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Association between vitamin B12 level and clinical peripheral neuropathy in type 2 diabetic patients on metformin therapy

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Abstract

Background: Vitamin B12 malabsorption is one of the side effects of long-term metformin intake. Prolonged vitamin B12 deficiency can lead to irreversible nervous system damage. So, the current study aimed to determine the association between serum vitamin B12 level and clinical peripheral neuropathy among type 2 DM patients who are on metformin in comparison to those not taking it. It is a cross-sectional study that was conducted in Egypt and recruited 100 type 2 diabetic patients who were divided into 2 groups: metformin treated, and non-metformin treated (50 subjects per group). The collected data included personal history, medical history, dietetic history that included frequency of eating food rich in vitamin B12 and clinical peripheral neuropathy assessment by Toronto Clinical Scoring System (TCSS). Blood samples were collected for assessment of HbA1c and vitamin B12 level.

Results: Vitamin B12 deficiency was present in 4% of metformin group and 2% of non-metformin group. The mean value of vitamin B12 between metformin users (624.3 ± 364.1 pg/ml) and non-metformin users (991.0 ± 489.9 pg/ml) showed a high significant difference, p value < 0.001. There was a significant difference between study groups regarding HbA1c level (p value = 0.03). Peripheral neuropathy was significantly associated with HbA1c (p value = 0.04) and female gender (p value = 0.001).

Conclusions: Vitamin B12 level was lower in type 2 diabetic patients on metformin compared to those on other oral anti-diabetic drugs, but without significant deficiency. Peripheral neuropathy was significantly associated with poor glycemic control and female gender, but was not associated with vitamin B12 deficiency.

Keywords: Metformin, Peripheral neuropathy, Type 2 diabetes mellitus, Vitamin B12 deficiency

Background

Diabetes mellitus (DM) is one of the four priority noncommunicable diseases (NCDs) that are targeted for action worldwide. The prevalence of type 2 diabetes mellitus (T2DM) has been rising over the past few decades especially in low and middle-income countries [1].

According to the international Diabetes Federation (IDF) Diabetes Atlas in 2019, the global diabetes prevalence was estimated to be 9.3% and expected to rise to 10.2% by 2030 and 10.9% by 2045. One in two (50.1%) people living with diabetes do not know that they have diabetes. Egypt is among the world's top 10 countries for number of adults with diabetes aging 20–79 years and was estimated by 8.9 million diabetes cases [2].

Metformin is considered the first-line of treatment for type 2 diabetes mellitus because of its effectiveness, safety and multiple metabolic and cardiovascular benefits [3]. Malabsorption of vitamin B12 is one of the reported side effects of long-term metformin treatment [4]. Different studies conducted on type 2 diabetic patients

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taking metformin have reported 5.8–33% vitamin B12 deficiency [5, 6] The results of a recent meta-analysis based on 29 studies with 8089 participants showed that patients receiving metformin therapy had odds ratio of 2.45 (95% CI 1.74–3.44, $p < 0.0001$) of developing vitamin B12 deficiency in comparison to the non-metformin users [4].

Peripheral neuropathy (PN) is one of the consequences of vitamin B12 deficiency and it rapidly improves with supplementation in the initial stages. Prolonged deficiency can lead to irreversible nervous system damage [7]. The recent American Diabetes Association (ADA) position statement on diabetic neuropathy in 2017 has highlighted the importance of excluding vitamin B12 deficiency in patients with diabetic neuropathy [8].

Different studies were conducted to investigate the effect of metformin-induced vitamin B12 deficiency to cause or worsen, PN in patients with diabetes [9]. A study that was conducted in Canada showed that type 2 DM patients on metformin therapy had more sever PN and it was significantly correlated with metformin dose [10]. While an Indian study showed that it was significantly correlated with metformin duration [11].

On the contrary, other studies showed no significant difference between vitamin B12 level and PN among metformin-treated type 2 DM patients [12–14]. There is scarcity in published data on metformin-induced vitamin B12 deficiency and PN in Egypt. So, the current study was designed to determine the association between serum vitamin B12 level and clinical PN among a sample of Egyptian type 2 DM patients who were treated with Metformin in comparison to patients who were not receiving it.

