

# Neuropathic pain is not associated with serum vitamin D but is associated with female gender in patients with type 2 diabetes mellitus

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## ABSTRACT

**Objective** Neuropathic pain is a common complication of diabetes mellitus (DM). Patients may complain of several neuropathic symptoms including impaired peripheral sensation, numbness, tingling, burning, and pain. Because these symptoms may cross with symptoms of vitamin D deficiency, we hypothesized that neuropathic pain and vitamin D deficiency may be associated in patients with type 2 DM.

**Research design and methods** This is a cross-sectional study that involved 239 participants with type 2 DM. Neuropathic pain was assessed using PainDETECT questionnaire. Serum 25-hydroxyvitamin D was measured by the electrochemiluminescence immunoassay, fasting blood glucose was measured by the hexokinase method and hemoglobin A1c was measured by the turbidimetric inhibition immunoassay.

**Results** The prevalence of neuropathic pain among type 2 DM participants was 26.8%. Vitamin D deficiency was reported in 67.8% of type 2 DM participants. The neuropathy score for females was significantly higher than that for males ( $p < 0.01$ ). There was no significant difference in serum vitamin D between type 2 DM participants according to their gender and according to their neuropathy status ( $p > 0.05$ ). Ordinal logistic regression analysis has shown that female gender was the only significant predictor of neuropathic pain among type 2 DM participants ( $p < 0.01$  with an OR (95% CI) of 2.45 (1.29 to 4.67)).

**Conclusions** Neuropathic pain was not associated with serum vitamin D but was associated with female gender in type 2 DM. Because our results were not consistent with other studies that used different neuropathy assessment tools, we suggest that further research should be conducted to check the validity of these tools in identifying subjects with neuropathy.

## INTRODUCTION

Neuropathic pain is a common complication of type 1 and type 2 diabetes mellitus (DM) with a lifetime prevalence of ~50%.<sup>1</sup> The proposed pathophysiological mechanism behind the development of neuropathic pain is almost due to the toxic effects of chronic hyperglycemia.<sup>2</sup> These include the formation of advanced glycation endproducts and

## Significance of this study

### What is already known about this subject?

▶ Neuropathic pain is a common complication of diabetes mellitus (DM). Vitamin D deficiency is very common among patients with type 2 DM and among the general population. There is increasing evidence suggesting that vitamin D deficiency could be associated with the development of neuropathic pain.

### What are the new findings?

▶ Neuropathic pain was not associated with serum vitamin D in patients with type 2 DM. Instead, it was associated with female gender.

### How might these results change the focus of research or clinical practice?

▶ Researchers should reconsider the association between vitamin D deficiency and neuropathic pain according to the method used for neuropathy determination. Further research could also be performed to confirm the association between neuropathic pain and gender in patients with type 2 DM.

reactive oxygen radicals, which can cause injuries in the microvasculature that supplies peripheral nerves.<sup>3–5</sup> Therefore, patients may complain of various neuropathic symptoms including impaired peripheral sensation, numbness, tingling, burning, and pain.<sup>6</sup> Unfortunately, the condition may deteriorate and lead to more serious problems such as foot ulcers and infections.<sup>7</sup>

Interestingly, there is a growing evidence suggesting vitamin D deficiency as a risk factor for diabetic neuropathy.<sup>8–13</sup> Although vitamin D is known to be involved in calcium homeostasis and bone remodeling, it also has other systemic functions that could be mediated by its action on vitamin D receptors (VDRs), which are expressed on various cell types.<sup>14</sup> So, vitamin D deficiency is not only involved in the pathogenesis of bone diseases but also it may be implicated in the development



