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ORIGINAL ARTICLE

The Prevalence of Vitamin B12 Deficiency in Type 2 Diabetes with Neuropathy; A Multi-Center Study

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ABSTRACT

Background: Vitamin B12 is essential to nerve-related functionalities and hematopoiesis. There may be neuropathy-like symptoms in case of deficiency as they resemble those caused by diabetic neuropathy. The risk factors involved are patients diagnosed with Type 2 Diabetes Mellitus (T2DM), especially those patients undergoing long term metformin treatment whose absorption abilities have been affected. This deficiency cannot be reversed and it is therefore important to detect it early enough before the damage caused on the nerves is permanent. A wide range of studies have shown that there is a high incidence of B12 deficiency among patients with diabetes, in particular the peripheral neuropathy population, reinforcing the importance of regular screening and possible supplementation.

Objective: To determine the prevalence of vitamin B12 deficiency in patients with type 2 diabetes mellitus (T2DM) presenting with neuropathy, and to assess its association with metformin usage and the severity of neuropathy.

Study design: A cross-sectional Multi-Center study:

Place and duration of study: Department of Endocrinology, MTI-HMC Peshawar; Timergara Teaching Hospital, Timergara and the District Headquarter Hospital, Dir Upper from January 2022 to June 2022.

Methods: 100 T2DM patients with clinically established neuropathy. Exclusion was based on chronic alcohol consumption, renal failure and B12 supplement during a short time. Quantification of vitamin B12 was carried out by chemiluminescent immunoassay. Measurement of neuropathy was done by Michigan Neuropathy Screening Instrument. The number of metformin dose and duration were charted. Statistical operation was done through SPSS; p-value 0.05 was to be regarded as significant.

Results: Of the 100 patients, 60 per cent were Vitamin B12 deficient (<200 pg./mL). The average patients age was 58.3 9.6 years. The deficiency of B12 in people on metformin longer than 5 years was much more frequent (p = 0.002). The low B12 levels were associated strongly with very high scores of neuropathies (p=0.01). In patients, there was less severe neuropathy among those with normal levels of B12. The prevalence of deficiency did not differ significantly according to gender (p = 0.28).

Conclusion: vitamin B12 deficiency is very common in individuals who have T2DM and neuropathy, as well as in individuals who are taking long-term metformin. Screening and early supplementation of Vitamin B12 in diabetic patients can reduce the risk of developing and graduating diabetic neuropathy, thus enhancing quality of life and translate to the issue of disability among diabetic patients.

Keywords: Vitamin B12, Type 2 Diabetes, Neuropathy, Metformin

INTRODUCTION

Diabetic peripheral neuropathy (DPN) is one of the most frequent and disabling T2DM complications that patients develop in a varied number across their disease process, as many as 50 percent of the patients[1,2]. The diversity of symptoms of DPN includes numbness, burning, tingling, and muscular weakness of the limbs, which very negatively affect the quality of life and raise the threat of foot ulcers and amputations[3]. Vitamin B12 (cobalamin) is indispensable in synthesis of DNA, development of red blood cells, and sustaining neurological activity[4]. The lack of Vitamin B12 may cause megaloblastic anemia and neurological disorders that are quite similar to diabetic neuropathy. Thus, B12 deficiency in diabetics may either worsen the preexisting neuropathic conditions or simulate diabetic neuropathy, in this case, the complication could occur without an appropriate differentiation unless a specific biochemical evaluation is made[5]. The popular antidiabetic oral drug metformin is a first-line diabetes drug known to affect Vitamin B12 absorption in the gastrointestinal tract[6]. A number of studies have also shown the association of the long-term consumption of metformin and decreased serum B12 levels especially when the therapeutic doses are high, and the duration of the use is beyond five years[7]. Although this is one of the known effects, B12 monitoring is not a regular procedure in clinical practice. Vitamin B12 deficiency is likely to be reversible and its early diagnosis and therapy may avert neuropathic lesion in diabetics or even reverse them. Nevertheless, due to the fact that diabetic neuropathy and B12-deficiency neuropathy have overlapping symptoms, there is a need to exercise more clinical awareness and laboratory screening. Limited regional studies have investigated the real prevalence of the B12 deficiency in T2DM patients who have neuropathy and more so in patients who are under long-term metformin. Such difference in the literature provides the necessity of the further study. The findings highlighting high prevalence of B12 deficiency in this subgroup can be used to indicate screening recommendations especially in resource poor settings that would otherwise not use B12 testing as a normal practice[8,9].