Methods

This was a cross-sectional study that was conducted in the outpatient Clinics of Ain Shams University Hospitals in Egypt during September 2019- September 2020. The study recruited 100 type 2 diabetic patients aging from 30 to 70 years old who were divided into 2 groups: the first group included 50 patients receiving metformin therapy and the other group included 50 patients receiving other anti-diabetic therapies.

The recruited patients fulfilled the following inclusion criteria: metformin group that included patients receiving at least 1 g metformin per day and for a duration ≥ 6 months. And non-metformin group that included patients receiving other anti-diabetic drugs than metformin for the last 6 months. While the exclusion criteria included those: who were taking vitamin B12 supplements in the last 3 months; those taking H2 blockers, antacids, or proton pump inhibitors; pregnant females, type 1 diabetes, strictly vegetarian or alcoholic patients. In addition, patients having pernicious anemia, renal insufficiency, HIV infection or malabsorption syndromes or other causes of malabsorption like gastric bypass, gastrectomy or any surgery involving the small intestine were excluded.

The study sample was calculated by Power and Sample Size Program, version 3.1 and based on the results of the recent study of *Osama and colleagues, 2016* conducted in Fayoum University Hospital outpatient clinics. The proportion of peripheral neuropathy in patients taking metformin: was 80%, the average of proportion of peripheral neuropathy in patients not taking metformin from two studies was 57% [15, 16]**.** The provisional sample size in every group was 49 patients to be able to reject the null hypothesis that the exposure rates for the 2 groups are equal, with power 80% and type 1 error 10% [17]. Uncorrected Chi-squared statistic was used to evaluate this null hypothesis.

The collected data included personal and medical history like age, gender, duration of diabetes, type of anti-diabetic medication taken by the patients, its dose and duration. In addition to dietetic history that included frequency of eating food rich in vitamin B12. These food items were selected according to Dietitian Canada Canadian Nutrient file [18] to evaluate its consumption by the study participants in term of frequency (daily, weekly, monthly and seasonal) and the portion in each time. These data were then analyzed by ESHA (Elizabeth Stewart Hands and Associates) Research Food Processor Nutrition and Fitness Software version 11.7 update in 2019 (Inc., Salem, OR, USA.) to calculate the estimated vitamin B12 daily intake of each participant. Then, it was compared to the RDA of vitamin B12 (2.4 mcg per day) to assess the adequacy of vitamin B12 daily consumption.

Neurological assessment was also done to detect clinical PN among the study participants. It was done using Toronto Clinical Scoring System (TCSS) which is a well validated tool. The score (total = 19) was divided into normal (0–5), mild (6–8), moderate (9–11) or severe (12–19) PN [19].

Finally, two blood samples were collected to measure HbA1c and serum vitamin B12 levels:

HbA1c assay: Two milliliters of venous blood were collected from each subject under complete aseptic conditions and placed into sterile EDTA vacutainer tube and whole blood was stored at 4 °C until processing (not more than 1 week). HbA1c determination was done by turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood on Cobas c311 automated analyzer (Roche Diagnostics GmbH, SandHofer Strasse 116, D-68305 Mannheim, Germany) with reference range of prediabetes 5.7–6.4%, Diabetic $\geq 6.5\%$ and good diabetes control $\leq 7.0\%$.

Serum vitamin B12 assay: Two milliliters of venous blood were collected from each subject under complete aseptic conditions and placed into sterile serum separation vacutainer tube and was left to clot for 30 min. Samples were centrifuged at 3000 rpm for 20 min for serum separation. The separated serum was stored at − 320 °C until used. Hemolyzed samples were discarded, repeated freezing and thawing was avoided. Vitamin B12 was measured by electrochemiluminescence immunoassay on Cobas e411 automated analyzer (Roche Diagnostics GmbH, SandHofer Strasse 116, D-68305 Mannheim, Germany) with serum reference range 197–771 pg./ml.

The research was conducted according to the declaration of Helsinki, the proposal and conduct of the study was ethically cleared by the Research Ethical Committee at the Faculty of Medicine, Ain Shams University, Cairo, Egypt. Informed written consent to participate in the study was obtained from the participants. All information provided by the participants was kept confidential. In addition, any information leading to identification of study participants was not included in the data collection tool.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 23 for Window (IBM Corp., Armonk, N.Y., USA. 2015) was used for data entry and analysis. Descriptive analyses of qualitative variables were done using frequencies and percentages while for quantitative variables both mean and standard deviation (SD) were applied. Group comparisons were performed using chi square (X^2) test for qualitative variables. For quantitative variables Student's *t*-test or Mann–Whitney *U* test when appropriate was done. Correlation coefficient test was performed to correlate between two quantitative variables. To determine the predictors of neuropathy score (TCSS score) and Vitamin B 12, stepwise linear regression model was done. *P* value < 0.05 was considered as significant and *p* value < 0.01 as highly significant.