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of other diseases including DM and cardiovascular diseases.<sup>15 16</sup> Several researches have shown that vitamin D deficiency may predispose subjects to hyperglycemia and thus sufficient intake of vitamin D may improve their glycemic control.<sup>17 18</sup> Additionally, complications of DM may be reduced or delayed by maintaining normal serum vitamin D levels.<sup>19 20</sup> Increasing evidence suggests a role for vitamin D supplementation in improving symptoms of diabetic neuropathy. For instance, Lee *et al*<sup>11</sup> suggested that vitamin D could be used as an analgesic for pain resulting from diabetic neuropathy. Nadi *et al*<sup>21</sup> has also shown that vitamin D supplementation combined with training can improve symptoms of sensorimotor neuropathy in women with type 2 DM. Many other studies have also reported an improvement of symptoms of painful diabetic neuropathy on vitamin D supplementation.<sup>22–24</sup> Regarding the association of vitamin D level with diabetic neuropathy, a recent meta-analysis has shown that vitamin D deficiency could be associated with the development of diabetic neuropathy in Caucasian patients with type 2 DM.<sup>13</sup> A number of other studies conducted on different populations have also shown an association between the levels of serum vitamin D and diabetic neuropathy.<sup>12 25–27</sup>

However, our preliminary data showed that healthy vitamin D-deficient subjects usually experience peripheral neuropathic sensation including numbness, tingling, burning in addition to widespread musculoskeletal pain that resolved by vitamin D supplementation. The similarity in the clinical picture of both vitamin D deficiency and diabetic neuropathy lead us to the hypothesis that the two conditions may be related. Therefore, the aim of this study was to provide evidence that neuropathic pain is associated with vitamin D deficiency in patients with type 2 DM. In addition, this study aimed to find the prevalence of neuropathic pain in patients with type 2 DM.

## MATERIALS AND METHODS

### Participants

This study involved type 2 DM participants who were recruited from the outpatient endocrine clinic of King Abdullah University Hospital (KAUH), Ramtha, Jordan, between January and December 2017. Patients with history of chronic renal impairment, chronic hepatic disease and/or who were on recent vitamin D supplement were excluded from the study. All participants had signed appropriate consent forms before they had been informed about the purpose of the study and after obtaining ethical approval.

### Assessment of neuropathy status

Neuropathy status was determined using the well-validated PainDETECT questionnaire<sup>28</sup> that uses a scale from 0 to 38 to define neuropathy. Participants with a neuropathy score from 0 to 12 were considered as nociceptive (a neuropathic pain component is unlikely), participants with a neuropathy score from 13 to 18 were considered as having unclear neuropathy status (a neuropathic

pain component can be present) and participants with a neuropathy score from 19 to 38 were considered as having neuropathic pain.<sup>28</sup>

### Study design and sample size calculation

This is a cross-sectional study that involved a cohort of type 2 DM participants (n=239). Sample size was calculated using the formula  $(n = (t)^2(p)(1-p)/(d)^2)$ ,<sup>29</sup> where t=1.96 (represents the 95% CI), p=0.20 (the approximate prevalence of neuropathy among patients with type 2 DM as determined by Ojo *et al*<sup>30</sup> using PainDETECT questionnaire<sup>28</sup>) and d=0.05 (the margin of error based on the 95% CI).<sup>29</sup>

### Data collection

Data about age, gender, duration of type 2 DM, smoking, history of vitamin D supplements, current treatment with neuropathy medications, history of chronic renal impairment and history of chronic hepatic diseases were collected from participants' medical records and by self-reporting. Body mass index (BMI) was calculated using the formula: BMI=weight (kg)/height (m<sup>2</sup>). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in mm Hg at rest using a mercury sphygmomanometer.