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METHODS

This study cross sectional multi centers study conducted in Department of Endocrinology, MTI-HMC Peshawar; Timergara Teaching Hospital, Timergara and the District Headquarter Hospital, Dir Upper from January 2022 to June 2022. A total of 100 patients with a confirmed diagnosis of type 2 diabetes mellitus and clinical and/or electrophysiological features of diabetic peripheral neuropathy were enrolled after obtaining informed consent. Data were collected on age, gender, duration of diabetes, metformin dosage and duration, and serum vitamin B12 levels. Serum vitamin B12 concentration was measured using chemiluminescent immunoassay. Based on B12 levels, patients were categorized as deficient (<200 pg/mL), borderline (200–300 pg/mL), or normal (>300 pg/mL). Statistical analysis was performed using SPSS version 24.0..

Inclusion Criteria: The inclusion criteria were T2DM of more than 6 Month of duration and confirmed peripheral neuropathy since they had MNSI score of 4 or more as well as clinical examination. Patients aged 30 years and more were included.

Exclusion Criteria: Patients with chronic kidney Diseases,

chronicle of abusing alcohol, thyroid problems, B12 supplement in the previous 6 months (or more), and other recognized reasons of neuropathy were not qualified to the study.

Ethical Approval Statement: The protocol of this study had been sanctioned by the Institutional Ethics Committee .Before their enrolment, informed consent was written by all the participants. The study was conducted based on the Declaration of Helsinki on biomedical study on human beings.

Data Collection: The examination of the patients were performed during an outpatient visit. Ancillula statement was used to format questions on demographics, history of diabetes, usage of metformin, and symptoms. Neuropathy was analyzed by clinical assessment and MNSI. The tests on serum B12 in blood were conducted with the following standard procedures in the central laboratory of the hospital.

Statistical Analysis: The SPSS version 24.0 was used to analyze data. The continuous variables have been reported as mean +/- standard deviation, whereas the categorical variable as a percentage. Associations were measured using chi-square and Student t-tests. A value of p< 0.05 was found to be significant. Level of B12 and duration of use of metformin to neuropathy scores were correlated.

RESULTS

One hundred patients with T2DM, who had proven diabetic neuropathy were recruited. The average age was 58.3 +/9.6 years and male to female ratio was 1.3:1. Sixty percent of the patients had Vitamin B12 deficiency (<200 pg./mL), 25 percent of them showed borderline levels (200-300 pg./mL), and a mere 15 percent possessed normal levels. On metformin utilization, 78.3 percent of B12 deficiency patients have been taking the drug more than 5 years compared to the 32 percent of the normal B12 group (p = 0.002). Longevity in the use of metformin had a strong relationship with lower levels of B12 (r = -0.41, p = 0.001). The neuropathy scores showed significantly higher scores in patients with B12 deficiencies based on MNSI (mean score 7.1 1.3) as against the neurological normal level (mean score 4.2 0.9) (p = 0.01). There was no indication of statistical significance in relation to the prevalence of B12 deficiency between males and females (p = 0.28). There were even not seriously high levels of B12 and even greater severity of the symptoms of diabetic neuropathy. Age and B12 relationships (p = 0.12).

Table 1: Demographic and Clinical Characteristics of Study Participants (N = 100)

Variable	Value
Mean Age (years)	60.3 ± 9.6
Gender (Male/Female)	40 / 43
Mean Duration of Diabetes (years)	9.8 ± 4.2
Mean Duration of Metformin Use (years)	6.7 ± 3.5
Mean Daily Metformin Dose (mg)	1430 ± 390
Mean MNSI Score	6.3 ± 1.9
Vitamin B12 Level (pg./mL) - Mean ± SD	201.4 ± 65.2

Table 2: Distribution of Vitamin B12 Status Among Participants

Vitamin B12 Status	Frequency (n)	Percentage (%)
Deficient (<200 pg./mL)	60	60%
Borderline (200–300 pg./mL)	25	25%
Normal (>300 pg./mL)	15	15%

Table 3: Association Between Vitamin B12 Status and Duration of Metformin Use

B12 Status	Metformin Use <5 yrs. (n=40)	Metformin Use ≥5 yrs. (n=60)	p-value
Deficient	13 (32.5%)	47 (78.3%)	0.002**
Borderline	15 (37.5%)	10 (16.7%)	
Normal	12 (30.0%)	3 (5.0%)	

Table 4: Correlation Between Vitamin B12 Level and Neuropathy Severity (MNSI Score)

B12 Status	Mean MNSI Score ± SD	p-value
Deficient	7.1 ± 1.3	
Borderline	5.9 ± 1.0	
Normal	4.2 ± 0.9	0.01**

Table 5: Correlation of Vitamin B12 Deficiency with Age and Gender

Variable	Deficient B12 (n=60)	Non-Deficient B12 (n=40)	p-value
Mean Age (years)	59.2 ± 9.8	56.8 ± 9.4	0.12
Male Gender	35 (58.3%)	22 (55.0%)	0.28
Female Gender	25 (41.7%)	18 (45.0%)	

