Type 2 diabetes mellitus (T2DM) is associated with systemic inflammation that results in insulin resistance. Increased oxidative stress and several other pathways cause inflammation, which is responsible for most of the complications of diabetes. Characteristics of systemic inflammation are increased levels of inflammatory biomarkers in the bloodstream, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-2, IL-6, IL-8, and IL-12. T2DM patients with diabetic polyneuropathy (DPN) have increased serum levels of inflammatory cytokines.

Inflammatory cytokines directly induce insulin resistance by interrupting insulin signal transduction. A review by Wilson and Wright stated several studies on the role of inflammatory mediators in diabetic neuropathy (DN), including TNF-α and IL-6 in animal models induced by streptozotocin and T2DM patients with or without DN. They also explained the role of IL-8 in T2DM patients compared with controls.

Treatment of aggressive hyperglycemia is not effective in T2DM. The treatment of DN should be developed outside of the treatment of hyperglycemia and, if possible, begin early in the course of the disease. Inhibition of the inflammatory response is effective in preventing DN. Human studies examining the effects of vitamin D supplementation on inflammatory biomarkers in subjects with or at
high risk of developing T2DM are scarce and produce conflicting results. Vitamin D supplementation or bioactive form 1,25(OH)2D improves insulin sensitivity by preventing the synthesis of excessive inflammatory cytokines.11

We sought to assess the modulation of IL-8 production by vitamin D supplementation in Indonesian patients with DPN.

METHODS

This study was a cohort prospective, randomized, placebo-controlled, double-blind trial. The study was approved by the Faculty of Medicine Universitas Sumatera Utara/Haji Adam Malik General Hospital Ethical Committee. All subjects signed informed consent before examination. Fifty subjects with T2DM in Haji Adam Malik General Hospital Medan were enrolled in this study from July 2018 to February 2019. They were divided into two groups, which were treated for 10 weeks with either placebo or vitamin D (D3) supplementation (Natrol 10 000 IU) of 50 000 IU/week. The placebo contained saccharum lactis with the same weight as vitamin D supplementation. They were evaluated by routine nerve conduction study (NCS) in the upper and lower limbs, and their serum vitamin 25-hydroxyvitamin D (25(OH)D) and IL-8 levels before and 10 weeks after placebo or vitamin D supplementation were measured.

Before the blood test, the patient was asked to fast for about eight hours. The subjects were randomized in a double-blind fashion; 25 subjects had placebo and 25 had vitamin D supplementation. The placebo or vitamin D supplementation was taken as three capsules a day, once a week, for 10 weeks. The selection of treatment group was done randomly using a random table. Patients and researcher did not know the patient got a placebo or vitamin D supplementation.

T2DM patients with mild to moderate DPN were included in the study. Patients who took vitamin D in the last three months, were taking anti-tuberculous drugs or chemotherapy, with renal, hepatic, gastrointestinal, stroke, malignancy or infection problems, pregnant, breastfeeding, or using oral contraceptives were excluded from the study. Patients allergic to vitamin D and did not regularly take medication were also excluded. NCS examination was performed by the same neurologist using Cadwell electroneuromyography machine. NCS consisted of distal latencies (DL), amplification, and nerve conduction velocity (NCV). Serum vitamin 25(OH)D status was evaluated using the chemiluminescent immunoassay method by DiaSorin Liaison, and IL-8 was evaluated using enzyme-linked immunoassay method by Quantikine HS ELISA.

Changes in the vitamin D and IL-8 levels were considered independent variables and NCS changes as a dependent variable. The mean difference was analyzed using T dependent test and correlation was analyzed using Pearson’s correlation. The role of IL-8 and vitamin D supplementation on NCSs was analyzed using linear regression. The results were considered significant at \( p < 0.050 \). The correlation coefficient (R) measures strength and direction. If \( R \) is negative, the direction is negative relationship. If \( R \) is positive, the direction is positive relationship. R value of 0.300 indicates weak strength, 0.500 is moderate strength, and 0.700 means strong strength. The statistical calculations were done using a computerized program.