### Blood sampling and lab assays

Appropriate fasting venous blood samples (10 mL) were collected in the biochemistry lab of KAUH by a qualified laboratory technician. Serum was prepared within 2 hours of blood collection by centrifugation at 2100×g for 8 min at room temperature using a high speed Jouan MR23i centrifuge (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Fasting blood glucose (FBG) was measured by the hexokinase method<sup>31</sup> using a Hitachi 902 auto-analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Hemoglobin A1c (HbA1c) was measured by the turbidimetric inhibition immunoassay<sup>32</sup> using a cobas b 101 system (Roche Diagnostics GmbH). 25-hydroxyvitamin D was measured by electrochemiluminescence immunoassay<sup>33</sup> using a Roche Modular E170 Analyzer (Roche Diagnostics GmbH). Participants were classified as having deficient vitamin D level (<20 ng/mL), insufficient vitamin D level (20–30 ng/mL) or sufficient vitamin D level (>30 ng/mL).<sup>34</sup>

### Statistical analysis

Data were analysed using the IBM SPSS statistics 20 software (IBM, Armonk, New York, USA). Continuous variables that were normally distributed were presented as mean±SD while continuous variables that were not normally distributed were presented as median (25th–75th percentiles). Qualitative variables were presented as frequency (%). Differences in the mean or median levels of continuous variables between male and female participants were determined using Student's t test or Mann-Whitney test, respectively. Differences in the mean or median levels of continuous variables between nociceptive, neuropathic and participants with unclear

**Table 1** General and biochemical characteristics of type 2 DM participants according to their gender

Variable	Gender		P value*
	Male	Female	
N (%)	99 (41.4)	140 (58.6)	–
Age (year)	57.39±9.58	55.90±8.61	0.21
BMI (kg/m <sup>2</sup> )	29.40±4.00	32.16±4.36	<0.01
Smoking			
Yes	39 (39.4)	4 (2.9)	<0.01
No	60 (60.6)	163 (97.1)	
Duration of type 2 DM (year)	7 (4–13)	5.50 (3–10)	0.08
FBG (mmol/L)	8.70 (6.90–12.60)	8 (6.5–12.1)	0.29
HbA1c (%)	8.29 (6.94–9.80)	7.53 (6.70–9.05)	0.05
SBP (mm Hg)	139.82±15.99	136.60±17.31	0.15
DBP (mm Hg)	84.50±10.02	81.88±10.27	0.05
25-hydroxyvitamin D (ng/mL)	14.80 (9.05–21.70)	14.29 (8.04–24.30)	0.79
On neuropathic pain medication (gabapentin)			
Yes	8 (8.1)	8 (5.7)	0.60
No	91 (91.9)	132 (94.3)	

\*Statistically significant differences ( $p < 0.05$ ) were determined using Student's t test or Mann-Whitney test for continuous variables and  $\chi^2$  test or Fisher's exact test for categorical variables. Data were expressed as frequency (%), mean±SD or median (25th–75th percentiles). BMI, body mass index; DBP, diastolic blood pressure; DM, diabetes mellitus; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; SBP, systolic blood pressure.

neuropathy were determined using one-way analysis of variance test with post hoc analysis or Kruskal-Wallis H test, respectively. Differences in categorical variables between male and female participants, between participants who were on neuropathy medications and who were not on neuropathy medications and between nociceptive, neuropathic and participants with unclear neuropathy were determined using  $\chi^2$  test or Fisher's exact test as appropriate. Ordinal logistic regression analysis was used to determine predictors of neuropathic pain. All p values were considered statistically significant at the level of <0.05.

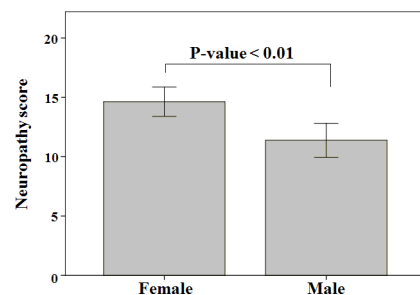
## RESULTS

### General and biochemical characteristics of type 2 DM participants

The mean age±SD was 56.51±9.03 years; the mean BMI±SD was 31.01±4.42; the median duration of type 2 DM (25th–75th percentiles) was 6 (3–10) years; the median FBG (25th–75th percentiles) was 8.55 (6.60–12.23) mmol/L; the median HbA1c (25th–75th percentiles) was 7.75 (6.81–9.43); the mean SBP±SD was 137.94±16.81 mm Hg; the mean DBP±SD was 82.97±10.23 mm Hg; the median 25-hydroxyvitamin D (25th–75th percentiles) was 14.77 (8.45–22.99) ng/mL and the percentage of current smoking was 18%. Characteristics of participants according to their gender are presented in table 1.