Results

A total of 100 type 2 DM patients; 50 patients were on metformin therapy and 50 patients were on other oral anti-diabetic drugs. The mean age was quite similar in metformin and non-metformin users (53.2 vs 53.3 years). The mean serum vitamin B12 was significantly lower in metformin group than non-metformin group, with no significant deficiency between them. Vitamin B12 daily intake was adequate in about 70% of both groups. The mean HbA1c level was significantly lower in metformin group compared to non-metformin group. The frequency of PN was high in both groups, with no significant difference between them. No statistically significant difference in age, sex, diabetes duration, vitamin B12 estimated daily intake, adequacy of vitamin B12 daily intake, or frequency and severity of PN between metformin and non-metformin users (Table 1).

Table 1 Characteristics of metformin and non-metformin groups

Variables	Non-metformin group *n* = 50	Metformin group *n* = 50	*P* value
Gender			
Female	39 (78%)	32 (64%)	0.123
Male	11 (22%)	18 (36%)	
Age (years)	53.3 ± 7.9	53.2 ± 7.2	0.926
Diabetes duration (years)	6.3 ± 5.0	7.3 ± 5.1	0.295
Estimated vitamin B12 daily intake (mcg)	7.1 ± 16.5	5.4 ± 4.5	0.535
Adequacy of vitamin B12 daily intake (≥ 2.4 mcg) *n* (%)	36 (72%)	35 (70%)	0.826
Serum vitamin B12 (pg/ml)	991.0 ± 489.9	624.3 ± 364.1	< 0.001
Vitamin B12 deficiency *n* (%)	1 (2%)	2 (4%)	1.000
HbA1c (%)	8.6 ± 2.3	7.6 ± 2.0	0.03
Peripheral Neuropathy frequency *n* (%)	32 (64%)	36 (72%)	0.391
TCSS neuropathy score	7.7 ± 3.7	7.8 ± 3.4	0.910
TCSS grade			
Normal (0–5)	18 (36%)	14 (28%)	
Mild (6–8)	12 (24%)	17 (34%)	0.452
Moderate (9–11)	14 (28%)	10 (20%)	
Sever (12–19)	6 (12%)	9 (18%)	

P value < 0.05 significant; *P* value < 0.01 highly significant

TCSS Toronto Clinical Scoring System

Regarding the relation between PN and the other study variables, PN was significantly associated with female gender, HbA1c level and metformin duration. However, after adjustment for confounders (age, diabetes duration, metformin dose, and serum vitamin B12 level), predictors of PN were female gender and high HbA1c (Tables 2 and 4).

Table 3 shows a high significant difference between gender and vitamin B12 level, it was higher in females than males. Vitamin B12 level was positively correlated with HbA1c level while there were no correlations with the other study variables like age, diabetes duration, metformin dose and duration, daily vitamin B12 intake and TCSS score. However, the adjusted model showed that predictors of vitamin B12 level were age, female gender, and HbA1c level (Table 4).

Discussion

The reported frequency of vitamin B12 deficiency related to metformin use varies in different studies. The present study showed that vitamin B12 deficiency was present in 4% of the metformin group and 2% in the non-metformin one with no statistically significant difference between the 2 groups. The United States National Health and Nutrition Examination Survey (1999- 2006) showed that vitamin B12 deficiency was present in 5.8% of diabetic patients using metformin compared with 2.4% of those not using it [5]. In another comparable study that was recently conducted on 351 type 2 DM patients in Botswana, most of them were metformin treated, about 6.6% of patients had vitamin B12 deficiency [20]. While a study done in South Africa among 121 metformin treated type 2 DM patients reported that 28.1% of the patients had vitamin B12 deficiency. This higher frequency was attributed to the very high mean of metformin dose (2400 mg) and duration (9.6 years) among the study participants compared to other studies [21].