RESULTS

All randomized subjects (n = 50) completed this study. Their characteristics data were similar in both groups at baseline \( (p > 0.050) \) as shown in Table 1.

There were significant differences between the mean vitamin 25(OH)D and IL-8 levels before and after receiving vitamin D supplementation [Table 2].

We found no significant correlation between vitamin 25(OH)D and IL-8 levels [Table 3]. There were significant correlations with negative direction and weak strength between IL-8 level with DL of sensory ulnar, amplification of sensory sural, and NCV of motor tibial nerves. On the other hand, we observed a positive direction and weak strength with NCV of the motor median nerve. There were significant correlations between changes in vitamin 25(OH)D levels with DLs, amplitudes, and NCVs of the nerves examined, where strength was moderate and negative direction with DLs, while positive direction with amplitudes and NCVs.

The role of IL-8 on NCSs among subjects who received placebo or vitamin D supplementation was not significant. The role of IL-8 on amplitudes of sensory sural nerve and NCV of motor tibial nerve were significant with regression coefficient values of
### Table 1: Characteristics data of the subjects.

| Characteristics                      | Placebo group | Vitamin D group | p-value |
|---------------------------------------|---------------|-----------------|---------|
| Gender                                |               |                 |         |
| Male, n (%)                           | 9 (36.0)      | 4 (16.0)        | 0.111   |
| Female, n (%)                         | 16 (64.0)     | 21 (84.0)       |         |
| Age, years, mean ± SD                 | 58.2 ± 9.4    | 54.5 ± 7.1      | 0.232   |
| Duration of DM, years, mean (min–max) | 5.0 (0.5–20.0) | 4.0 (0.5–28.0) | 0.539   |
| HbA1c, %, mean ± SD                   | 8.3 ± 1.5     | 9.2 ± 2.7       | 0.130   |
| 25(OH)D level at baseline, ng/mL, mean ± SD | 18.5 ± 5.1 | 16.0 ± 5.0      | 0.091   |
| IL-8 level at baseline, pg/mL, mean ± SD | 54.3 ± 54.8 | 52.1 ± 31.4     | 0.866   |

SD: standard deviation; DM: diabetes mellitus; HbA1c: hemoglobin A1c; IL: interleukin.

### Table 2: The differences between mean vitamin 25(OH)D and interleukin-8 (IL-8) levels before and after vitamin D supplementation.

| Levels                      | Before          | After           | p-level |
|-----------------------------|-----------------|-----------------|---------|
| 25(OH)D, ng/mL              | 16.0 ± 5.0      | 36.0 ± 12.3     | 0.001*  |
| IL-8, pg/mL                 | 52.1 ± 31.4     | 29.7 ± 23.1     | 0.002*  |

T dependent test. *p < 0.050.