### Prevalence of neuropathic pain among type 2 DM participants

The mean neuropathy score±SD for type 2 DM participants ( $n=239$ ), as determined by the PainDETECT questionnaire,<sup>28</sup> was 13.29±7.48 (range is 0–38). According to the questionnaire classification criteria, 26.8% of participants were classified as having neuropathy, 49% of participants were classified as nociceptive and 24.3% of participants were classified as having unclear neuropathy score. As shown in figure 1, neuropathy score was significantly higher in female participants compared with male participants ( $p < 0.01$ ).



**Figure 1** Difference in neuropathic score between male and female type 2 diabetes mellitus participants. P-value was determined using Student's t-test (significance level was set at < 0.05). Bars represent mean neuropathy score + Standard Deviation (SD).

**Table 2** Differences in study variables according to neuropathy status among participants with type 2 DM

Variable	Neuropathy status			P value*
	Nociceptive (score ≤12, n=117)	Unclear neuropathy (score 13–19, n=58)	Neuropathy (score ≥19, n=64)	
Age (year)	55.41±8.26†	59.79±9.27‡	55.55±9.57‡	<0.01
Gender				
Male	59 (50.4)	25 (43.1)	15 (23.4)	<0.01
Female	58 (49.6)	33 (56.9)	49 (76.6)	
BMI (kg/m <sup>2</sup> )	30.42±3.94	31.17±5.14	31.95±4.44	0.08
Smoking				
Yes	21 (17.9)	11 (19)	11 (17.2)	0.97
No	96 (82.1)	47 (81)	53 (82.8)	
Duration of DM (year)	6 (2–10)	6.5 (4–10)	6 (3–11)	0.62
FBG (mmol/L)	8.6 (6.6–12.28)	8.5 (6.98–11.05)	8.5 (6–13.05)	0.91
HbA1c (%)	7.68 (6.79–9.20)	7.59 (6.84–9.48)	8.02 (6.77–9.64)	0.79
SBP (mm Hg)	138.67±18.08	139.67±17.41	135±13.38	0.25
DBP (mm Hg)	83.75±10.52	82.43±11.11	82.03±8.79	0.51
25-hydroxyvitamin D (ng/mL)	14.34 (8.86–21.65)	15.58 (8.29–25.73)	13.77 (7.43–23.85)	0.83
Vitamin D status				
Deficient (<20 ng/mL)	82 (70.1)	36 (62.1)	44 (68.8)	0.55
Insufficient (20–30 ng/mL)	22 (18.8)	17 (29.3)	12 (18.8)	
Sufficient (>30 ng/mL)	13 (11.1)	5 (8.6)	8 (12.5)	
On neuropathic pain medication (Gabapentin)				
Yes	5 (4.3)	6 (10.3)	5 (7.8)	0.26
No	112 (95.7)	52 (89.7)	59 (92.2)	

\*Statistically significant differences ( $p < 0.05$ ) were determined using one way analysis of variance test with post hoc analysis or Kruskal-Wallis H test for continuous variables and  $\chi^2$  test or Fisher's exact test for categorical variables. Data were expressed as frequency (%), mean±SD or median (25th–75th percentiles). Neuropathy status was determined using PainDETECT questionnaire.<sup>21</sup>

†Post-hoc analysis revealed significant difference in age between nociceptive and unclear neuropathy groups.

‡Post-hoc analysis revealed significant difference in age between unclear neuropathy and neuropathy groups.

BMI, body mass index; DBP, diastolic blood pressure; DM, diabetes mellitus; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; SBP, systolic blood pressure.

