	Retrospective case-control	200	89	111	NR	NR	NR	NR	ECLIA
Cui et al., 2015[b]	Retrospective case-control	200	107	93	NR	NR	NR	NR	ECLIA
Wang Q et al., 2016	Prospective case-control	230	123	107	NR	NR	NR	NR	ECLIA
Wang N et al., 2016	Prospective case-control	101	52	49	NR	NR	NR	NR	NR

Bilir et al., 2016	Prospective case-control	103	37	33	33	NR	NR	EP	UPLC
Ozuguz et al., 2016	Cross-sectional	96	26	70	NR	DM with DN: 38 ±12 DM without DN: 28 ±9	DM with DN: 18 ± 8 DM without DN: 11 ± 6	EP + MNSI + MFT	RIA
He et al., 2017	Cross-sectional	861	527	334	NR	DM with DN: 65.77 ±9.31 DM without DN: 57 ±11	DM with DN: 12 ±7 DM without DN: 8.55±5.98	PE + EMG	ECLIA
Zambelis et al., 2017 (Greek)	Prospective case-control	101	59	42	NR	49.18	7 ±6	NSS + NDS+ NCS+ QST	ECLIA
Zambelis et al., 2017 (Bangladeshi)	Prospective case-control	111	53	58	NR	47.35	6 ±5	NSS + NDS+ NCS+ QST	ECLIA
Abdelsadek et al., 2018	Prospective case-control	80	40	20	20	Healthy control:	9 ±5	NDS+ NCS	EIA

						46 ±2 DM without DN: 47 ±4			
Fan et al., 2018	Prospective case-control	287	164	123	NR	59.7±10.3	8 ±7	PE	ELISA
Shillo et al., 2019	Prospective case-control	59	31	14	14	NR	NR	DN4+ NIS(LL) + NCS	ECLIA
Niu et al, 2019	Cross-sectional	1461	478	983	NR	61 ±12	DM with DN: 12 DM without DN: 8	NR	ECLIA

DM: diabetes mellitus, DN: diabetic neuropathy, MNSI: Michigan Neuropathy Screening Instrument, NCS: nerve conduction studies, NSS: Neuropathy Symptom Score, NDS Neuropathy Disability Score, QST: Quantitative Sensory Test, QNE: Quantitative Neurological Examination, DN4: Douleur Neuropathique-4, NIS (LL): Neuropathy Impairment Score Lower Limb, UKSS: United Kingdom Screening Score, MFT: Monofilament Test, EP: Electrophysiological, PE: Physical Examination, DNES: Diabetic Neuropathy Examination Score, RIA: radioimmunoassay, ECLIA: electrochemiluminescence immunoassay, UPLC: ultra-performance liquid chromatography, LC/MS: Liquid chromatography- mass spectrometry, ELISA: enzyme-linked immunosorbent assay, RIDK: radio immunologic determination kit, EIA: enzyme immunoassays, NR: not reported

Table 2. Joanna Briggs Institute Critical Appraisal Checklist for Cross-Sectional Studies

Items	Skalli et al., 2011	Shehab et al., 2012	Soderstrom et al., 2012	Ahmadieh et al., 2013	Jung et al., 2015	Ozuguz et al., 2016	He et al., 2017	Niu et al., 2019
1. Were the criteria for inclusion in the sample clearly defined?	Unclear	Yes	Yes	Unclear	Yes	Yes	Yes	Yes
2. Were the study subjects and the setting described in detail?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3. Was the exposure measured in a valid and reliable way?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4. Were objective, standard criteria used for measurement of the	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

condition?								
5. Were confounding factors identified?	No	Yes	Yes	Yes	Yes	Unclear	Yes	Yes
6. Were strategies to deal with confounding factors stated?	No	Yes	Yes	Yes	Yes	Unclear	Yes	Yes
7. Were the outcomes measured in a valid and reliable way?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8. Was appropriate statistical analysis used?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Total of ''Yes''	5	8	8	7	8	6	8	8

Table 3. Joanna Briggs Institute Critical Appraisal Checklist for Case Control Studies

Checklist	Yoho et al, 2009	Chaychi et al., 2011	Bajaj et al., 2014	Kheyami et., al 2014	Celikbilek et al., 2015	Alamdari et al., 2015	Celikbilek et al., 2015	Bilir et al., 2016	AbdelSadek, 2018	Zambelis et al., 2017	Abdelsadek et al., 2018	Fan et al., 2018	Shillo et al., 2019
9. Were the groups comparable other than the presence of disease in cases or the absence of disease in controls?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

10. Were cases and controls matched appropriately?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11. Were the same criteria used for identification of cases and controls?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	yes
12. Was exposure measured in a	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

standard, valid and reliable way?													
13. Was exposure measured in the same way for cases and controls?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14. Were confounding factors identified?	Unclear	Yes	Unclear	No	Unclear	Yes	Yes	No	Yes	Unclear	Unclear	Unclear	Yes