### Table 3: The correlation between changes in vitamin 25(OH)D, interleukin-8 (IL-8) levels, and NCS.

| Variables                              | IL-8 | 25(OH)D |
|----------------------------------------|------|---------|
|                                        | R    | p-value | R    | p-value |
| Distal latency, ms                     |      |         |      |         |
| Motor median nerve                     | -0.026 | 0.856 | -0.362 | 0.010* |
| Motor ulnar nerve                      | 0.058 | 0.691 | -0.514 | < 0.001** |
| Sensory median nerve                   | -0.028 | 0.849 | -0.056 | 0.698 |
| Sensory ulnar nerve                    | -0.340 | 0.016* | -0.513 | < 0.001** |
| Motor peroneal nerve                   | 0.066 | 0.649 | -0.469 | 0.001* |
| Motor tibial nerve                     | -0.071 | 0.623 | -0.537 | < 0.001** |
| Sensory sural nerve                    | 0.000 | 0.999 | -0.132 | 0.361 |
| Amplitude, mV                          |      |         |      |         |
| Motor median nerve                     | -0.126 | 0.383 | 0.625 | < 0.001** |
| Motor ulnar nerve                      | -0.050 | 0.728 | 0.551 | < 0.001** |
| Sensory median nerve                   | -0.184 | 0.202 | 0.493 | < 0.001** |
| Sensory ulnar nerve                    | -0.092 | 0.527 | 0.494 | < 0.001** |
| Motor peroneal nerve                   | -0.131 | 0.364 | 0.564 | < 0.001** |
| Motor tibial nerve                     | -0.037 | 0.801 | 0.520 | < 0.001** |
| Sensory sural nerve                    | -0.282 | 0.047* | 0.461 | 0.001* |
| Nerve conduction velocity, m/s         |      |         |      |         |
| Motor median nerve                     | 0.289 | 0.042* | 0.525 | < 0.001** |
| Motor ulnar nerve                      | 0.059 | 0.686 | 0.438 | 0.001* |
| Sensory median nerve                   | -0.246 | 0.086 | 0.257 | 0.072 |
| Sensory ulnar nerve                    | -0.008 | 0.958 | 0.527 | < 0.001** |
| Motor peroneal nerve                   | 0.073 | 0.377 | 0.494 | < 0.001** |
| Motor tibial nerve                     | -0.378 | 0.007* | 0.235 | 0.101 |
| Sensory sural nerve                    | -0.074 | 0.611 | 0.231 | 0.106 |

NCS: nerve conduction study; R: correlation coefficient; Pearson’s correlation test. *p < 0.050; **p < 0.001.
-0.009 and -0.027. This means an increase in IL-8 resulted in a decrease on amplitudes of sensory sural nerve and NCV of motor tibial nerve by 0.009 and 0.027 [Table 4].

The role of vitamin D supplementation on NCSs was significant, except for DLs and NCVs of sensory median and sural nerves, and NCV of motor tibial nerve [Table 5].

**DISCUSSION**

Interleukins, such as IL-8, play an important role in inflammation because they mediate inflammatory cells in the acute and chronic inflammatory processes. Early inflammatory stimulus leads to the release of IL-8. Interleukins, such as IL-8, play an important role in inflammation because they mediate inflammatory cells in the acute and chronic inflammatory processes. Early inflammatory stimulus leads to the release of IL-8.

In this study, there were significant differences between mean vitamin D25 (OH) and IL-8 levels before and after receiving vitamin D supplementation (p = 0.001 and p = 0.002). The Endocrine Society Clinical Practice Guidelines recommends that adults with vitamin D deficiency are given vitamin D2 or D3 50 000 IU/week for eight weeks to reach vitamin 25(OH)D level > 30 ng/mL, followed by a maintenance dose of 1500–2000 IU/day. Supplementation of vitamin D at a dose of 30 000 IU/week increased vitamin 25(OH)D level by 2.26–2.92 ng/week for the first eight weeks and followed by a slight increase of 1.64–1.73 ng/week over 12 weeks.

Vitamin D metabolites and their analogs could reduce IL-8 as hyperinflammatory macrophages. Glucose regulates IL-8 production at the transcription level. Vitamin D deactivates NF-κB, which regulates transcription of pro-inflammatory cytokine encoding genes.