### Differences in 25-hydroxyvitamin D, glycemic measures and other variables according to neuropathy status among type 2 DM participants

Vitamin D deficiency and insufficiency were detected in 67.8% and 21.3% of type 2 DM participants ( $n=239$ ), respectively. As shown in [table 2](#), there were no significant differences in serum 25-hydroxyvitamin D, FBG, HbA1c, duration of type 2 DM, BMI, smoking status, SBP and DBP between participants who were classified as nociceptive, with unclear neuropathy score or with neuropathy ( $p > 0.05$ ). As well, there was no significant association between vitamin D status and neuropathy status ( $p=0.55$ ). The age of type 2 DM participants with unclear neuropathy was significantly higher than the age of both nociceptive and neuropathic participants ( $p < 0.01$ ). The neuropathic status was only associated with the gender of type 2 DM participants ( $p < 0.01$ ); 76.6% of neuropathic participants were of female gender while the rest were males (23.4%).

### Predictors of neuropathic pain among type 2 DM participants

Predictors of neuropathic pain were investigated using ordinal logistic regression model that involved ordinal neuropathic status (nociceptive, unclear neuropathy and neuropathy) as a dependent variable and other variables including age, gender, BMI, smoking, duration of type 2 DM, FBG, HbA1c, SBP, DBP and 25-hydroxyvitamin D as independent variables. As shown in [table 3](#), female gender was the only significant predictor for neuropathic pain in participants with type 2 DM ( $p < 0.01$ ). For females, the odds of neuropathy category versus the combined unclear neuropathy and nociceptive categories were 2.45 times higher than that for males, adjusted to other variables in the model. Likewise, the odds of the combined neuropathy and unclear neuropathy categories versus nociceptive category were 2.45 times higher for females compared with males, adjusted to other variables in the model.

**Table 3** Ordinal logistic regression analysis for predictors of neuropathic pain among type 2 DM participants

Variable	Estimate	SE	Wald	OR (95% CI)	P value
Neuropathy category 1	2.61	1.84	2.004.20	13.59 (0.37 to 504.87)	0.16
Neuropathy category 2	3.80	1.85	4.20	44.70 (1.18 to 1691.22)	0.04
Age (years)	0.02	0.02	2.33	1.02 (0.99 to 1.06)	0.13
Female gender and male gender (Ref.)	0.90	0.33	7.45	2.45 (1.29 to 4.67)	<0.01
BMI (kg/m <sup>2</sup> )	0.04	0.03	1.51	1.04 (0.98 to 1.10)	0.22
Smoking and non-smoking (Ref.)	0.51	0.40	1.64	1.66 (0.76 to 3.62)	0.20
Duration of DM (years)	0.02	0.02	0.78	1.02 (0.98 to 1.07)	0.38
FBG (mmol/L)	-0.06	0.04	2.81	0.94 (0.87 to 1.01)	0.09
HbA1c (%)	0.18	0.09	3.79	1.19 (1.00 to 1.43)	0.05
SBP (mm Hg)	-0.01	0.01	0.92	0.99 (0.97 to 1.01)	0.34
DBP (mm Hg)	<-0.01	0.02	0.01	1.00 (0.97 to 1.03)	0.91
25-hydroxyvitamin D (ng/mL)	<0.01	0.01	0.03	1.00 (0.98 to 1.03)	0.86

Model fitting information: -2 Log likelihood=468.08 (intercept only) and 447.66 (final);  $\chi^2=20.42$ ;  $p=0.03$ . Goodness of fit information: Pearson  $\chi^2=438.01$ ; Pearson  $p$  value=0.41; deviance  $\chi^2=447.66$ ; deviance  $p$  value=0.29. Neuropathy category 1: neuropathy score  $\leq 12$ . Neuropathy category 2: neuropathy score 13–19. Neuropathy category 3: neuropathy score  $\geq 19$ .  $P < 0.05$  was considered statistically significant.

BMI, body mass index; DBP, diastolic blood pressure; DM, diabetes mellitus; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; Ref, reference; SBP, systolic blood pressure.

