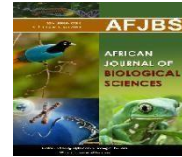


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### **CORRELATION BETWEEN VITAMIN B12 , SERUM PHOSPHATE, SERUM MAGNESIUM AND SEVERITY OF PERIPHERAL NEUROPATHY IN PATIENTS WITH TYPE II DIABETES MELLITUS.**

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[doi: 10.48047/AFJBS.6.15.2024.1505-1516](https://doi.org/10.48047/AFJBS.6.15.2024.1505-1516)**ABSTRACT**

**Introduction:** Diabetic peripheral neuropathy (DPN) is a common microvascular complication that affects nearly 50% of people with diabetes within their lifetime. The pathogenesis of diabetic peripheral neuropathy (DPN) is multifactorial, involving both metabolic and neurovascular mechanisms. Among the various metabolic factors implicated, deficiencies in vitamin B12, serum magnesium, and serum phosphate have emerged as potential contributors to nerve damage in diabetic patients.

**Aim:** To explore the association between vitamin B12, serum magnesium, serum phosphate levels, and the severity of peripheral neuropathy in patients with type 2 diabetes mellitus.

**Methods:** The present study was a hospital-based observational study. Patients aged more than 18 years with a confirmed diagnosis of type 2 diabetes mellitus were included in the study. A total of 150 patients with type 2 diabetes mellitus were selected from the Outpatient department for the study using consecutive sampling. The severity of diabetic peripheral neuropathy was assessed with the help of the Toronto Clinical Scoring System (TCSS) which is a validated 5-scoring system for DPN.

**Results:** The severity of peripheral neuropathy among the type 2 diabetes mellitus using the TCSS scale found that 28.0% of patients had mild neuropathy, 24.0% had moderate neuropathy and 12.0% had severe neuropathy. It was found that 28.0% had low serum vitamin B12 levels, 42.6% had low serum magnesium levels and 37.0% had low serum phosphate levels. There was a significant association found between serum vitamin B12, magnesium, phosphate level, and severity of peripheral neuropathy ( $p < 0.001$ ).

**Conclusion:** Vitamin B12 deficiency is found to be a potential comorbidity in type 2 diabetes mellitus patients. It significantly worsens peripheral neuropathy in these patients. It was concluded that an adequate magnesium and phosphate level in patients with glycemic control can prevent the development of diabetic peripheral neuropathy.

**Keywords:** Diabetic peripheral neuropathy (DPN), Vitamin B12 deficiency, serum magnesium, serum phosphate

**Introduction**

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from insulin resistance and inadequate insulin secretion. It is associated with a myriad of complications, among which peripheral neuropathy (PN) is one of the most prevalent and debilitating<sup>1</sup>. Peripheral neuropathy refers to damage to the peripheral nerves that leads to symptoms such as pain, numbness, tingling, and impaired sensation in the extremities, significantly impacting patients' quality of life<sup>2</sup>.

Diabetic peripheral neuropathy (DPN) is a common microvascular complication that affects nearly 50% of people with diabetes within their lifetime. It predominantly affects the hands and lower limbs and leads to loss of protective sensation, resulting in continuous injury to insensitised feet. The prevalence of DPN varies widely across different regions and populations, ranging from 6% to 51% of diabetics. This variation may be due to differences in the diagnostic criteria, methods, and instruments used to assess DPN, as well as the characteristics and risk profiles of the study populations<sup>3</sup>.

The pathogenesis of diabetic peripheral neuropathy (DPN) is complex and multifactorial, involving metabolic, vascular, and neurodegenerative mechanisms. Chronic hyperglycemia is considered a major contributor, leading to oxidative stress, microvascular damage, and impaired nerve conduction. However, emerging evidence suggests that deficiencies in certain micronutrients, such as vitamin B12, magnesium, and phosphate, may also play crucial roles in the development and progression of neuropathic complications in diabetes<sup>4</sup>.

Vitamin B12, also known as cobalamin, is essential for the maintenance of myelin sheaths and the proper functioning of nerve cells. Deficiency in vitamin B12 can lead to neuropathy characterized by sensory and motor disturbances. In individuals with T2DM, vitamin B12 deficiency may exacerbate neuropathic symptoms by impairing nerve regeneration and contributing to nerve damage<sup>5</sup>.