DISCUSSION

Vitamin B12 deficiency was high (60%) in patients with Type 2 Diabetes Mellitus (T2DM) accompanied by peripheral neuropathy and mainly among patients taking metformin long-term. Our results give support to the other literature that has highlighted similar relationships between long-term metformin and reduced serum Vitamin B12 levels, and worsening of the neuropathic symptoms in people with diabetes. Metformin being one of the most pivotal drugs in T2DM treatment has been found to disrupt absorption of Vitamin B12 through the calcium-dependent effect on membrane action in the ileum[10]. Past studyes have seen the prevalence of metformin-related B12 deficiency at between 10 and 30 percent depending on the dosing and duration[11]. The prevalence of our study of 60-percent was found to agree with top end estimates, especially due to the fact that we worked with a CCG cohort of patients with established neuropathy which is likely to demonstrate more severe deficiencies. Diabetes Prevention Program Outcomes study (DPPOS) noted that patients on long-term metformin (4 years and above) showed 19 percent lower Vitamin B12 level than placebo and 5.8 percent patients developed overt deficiency[12]. Equally, Ting et al. noted that patients on metformin were found to have less B12 in their body and higher risk of peripheral neuropathy, when they exceed 5 years on the treatment[13]. We have found similarities with these findings as we had a significant correlation between metformin 1-5 year and B12 deficiency (p = 0.002) and negative correlation between B12 and increase severity of neuropathy. Diagnosis is usually complicated by the clinical similarity of diabetic neuropathy and neuropathy caused by Vitamin B12 deficiency. Both are characterized by symptoms of numbness, burning and tingling, however, B12-related neuropathy can also be associated with ataxia and proprioceptive loss[14]. Such cross-sectional indicators point to the necessity to check the deficiency of Vitamin B12 in diabetic patients with neuropathy, particularly medicines taking long-lasting metformin intake. Patients with B12 deficiency reported a higher MNSI score than those with normal levels (7.1 vs. 4.2, p = 0.01) and we concluded that it was more pronounced neuropathy. This agrees with the findings of Ahmed et al., who discovered that diabetics without B12 scored 3.6 higher on neuropathy scale and had worse nerve conduction velocity compared to the other diabetics with sufficient quantity of B12[15]. On the same note, reinstate et al. reported that U.S. adults with diabetes exhibited borderline or deficient levels of B12, which was independently associated with peripheral neuropathy[16]. Others in the scientific community state that having B12 levels as low as in borderline B12 cases (200-300 pg./mL), representing a quarter of our sample, may also not be sufficient to support good neurological health. It was noted by Singleton et al. that even sub clinically B12 deficient patient can be characterized by disturbed levels of methylmalonic acid (MMA) and premature nerve damage[17]. Hence, serum B12 is subject to false negatives and thus functional markers like MMA or homocysteine can better add to the diagnosis. Our results can also be used to recommend that diabetic patients, particularly those with complaints of neuropathies and exposed to metformin, be monitored regularly by B12. The American Diabetes Association (ADA) recommends the use of B12 levels tests in individuals who use metformin in the long run and especially in case of the presence of neuropathy[18]. Nevertheless, compliance with this guideline is not at its optimum in most clinical practices. It is interesting to note that gender and age did not have any significant relationship with B12 deficiency in this study which is similar to the studies of Hermida et al., who showed no statistically significant sex-difference in the B12 state of diabetic groups[18]. This fact implies that length and dosage of metformin are

the risk factors of B12 depletion in the first place.

CONCLUSION

Deficiency in vitamin B12 is commonly experienced in T2DM patients experience neuropathy, particularly in patients under the long-term use of metformin. Obtained neuropathy cannot be worsened by routine screening and early supplementation to the point of improving the outcomes. It is critical to differentiate between diabetic and B12- deficiency neuropathy in order to manage the given disease and prevent the irreversible nerve loss.

Limitations: The study was cross-sectional and it took place at a Multi Centers. There was no measurement of functional biomarkers like methylmalonic acid and homocysteine. The results of the assessment of neuropathy were clinical, and nerve conduction tests to support and differentiate between diabetic neuropathy and B12-deficiency neuropathy lacked.

Future Findings: future studies should be in the form of multicentered and longitudinal study using larger populations with a view to determining causality. The addition of functional B12 biomarkers and nerve conduct tests can enhance diagnosis. Interventional study on B12 supplementation can provide an assessment of its effects on the progression of neuropathy and design uniform screening and therapeutic guidelines.

Abbreviations

1.	T2DM	Type 2 Diabetes Mellitus
2.	DPN	Diabetic Peripheral Neuropathy
3.	B12	Vitamin B12
4.	MNSI	Michigan Neuropathy Screening Instrument
5.	SD	Standard Deviation
6.	SPSS	Statistical Package for the Social Sciences
7.	MMA	Methylmalonic Acid
8.	ADA	American Diabetes Association
9.	DPPOS	Diabetes Prevention Program Outcomes Study
10.	IEC	Institutional Ethics Committee

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Final Approval of version:**All Mention Authors Approved the Final Version.**

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