### Association between vitamin D status and treatment with neuropathic pain medications among participants with type 2 DM

As shown in table 4, there were only 16 participants (6.69%) who were treated for neuropathy by gabapentin medication. Statistical analysis did not show any significant association between vitamin D status and treatment for neuropathic pain ( $p=0.78$ ).

### DISCUSSION

In the current study, the prevalence of neuropathic pain among participants with type 2 DM was 26.8%. This was slightly higher than the prevalence of neuropathic pain among type 2 DM participants (21.6%) that was determined by Ojo *et al*<sup>30</sup> using the PainDETECT

questionnaire<sup>28</sup>; the same assessment method used in our study. Importantly, figure 1 has shown that the neuropathy score in female participants was significantly higher than that for male participants. As well, table 1 has shown that 76.6% of neuropathic participants were females while 23.4% were males. This was almost similar to findings of Ojo *et al*'s<sup>30</sup> study, in which 66.7% of neuropathic participants were females while 33.3% were males. These slight differences could be due to the differences between various populations as Ojo *et al*'s<sup>30</sup> study was conducted in Nigeria and our study was conducted in Jordan. Therefore, these findings suggest that female patients with type 2 DM are more likely to complain of peripheral neuropathic symptoms compared with males.

The current study was also interested in investigating the association between vitamin D deficiency and peripheral neuropathic pain in participants with type 2 DM. The relative similarity in the clinical symptomatology of both conditions especially the feeling of tingling and numbness has driven us to the hypothesis that the two conditions could be associated. Notably, this study did not find any significant difference in vitamin D levels between type 2 DM participants who were nociceptive, with unclear neuropathy score and with neuropathy. Additionally, there was no significant association between vitamin D status (deficient, insufficient and sufficient vitamin D) and neuropathy status (nociceptive, unclear neuropathy and neuropathy) in participants with type 2 DM (table 2). Our findings were inconsistent with the results of the few studies reported in the literature that were interested in finding association between diabetic neuropathy and vitamin D deficiency. For instance, Orabi *et al*'s study<sup>35</sup> has reported that patients with diabetic neuropathy were having significantly lower vitamin D levels compared with

**Table 4** Association between vitamin D status and taking neuropathic pain medications among participants with type 2 DM

	Participants on neuropathic pain medications		P value
	Yes (n=16)	No (n=223)	
Vitamin D status			0.78
Deficient (<20 ng/mL)	10 (62.5)	152 (68.2)	
Insufficient (20–30 ng/mL)	4 (25.0)	47 (21.1)	
Sufficient (>30 ng/mL)	2 (12.5)	24 (10.8)	

\*Gabapentin was the only medication that was used to treat neuropathy.

†Statistically significant difference ( $p < 0.05$ ) was determined using Fisher's exact test. Data were expressed as frequency (%). DM, diabetes mellitus.

controls. Likewise, Shehab *et al*'s study<sup>8</sup> had reported that patients with diabetic neuropathy were having significantly lower vitamin D levels compared with patients with type 2 DM without neuropathy. As well, Shillo *et al*'s study,<sup>12</sup> has reported a significant lower vitamin D level in type 2 DM white Europeans with neuropathy compared with controls. This inconsistency could be due to the small sample size of Orabi *et al*'s<sup>35</sup> and Shillo *et al*'s<sup>12</sup> studies and the different neuropathy assessment methods used in our study. Unfortunately, there was no study in the literature that used the PainDETECT questionnaire<sup>28</sup> to assess neuropathic pain in association with vitamin D deficiency although the questionnaire is well validated and was used by other researchers like Ojo *et al*'s<sup>30</sup> to investigate the prevalence of neuropathic pain in patients with type 2 DM as mentioned above. Even though, we believe that further studies are required to assess the relation between diabetic neuropathy and vitamin D levels using different methods of neuropathy assessment including both neuropathy questionnaires and clinical neuropathy assessment. This will expose the validity of each method in categorizing patients with neuropathy in relation to vitamin D status.