Magnesium is involved in numerous biochemical processes, including nerve conduction and neurotransmitter release. Low serum magnesium levels have been associated with increased neuropathic pain and altered nerve function<sup>6</sup>. In diabetes, magnesium deficiency may exacerbate neuropathy through its role in insulin resistance and inflammation, both of which are implicated in the pathogenesis of DPN<sup>7</sup>.

Phosphate is crucial for energy metabolism and bone health but its role in neuropathy is less understood compared to vitamin B12 and magnesium. Recent studies have suggested that abnormal phosphate metabolism may contribute to nerve dysfunction and neuropathic symptoms, particularly in the context of metabolic disturbances such as diabetes<sup>7,8</sup>.

The adverse effects of Diabetic peripheral neuropathy (DPN) are compounded by poor foot hygiene, improper footwear, and frequent barefoot walking, in such circumstances complications of foot infections and gangrene are a common cause of hospital admissions. The early detection of DPN using an objective screening test followed by its appropriate management is important, as up to 50% of diabetic peripheral neuropathies may be asymptomatic<sup>9</sup>.

Despite the growing recognition of the potential role of vitamin B12, magnesium, and phosphate in diabetic neuropathy, there remains a significant gap in understanding their individual and collective impacts on the severity and progression of peripheral neuropathy in T2DM patients. Investigating the associations between these micronutrients and neuropathic outcomes could provide valuable insights into novel therapeutic strategies or preventive measures for managing diabetic complications.

**Aim:** To explore the association between vitamin B12, serum magnesium, serum phosphate levels, and the severity of peripheral neuropathy in patients with type 2 diabetes mellitus.

**Objectives:**

To assess the status of vitamin B12 deficiency, hypomagnesemia, and abnormal phosphate levels in T2DM patients with varying degrees of peripheral neuropathy severity.

To investigate whether deficiencies in vitamin B12, magnesium, or phosphate are independently associated with the severity of peripheral neuropathy in T2DM patients.

### **Methodology:**

#### **Study Design and Setting:**

The present study was a hospital-based observational study, to investigate the association between vitamin B12, serum magnesium, serum phosphate levels, and the severity of peripheral neuropathy in patients with type 2 diabetes mellitus (T2DM). The study was conducted at the General Medicine Department in Chettinad Hospital and Research Institute. The study period was between May to July 2024.

#### **Study population, Inclusion and Exclusion criteria:**

Patients aged more than 18 years with a confirmed diagnosis of type 2 diabetes mellitus were included in the study. The patients with gestational diabetes mellitus, diagnosed cases of type 1 diabetes mellitus, maturity-onset diabetes of young, pancreatic or endocrine causes of diabetes, patients on a strict vegetarian diet, Presence of other significant neurological disorders unrelated to diabetic neuropathy, and Severe systemic illnesses or conditions affecting vitamin B12, magnesium, or phosphate metabolism independently of diabetes were excluded from the study.

#### **Sample size and Sampling method:**

A total of 150 patients with type 2 diabetes mellitus were selected from the Outpatient department for the study using consecutive sampling.

#### **Data variables:**

The following variables were collected: age, gender, duration of diabetes, risk factors of diabetes, previous diagnosis of diabetic neuropathy by clinical record, and previous nerve conduction study or by MNSI. Routine biochemical investigations were done to assess glycemic control, serum vitamin B12, Serum magnesium, Serum phosphate, and coexisting nephropathy.

#### **Study Tool:**

The Michigan Neuropathy Screening Instrument (MNSI) was used to diagnose diabetic neuropathy clinically. The test is administered by a symptomatic initial questionnaire and a

physical examination component. The severity of diabetic peripheral neuropathy was assessed with the help of the Toronto Clinical Scoring System (TCSS) which is a validated 5-scoring system for DPN. The criteria of classification were: 0 to 5 points without DPN, 6 to 8 points mild DPN, 9 to 11 points moderate DPN, and 12 to 19 points severe DPN.