In cell culture experiments, there was a decrease in IL-8 by vitamin D metabolites and its analogs.
only if high vitamin D concentrations were achieved. Normal vitamin D levels for a tropical country were 54–90 ng/mL. In our study, the mean vitamin 25(OH)D level after receiving vitamin D supplementation was 36.0±12.3 ng/mL. So, vitamin 25(OH)D level still has not reached normal or adequate levels. Therefore, this study found no significant correlation between vitamin 25(OH)D and IL-8 levels. Inflammatory mechanisms have an important role in neuropathy. Pro-inflammatory cytokines affect glia cells and neurons involved in the pathological process of DN. Pro-inflammatory cytokines, such as TNF-α, IL-1, IL-6, IL-8, monocyte chemoattractant protein 1, and C-reactive protein. Our study found that significant correlations with negative direction and weak strength between IL-8 levels with DL of sensory ulnar, amplitudes of sensory sural, and NCV of motor tibial nerves. On the other hand, we found a positive direction and weak strength with NCV of motor median nerve. The increase in IL-8 levels resulted in the decrease in DL of sensory ulnar, amplitude of sensory sural, and NCV of motor tibial nerves. Myelin damage affects nerve conduction, resulting in prolonged DL and slowing NCV. The most common compression neuropathies in DM patients are carpal tunnel syndrome (30%) and ulnar neuropathy (2.1%).

We found significant correlations between changes in vitamin 25(OH)D levels with DLs, amplitudes, and NCVs of the nerves examined, where strength was moderate and negative direction with DLs, while positive direction with amplitude and NCVs. A decrease in vitamin 25(OH)D levels resulted in the increase in DLs, whereas amplitude and NCVs decreased.

Abdelsadek et al. also found significant correlations between vitamin 25(OH)D level and amplitudes of motor peroneal, sensory median, and sensory sural nerves and the NCV of the sensory sural nerves \( (p < 0.001) \). Lower levels of vitamin 25(OH)D were associated with a decrease in NCV of peroneal and sural nerves. The severity of NCV abnormality was closely associated with serum 25(OH)D concentrations.

Our study found no significant correlations between changes in vitamin 25(OH)D levels with DL and NCV of sensory median, NCV of motor tibial, and the NCV of sensory sural nerves. DPN patients show varying degrees of nerve regeneration and degeneration. Axonal regeneration is the body’s natural response to compensate for damage caused by DM, but only partial regeneration is an important component of DN development. Regeneration of nerve fibers is delayed in the tibial (motor) and sural (sensory) nerves.

The role of IL-8 on NCSs among patients who received placebo or vitamin D supplementation was not significant. The role of IL-8 on the amplitude of the sensory sural nerve and NCV of motor tibial nerve were significant with regression coefficient values of -0.009 and -0.027. Immunohistochemical detection of IL-8 was used as a diagnostic marker of traumatic axonal lesions. Axonal degeneration results in a decrease in sensory and motor amplitude. There is a linear relationship between the number of missing axons and sensory amplitude. Motor amplitude is less sensitive at the onset of axonal lesions. The role of vitamin D supplementation on NCSs was significant. That means vitamin D supplementation resulted in decreased DLs, increased amplitudes and NCVs (except for DLs and NCVs of sensory median and sural nerves, and NCV of the motor tibial nerve). These could be related to the pathophysiology underlying DPN, where the duration of DM was prolonged and the pathophysiology was mainly axonal, and in which case would take more than eight weeks for nerves to regenerate or correct the effects of vitamin D deficiency. In this study, vitamin D supplementation was for 10 weeks, so some nerves did not experience significant improvement.

We did not document the patients’ diets and their duration of sun exposure, which contributes as a limitation of this study. Further studies should be conducted on a large number of patients...
before coming to definitive conclusions, especially modulation of IL–8 by vitamin D supplementation.

CONCLUSION

Higher IL–8 levels were correlated with poorer amplitudes of sensory sural nerves and NCV of motor tibial nerves. Lower vitamin 25(OH)D levels were correlated with poorer DLs, amplitudes, and NCVs. There was no significant correlation between vitamin 25(OH)D and IL–8 levels. Thus, no sufficient evidence that vitamin D supplementation modulates IL–8 in Indonesian patients with DPN. Our results show that a weekly dose of 50 000 IU of vitamin D₃ for 10 weeks was effective at improving the manifestation of polyneuropathy (NCs) in diabetic patients.