To find predictors of neuropathic pain among participants with Type 2 DM, ordinal logistic regression analysis has shown that female gender was the only significant predictor for neuropathic pain while vitamin D level, age, BMI, FBG, duration of type 2 DM, SBP and DBP were not. The significance level for the HbA1c in the model was on the borderline with a p value of 0.05 and OR (95% CI) of 1.19 (1.00 to 1.43). These results were interesting to us because most of other previous studies had reported different predictors for diabetic neuropathy including the long duration of DM, increased age and elevated HbA1c.<sup>36–38</sup> As well, these studies did not find any gender-related differences in neuropathic pain among patients with type 2 DM.<sup>36–38</sup> Again, the inconsistency between our results and other previous studies<sup>36–38</sup> is almost because these studies used different methods to assess neuropathy. Interestingly, Gryz *et al*'s<sup>36</sup> tried to assess predictors of diabetic neuropathy in relation to different criteria of its diagnosis and they found that the predictors vary according to the criteria that were used for diagnosis. The only study that used PainDETECT questionnaire to assess neuropathy in patients with type 2 DM was Ojo *et al*'s study.<sup>30</sup> As mentioned above, results of Ojo *et al*'s study<sup>30</sup> were almost similar to our results in regard to the prevalence of diabetic neuropathy, frequency of neuropathy in females compared with males and the lack of any significant difference in FBG, HbA1c and duration of type 2 DM between patients with neuropathy and those without neuropathy. In contrast to Ojo *et al*'s study,<sup>30</sup> age was not a predictor for neuropathic pain in our study. As mentioned above, this could be due to differences in the study populations as both studies were conducted on different populations.

In summary, this study did not find any association between neuropathic pain and vitamin D levels in

participants with type 2 DM. So, this finding rejects our hypothesis that both vitamin D deficiency and neuropathic pain could be related. Instead, the current study has found that neuropathic pain in participants with type 2 DM can be predicted from the female gender but not from age, DM duration, FBG or HbA1c. The strengths of the current study comes from its suitable sample size and the method of neuropathy assessment which was well validated and used by other researchers to assess neuropathic pain.<sup>30</sup> On the other hand, the current study has also some limitations that may affect its findings. For example, the neuropathy status was not determined clinically but was determined by self-reporting using the PainDETECT questionnaire.<sup>28</sup> As well, we only used one assessment tool for neuropathy and we did not compare the validity of this tool compared with other questionnaires that were used previously to assess neuropathy.<sup>36–38</sup> So, the comparison of our results with these studies<sup>36–38</sup> could not be appropriate because of the differences in the methodology. Another possible limitation to the association between neuropathic pain and female gender is that females may have lower pain threshold and tolerance levels compared with males.<sup>39</sup> Unfortunately, the difference in the pain threshold between males and females was not taken in consideration in the method that we used to assess neuropathic pain. Despite of these limitations, we believe that the current study is the first report that did not find any association between vitamin D levels and neuropathic pain in participants with type 2 DM. As well, this is the second study that used the PainDETECT questionnaire<sup>28</sup> along with Ojo *et al*'s study<sup>30</sup> to assess neuropathic pain in patients with type 2 DM. Because of the similarity in our findings and results of Ojo *et al*'s study<sup>30</sup> and the inconsistency with other studies<sup>36–38</sup> that used different neuropathy assessment methods, we think that it will be wrathful to do further research to compare the various neuropathy assessment tools on same populations and to compare their results with results of the clinical neuropathy assessment methods.

## CONCLUSIONS

Neuropathy was not associated with serum vitamin D but was associated with female gender in participants with type 2 DM. This suggests that female patients with type 2 DM are more likely to complain of peripheral neuropathic symptoms compared with males. Because the lack of association between neuropathy and serum vitamin D was not consistent with other studies that used different neuropathy assessment tools, we suggest that further research should be conducted to check the validity of these tools in identifying subjects with neuropathy.

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**Patient consent for publication** Obtained.

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**Data sharing statement** Data are available upon reasonable request.

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