Vitamin B12 levels were classified as low if they were less than 200 pg/mL, borderline if they were between 200 and 300 pg/mL, and normal if they were higher than 300 pg/mL, taking into account that normal values are based on one of the largest studies derived from the Diabetes Prevention Program Outcomes Study but are not standardized. The Cutoff values for serum Magnesium levels are normal plasma Magnesium: 1.5 - 2.5 mEq/L and Hypomagnesemia with <1.5 mEq/L. The Cutoff values for serum Phosphate levels were Normal value- 2.5- 4.5 mg/dL and low serum phosphate value-<2.5 mg/dL.

Data analysis:

The data was entered in MS EXCEL 2019 and analyzed using SPSS Statistics 16.0. Quantitative variables were expressed in mean standard deviation and qualitative variables were expressed in proportions. The differences between proportions were analyzed using the Chi-Square test. A probability value of less than 0.05 was considered to be statistically significant.

### Results:

A total of 150 patients with type 2 diabetes mellitus who attended the outpatient department were included in the study. The majority of the study participants belong to the 51-60 years age group (38.7%) followed by 41-50 years (34.7%). Most of the study participants were males (54.6%) and females were 45.4%. It was found that the majority of the participants had 5-10 years (44.7%) of duration of diabetes followed by more than 10 years (43.3%) and 12.0% had less than 5 years. Regarding the risk factors of diabetes, 22.7% were smokers, alcohol drinking (18.0%) and 30.7% did regular exercises.

**Table 1: Socio-demographic profile and risk factors of the study subjects (n=150)**

| Variable          | Number of Subjects | Percentage |
|-------------------|--------------------|------------|
| <b>Gender</b>     |                    |            |
| Male              | 82                 | 54.6       |
| Female            | 68                 | 45.4       |
| <b>Age Groups</b> |                    |            |
| ≤ 30 years        | 4                  | 2.6        |

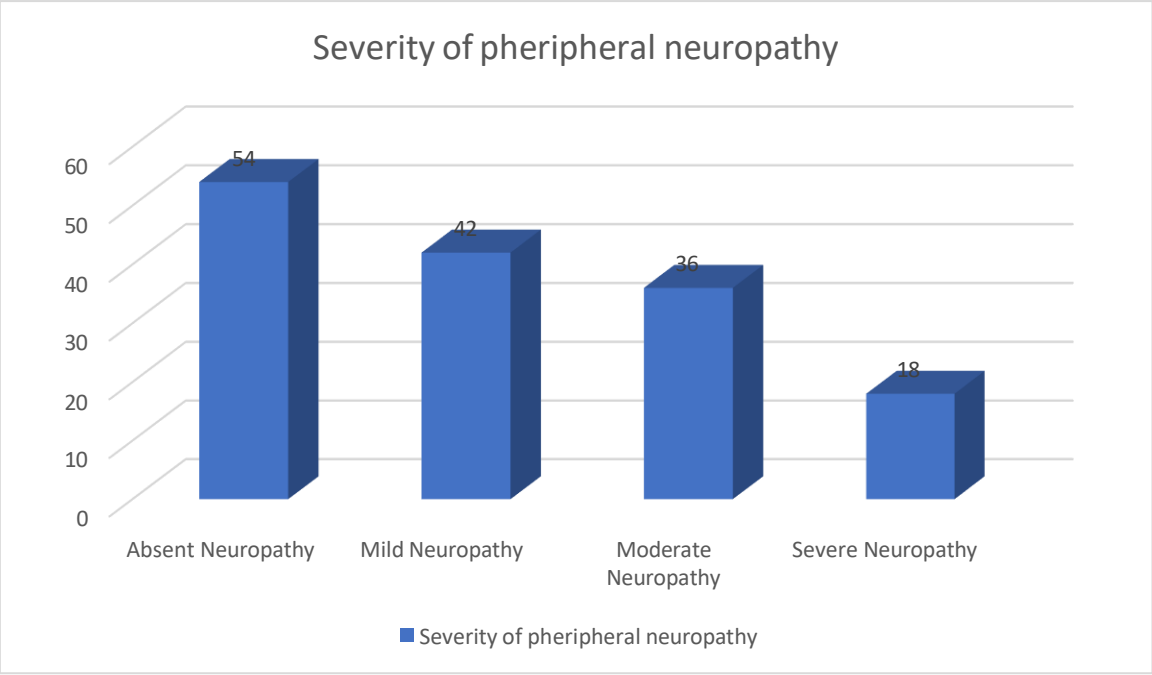
|                                 |     |      |
|---------------------------------|-----|------|
| 31-40 years                     | 28  | 18.7 |
| 41-50 years                     | 52  | 34.7 |
| 51-60 years                     | 58  | 38.7 |
| ≥60 years                       | 8   | 5.3  |
| <b>Duration of diabetes</b>     |     |      |
| <5 years                        | 18  | 12.0 |
| 5-10 years                      | 67  | 44.7 |
| >10 years                       | 65  | 43.3 |
| <b>Risk factors of Diabetes</b> |     |      |
| <b>Smoking</b>                  |     |      |
| Yes                             | 34  | 22.7 |
| No                              | 115 | 77.3 |
| <b>Alcohol</b>                  |     |      |
| Yes                             | 27  | 18.0 |
| No                              | 123 | 82.0 |
| <b>Physical activity</b>        |     |      |
| Yes                             | 46  | 30.7 |
| No                              | 104 | 69.3 |