Disclosure

The authors declared no conflicts of interest. No funding was received for this study.

REFERENCES

1. Vanherweghem A, Gyselens C, Mathieu C, Vitamin D and diabetes. In: Feldman D, Pike JW, Bouillon R, Giovannucci E, Goltzman D, Hewison M, editors. Vitamin D: health, disease, and therapeutics. 4th ed. Academic Press Elsevier; 2018. p. 969-989.

2. Rachana ST. Diabetic neuropathy: its pathogenesis and therapeutic drug targets. J Cell Sci Molec Biol. 2014;1(1):102.

3. Calton EK, Keane KN, Newsholme P, Soares MJ. The impact of vitamin D levels on inflammatory status: a systematic review of immune cell studies. PLoS One 2015 Nov;10(11):e0141770.

4. Doupis J, Lyons TE, Wu S, Gannaradli C, Dinh T, Vees A. Microvascular reactivity and inflammatory cytokines in painful and painless peripheral diabetic neuropathy. J Clin Endocrinol Metab 2009 Jun;94(6):2157-2163.

5. Magrinielli F, Briani C, Romano M, Ruggero S, Toffanin E, Triolo G, et al. The association between serum cytokines and damage to large and small nerve fibers in diabetic peripheral neuropathy. J Diabetes Res 2015;2015:547834.

6. Prattichizzo F, De Nigris V, Spiga R, Mancuso E, La Sala L, Antonicelli R, et al. Inflammageing and metaflammation: the yin and yang of type 2 diabetes. Ageing Res Rev 2018 Jan;41:1-17.

7. Nguyen DV, Shaw LC, Grant MB. Inflammation in the pathogenesis of microvascular complications in diabetes. Front Endocrinol (Lausanne) 2012 Dec;3(170):170.

8. Wilson N, Wright D. Inflammatory mediators in diabetic neuropathy. J Diabetes Metab. 2011 [cited 2019 August 6]. Available from: https://www.longdom.org/open-access/safety-and-efficacy-of-weekly-30000-iu-vitamin-d-supplementation-as-aslower-dosing-admistration-compared-to-a-daily-maint-2329-6887-1000233.pdf.

9. Zhou J, Zhou S. Inflammation: therapeutic targets for diabetic neuropathy. Mol Neurobiol 2014 Feb;49(1):536-546.
performance in peripheral artery disease. Vasc Med 2012 Oct;17(5):294-302.

27. Ongun N, Erdogan C, Topsakal S, Oncel C. Comparison of vitamin D levels among patients with diabetes with or without polyneuropathy. J Neuropsychiatr Neurol Disord 2017;4(102):1-4.

28. Hur J, Sullivan KA, Callaghan BC, Pop-Busui R, Feldman EL. Identification of factors associated with sural nerve regeneration and degeneration in diabetic neuropathy. Diabetes Care 2013 Dec;36(12):4043-4049.

29. Hayashi T, Ago K, Nakamae T, Higo E, Ogata M. Interleukin (IL)-8 immunoreactivity of injured axons and surrounding oligodendrocytes in traumatic head injury. Forensic Sci Int 2016 Jun;263:48-54.

30. Kakrani AL, Gokhale VS, Vohra KV, Chaudhary N. Clinical and nerve conduction study correlation in patients of diabetic neuropathy. J Assoc Physicians India 2014 Jan;62(1):24-27.

31. Chabas JF, Stephan D, Marqueste T, Garcia S, Lavaut MN, Nguyen C, et al. Cholecalciferol (vitamin D3) improves myelination and recovery after nerve injury. PLoS One 2013 May;8(5):e65034.

32. Shehab D, Al-Jarallah K, Abdella N, Mojiminiyi OA, Al Mohamedy H. Prospective evaluation of the effect of short-term oral vitamin d supplementation on peripheral neuropathy in type 2 diabetes mellitus. Med Princ Pract 2015;24(3):250-256.