15. Were strategies to deal with confounding factors stated?	Yes	No	No	No	No	Yes	No	No	Yes (	No	No	No	Yes
16. Were outcomes assessed in a standard, valid and reliable way for cases and controls?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

17. Was the exposure period of interest long enough to be meaningful?	Yes	Unclear	Yes	Unclear	Unclear	Unclear	Yes	Unclear	Yes	Unclear	Yes	Unclear	Unclear
18. Was appropriate statistical analysis used?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Total of ‘’’Yes’’,	9	8	8	7	7	9	9	7	10	7	8	7	9

Table 4. Primary and secondary outcomes

Studies	Mean 25(OH)D in diabetic subjects with DN (ng/ml)	Mean 25(OH)D in diabetic subjects w/out DN (ng/ml)	Mean 25(OH)D in healthy subjects (ng/ml)	Prevalence vitamin D insufficiency with DN	Prevalence vitamin D insufficiency w/out DN	Prevalence vitamin D deficiency with DN	Prevalence vitamin D deficiency w/out DN	Normal (sufficient) level of 25(OH)D (ng/ml)	Cut-off value for 25(OH)D deficiency (ng/ml)	Cut-off value for 25(OH)D insufficiency (ng/ml)
Yoho et al, 2009	14 ±5	17 ±8	27 ±9	NR	NR	NR	NR	>30	<10	10-30
Chaychi et al., 2011	21 ±9	36 ±7.5	NR	NR	NR	NR	NR	NR	NR	NR
Skalli et al., 2011	10 ±5	14 ±7	NR	NR	NR	NR	NR	NR	NR	NR
Shehab et al., 2012	15 ±16	23 ±24	NR	NR	NR	NR	NR	NR	NR	NR
Soderstrom et al., 2012	NR	NR	NR	243 (85.5%)	234 (77.0%)	NR	NR	>30	NR	<30
Ahmadieh et al., 2013	16 ±10	23.5 ±14.5	22.5 ±12	NR	NR	NR	NR	NR	NR	NR
Bajaj et al.,	17 ±4	23 ±6	NR	NR	NR	43	147	>30	<20	20-29

2014						(76.8%)	(51.2%)			
Xiao LF et al., 2014	12 ±4	24 ±6	NR	NR	NR	NR	NR	NR	NR	NR
Zhang JP et al., 2014	13 ±5	18 ±5	NR	NR	NR	NR	NR	NR	NR	NR
Kheyami et., al 2014	23 ±5	29 ±2.5	22± 4	NR	NR	NR	NR	NR	NR	NR
Alamdari et al., 2015	13.5 ±5	21 ±11.5	NR	NR	NR	NR	NR	NR	NR	NR
Celikbilek et al., 2015	23 ±8	33 ±7	43 ±9	NR	NR	NR	NR	NR	NR	NR
Jung et al., 2015 (111 males)	12.7	18.7	NR	7/60 (11.6%)	NR	9/21 (42.8%)	NR	>20	<10	10-20
Jung et al., 2015 (146 females)	9.3	13	NR	26/72 (36%)	NR	17/63 (27%)	NR	NR	NR	NR
Wang YF et al., 2015	12 ±4	24 ±6	NR	NR	NR	NR	NR	NR	NR	NR
Cui et al., 2015	15± 3	23± 4	NR	NR	NR	NR	NR	NR	NR	NR

Cui et al., 2015 (for elderly)	16 ± 9.5	22 ±10	NR	NR	NR	NR	NR	NR	NR	NR
Wang Q et al., 2016	9.±4	12 ±6	NR	NR	NR	NR	NR	NR	NR	NR
Wang N et al., 2016	12 ±6	15 ±6	NR	NR	NR	NR	NR	NR	NR	NR
Bilir et al., 2016	10 ±5	14 ±6	18 ±3.5	NR	NR	NR	NR	NR	NR	NR
Ozuguz et al., 2016	11 ±5	13.5 ±5	NR	NR	NR	NR	NR	NR	NR	NR
He et al., 2017	16 ±8	18 ±7	NR	141	109 (32.6%)	354	206 (61.7%)	>30	<20	21-29
Zambelis et al., 2017 (Greek)	23 ±12	19 ±12	NR	NR	NR	NR	NR	NR	NR	NR
Zambelis et al., 2017 (Bangladeshi)	12 ±6	11 ±5	NR	NR	NR	NR	NR	NR	NR	NR
Abdelsadek et al., 2018	21 ±8	31 ±15	30 ± 10	NR	NR	35 (87.6%)	9 (45%)	>30	<20	21-29

Fan et al., 2018	16 ±4	19 ±5	NR	29 (17.7%)	48 (39%)	135 (82.3%)	70 (56.9%)	30-60	<20	20-30
Shillo et al., 2019	14 ±2	20 ±2	25± 3	NR	NR	NR	NR	NR	NR	NR
Nui et al, 2019	17 ±8	18 ±7	NR	NR	NR	NR	NR	NR	NR	NR