Table 2: Statistics of different parameters of the study subjects (n=150)

| Parameters           | Mean   | Standard deviation | Minimum | Maximum |
|----------------------|--------|--------------------|---------|---------|
| FBS                  | 163.65 | 45.53              | 83.0    | 223.0   |
| PPBS                 | 238.45 | 63.18              | 145.0   | 416.0   |
| HbA1C                | 9.61   | 1.62               | 5.3     | 13.4    |
| Vitamin B 12 (pg/mL) | 412.3  | 154.1              | 978.0   | 97.0    |
| Magnesium            | 1.51   | 0.35               | 1.10    | 2.50    |
| Phosphate            | 2.13   | 0.46               | 0.54    | 4.3     |

The mean, Standard deviation, and range of the parameters are shown in this table

Figure 1: Severity of peripheral neuropathy among the study subjects (n=150)



The severity of peripheral neuropathy among the type 2 diabetes mellitus using the TCSS scale found that 54 patients had absent neuropathy, 42 patients had mild neuropathy, 36 patients had moderate neuropathy and 18 patients had severe neuropathy.

Table 3: Association between Vitamin B 12 level and Severity of Peripheral Neuropathy in Type 2 DM patients (n=150)

| Vitamin B 12 status | Severity of Peripheral Neuropathy |             |                 |               | p-value |
|---------------------|-----------------------------------|-------------|-----------------|---------------|---------|
|                     | Absent (n=54)                     | Mild (n=42) | Moderate (n=36) | Severe (n=18) |         |
| Low                 | 3                                 | 8           | 18              | 13            | <0.0001 |
| Borderline          | 13                                | 18          | 16              | 4             |         |



|        |    |    |   |   |  |
|--------|----|----|---|---|--|
| Normal | 38 | 16 | 2 | 1 |  |
|--------|----|----|---|---|--|

The majority of the study participants had normal vitamin B12 levels (34.0%) followed by 38.0% had borderline and 28.0% had low vitamin b12 levels. There was a statistically significant association found between Vitamin B12 level and Severity of Peripheral Neuropathy ( $p<0.001$ ). The low serum magnesium level was found in 64 patients (42.6%). There was a significant association found between serum magnesium level and severity of peripheral neuropathy ( $p<0.001$ ). The majority of the patients (62.7%) had normal serum phosphate levels and 56 patients (37.0%) had low serum phosphate levels.

**Table 4: Association between serum magnesium and phosphate and Severity of Peripheral Neuropathy in type 2 DM patients (n=150)**

| Variable               | Severity of Peripheral Neuropathy |                    |                    |                  | p-value |
|------------------------|-----------------------------------|--------------------|--------------------|------------------|---------|
|                        | Absent<br>(n=54)                  | Mild<br>(n=42<br>) | Moderate<br>(n=36) | Severe<br>(n=18) |         |
| <b>Serum magnesium</b> |                                   |                    |                    |                  |         |
| Low                    | 11                                | 16                 | 21                 | 16               | <0.0001 |
| Normal                 | 43                                | 26                 | 15                 | 2                |         |
| <b>Serum phosphate</b> |                                   |                    |                    |                  |         |
| Low                    | 8                                 | 19                 | 16                 | 13               | <0.001  |
| Normal                 | 46                                | 23                 | 20                 | 5                |         |

There was an association between serum magnesium and phosphate and the severity of peripheral neuropathy in T2DM, which was statistically highly significant.

## Discussion

Diabetic Peripheral neuropathy (DPN) is a prevalent and debilitating complication of type 2 diabetes mellitus (T2DM), affecting a significant proportion of patients and often leading to reduced quality of life and increased healthcare costs.

In the present study, the majority of the study participants belong to the 51-60 years age group (38.7%) followed by 41-50 years (34.7%) and most of them were males (54.6%). The mean age of the study participants was  $45.16 \pm 8.34$ . Similarly, a study by Basu DA et al<sup>10</sup> found that out of 57 patients with DPN 35 (61.42%) were male and 22 (38.58%) were female. A study by Muhurdaroglu M et al<sup>11</sup> reported that the mean age of the patients was  $60.6 \pm 10.4$  (min: 31-max: 94) years.

In the current study, the severity of peripheral neuropathy among type 2 diabetes mellitus using the TCSS scale found that 28.0% of patients had mild neuropathy, 24.0% had moderate neuropathy and 12.0% had severe neuropathy. It was found that the majority of the participants had 5-10 years (44.7%) of duration of diabetes followed by more than 10 years (43.3%) and 12.0% had less than 5 years. Similarly, a study by Negussie YM et al<sup>12</sup> observed that 76.1% of the participants had less than 5 years duration of diabetes and 23.9% had more than 5 years of diabetes.

In the present study, vitamin B12 level was found to be low for 28.0% of patients and 38.0% had borderline vitamin b12 levels. There was a statistically significant association found between Vitamin B12 level and Severity of Peripheral Neuropathy ( $p < 0.001$ ). A study conducted by Pflipsen MC et al<sup>13</sup> found 22.0% of type 2 diabetic patients to have metabolically confirmed vitamin B12 deficiency which is comparable to our study. Similarly, a study conducted by Beitzke et al<sup>14</sup> found a positive association between vitamin B12 deficiency and the severity of peripheral neuropathy. In studies conducted by Wile DJ et al<sup>15</sup> and Singh AK et al<sup>16</sup>, it was observed that the TCSS score was higher in patients with lower serum vitamin B12 levels. Similar findings have been reported in our study.

In this study, it was found that 42.6% of patients had low serum magnesium levels. There was a significant association found between serum magnesium level and severity of peripheral neuropathy ( $p < 0.001$ ). A study by Jamali AA et al<sup>17</sup> found that 56.45% of subjects with T2DM had hypomagnesemia and Diabetic peripheral neuropathy was observed in 58 (49.2%) of subjects with normal magnesemia and 81 (52.9%) subjects with hypomagnesemia. Low serum magnesium in patients with peripheral diabetic neuropathy could be the free interpreter of disease progression. Constant low levels of magnesium lead to higher blood sugar levels. The extent of magnesium reduction and insulin resistance is associated positively with the development and worsening of peripheral neuropathy.

In the present study, 37.0% of patients had low serum phosphate levels. There was an association found between serum magnesium level and severity of peripheral neuropathy ( $p < 0.001$ ). A study by Muhurdaroglu M et al<sup>11</sup> found that the patients with polyneuropathy had low serum phosphate levels. However, there was no significant association found between them ( $p = 0.83$ ). Similarly, a study by Zhang Q et al<sup>7</sup> found that Serum phosphate and serum magnesium levels were significantly

lower in patients with diabetic peripheral neuropathy (DPN) ( $P < 0.01$ ) and the percentages of DPN patients were lower in high tertile of serum phosphate and serum magnesium ( $P < 0.05$ ).

### Conclusion:

Vitamin B12 deficiency is found to be a potential comorbidity in type 2 diabetes mellitus patients. It significantly worsens peripheral neuropathy in these patients. The prevalence of hypomagnesemia and hypophosphatemia was increased in Type 2 diabetes mellitus patients suffering from peripheral neuropathy. Patients with diabetic neuropathy had considerably decreased serum values of magnesium and phosphate. This recommends that intermittent screening of serum vitamin B12 levels, magnesium levels, and phosphate in diabetic subjects can be helpful in early detection and better-quality management of diabetic neuropathy.

**Conflict of interest:** None

### References:

1. World Health Organization (WHO). Factsheets, Diabetes. 2021. <https://www.who.int/news-room/fact-sheets/detail/diabetes>. Accessed 24 Nov 2021.
2. International Diabetes Federation, author. *IDF diabetes atlas*. 2013. 22nd May 2016 Available from: [https://www.idf.org/sites/default/files/EN\\_6E\\_Atlas\\_Full\\_0.pdf](https://www.idf.org/sites/default/files/EN_6E_Atlas_Full_0.pdf).
3. Agarwal P et al. A comparative study of levels of vitamin B12 in patients of type 2 diabetes mellitus on metformin and not on metformin at tertiary care centre. *Int J Adv Med*. 2016 Aug;3 (3): 759-763.
4. Yagihashi S, Mizukami H, Sugimoto K. Mechanism of diabetic neuropathy: Where are we now and where to go? *J Diabetes Investig*. 2011 Jan 24;2(1):18-32.
5. Ahmed MA, Muntingh GL, Rheeder P. Perspectives on peripheral neuropathy as a consequence of metformin-induced vitamin B12 deficiency in T2DM. *Int J Endocrinol*. 2017;2017:2452853.
6. Gommers LM, Hoenderop JG, Bindels RJ, de Baaij JH. Hypomagnesemia in type 2 diabetes: A vicious circle? *Diabetes*. 2016;65(1):3–13.
7. Zhang Qi, Ji L, Zheng H, Li Q, Xiong Q, Sun W, et al. Low serum phosphate and magnesium levels are associated with peripheral neuropathy in patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract*. 2018;146:1–7.
8. Chung T, Prasad K, Lloyd TE. Peripheral neuropathy: clinical and electrophysiological considerations. *Neuroimaging Clin N Am*. 2014; 24(1):49-65.
9. Davies M, Brophy S, Williams R, Taylor A. The prevalence, severity, and impact of painful diabetic peripheral neuropathy in type 2 diabetes. *Diabetes*

- Care. 2006;29(7):1518.
10. Basu DA, Das S. Evaluation of severity of peripheral neuropathy in type 2 diabetes mellitus patients with special reference to vitamin b12 deficiency. IJSR. 2019; 8(3): 37-39.
  11. Muhurdaroglu M, Agadayi E, Kosem A. Low Magnesium Levels May Arise as a Risk Factor for the Development of Polyneuropathy in Patients with Type 2 Diabetes. Konuralp Medical Journal 2021;13(2): 169-174.
  12. Mekuria Negussie Y, Tilahun Bekele N. Diabetic peripheral neuropathy among adult type 2 diabetes patients in Adama, Ethiopia: health facility- based study. Sci Rep. 2024 Feb 15;14(1):3844.
  13. Pflipsen M, Oh RC et al. The Prevalence of Vitamin B12 Deficiency in Patients with Type 2 Diabetes: A Cross-Sectional Study. J Am Board Fam Med 2009; 22(5): 528-534.
  14. Beitzke M, Pfister P, Fortin J et al. Autonomic dysfunction and hemodynamics in vitamin B12 deficiency. Autonomic Neuroscience 2002; 97(1): 45-54.
  15. Wile DJ, Toth C. Association of Metformin, Elevated Homocysteine, and Methylmalonic Acid Levels and Clinically Worsened Diabetic Peripheral Neuropathy. Diabetes Care. 2010 Jan; 33(1): 156-161.
  16. Singh AK, Kumar A, Karamakar D, Jha RK. Association of B12 deficiency and clinical neuropathy with metformin use in type 2 diabetes patients. J Postgrad Med. 2014 Jan- Mar; 60 (1):99.
  17. Jamali, A.A., Ja mali, G.M., Tanwani, B.M., Jamali, A.A., Tanwani, Y. and Jamali, N.M. (2018) Association of Hypomagnesemia in Type 2 Diabetic Patients with and without Peripheral Neuropathy. Journal of Diabetes Mellitus, 8, 27-42.