The present study showed no significant association between vitamin B12 deficiency and estimated vitamin B12 daily intake or metformin use. However, metformin users had lower mean of serum vitamin B12 level 624.3 (SD 364.1) than the non-metformin users 991.0 (SD 489.6), p value less than 0.01. This agrees with a case control study done in Pakistan among 114 metformin treated and 105 non-metformin treated T2DM patients, in which the mean of vitamin B12 level was 311 (SD 194.4) in metformin group and 414 (SD 223.5) in non-metformin group (p value 0.03) [22].

The glycemic status of those taking metformin was not fully controlled (HbA1c 7.5%) but better than non-metformin users who's mean HbA1c was 8.6% (SD 2.3), with a significant difference (p value 0.03). Vitamin B12

Table 2 The relation between peripheral neuropathy and different study variables

Variable	Peripheral neuropathy		
	Yes	No	*P* value
Age (years)	52.8 ± 8.2	54.3 ± 5.9	0.334
Sex frequency (%)			
Males	13 (19.1)	16 (50.0)	0.001
Females	55 (80.9)	16 (50.0)	
Diabetes duration (years)	6.9 ± 5.4	6.6 ± 4.1	0.806
Metformin dose (among metformin group, *n* = 50)	1420.8 ± 525.0	1478.6 ± 390.6	0.711
Metformin duration (among metformin group, *n* = 50)	4.9 ± 4.3	2.6 ± 2.3	0.021
HbA1c Level (%)	8.4 ± 2.3	7.5 ± 1.9	0.04
Vitamin B12 Level (pg/ml)	803.5 ± 445.5	816.4 ± 517.5	0.912
Vitamin B12 dietary intake	7.2 ± 14.4	4.4 ± 3.7	0.329

P value < 0.05 significant; *P* value < 0.01 highly significant

Table 3 Comparison between males and females as regards serum vitamin B12

Groups	Gender	Serum vitamin B12	
		Mean ± SD	*P* value*
Both groups (n = 100)	Males	595.3 ± 368.2	0.001
	Females	894.4 ± 477.6	
Metformin group (n = 50)	Males	512.4 ± 258.2	0.140
	Females	687.3 ± 402.0	
Non-metformin group (n = 50)	Males	731.0 ± 483.3	0.045
	Females	± 471.8	

* Mann–Whitney *U* test

P value < 0.05 significant; *P* value < 0.01 highly significant

Table 4 Predictors of neuropathy score (TCSS score) and vitamin B 12—final models (stepwise linear regression)

Predictors	ß	*t*-test	*P* value	Model P value (using ANOVA)
I) Predictors of TCSS score				
1-Sex	2.01	2.75	0.007	0.003
2-HBA1c	0.28	1.90	0.06	
II) Predictors of serum Vitamin 12				
1-Sex	269.6	2.88	0.005	< 0.001
2-Age	13.5	2.34	0.021	
3-HBA1c	63.5	3.26	0.002	

P value < 0.01 highly significant

ß regression coefficient

ANOVA analysis of variance

TCSS Toronto Clinical Scoring System

level was higher in females and patients with poor glycemic control. The study of *Ahmed and colleagues* (2016) reported the same association between HbA1c levels and vitamin B12, and they assumed that adverse gastrointestinal effects of metformin could lead to non-compliance in metformin users. So, patients with poor glycemic control may have bad compliance to metformin and hence have good vitamin B12 levels [21]. In addition, our study showed that vitamin B12 levels were higher with increasing age. This is unlike the fact that vitamin B12 deficiency is common in elderly due to inadequate intake or protein bound malabsorption and atrophic gastritis. This might be explained by, that the old patients who had higher levels of vitamin B12 had recent vitamin B12 supplements which they did not recall. Synthetic vitamin B12 could be absorbed as it is not protein bound [23].

Regarding the relation between PN and the other variables, the current study reported a significant association with female sex, unlike the study of Jordan that reported more prevalent PN among males [14]. Also, PN was significantly associated with poor glycemic control which is known to have a major role in the pathogenesis of diabetic PN [24, 25].

The prevalence of PN in metformin group and non-metformin group was 72% and 64%, respectively, with no significant association. This agrees with the study of *Russo and colleagues* (2016) who reported that PN was neither associated with metformin use nor vitamin B12 deficiency. Metformin induced B12 deficiency usually starts early as the 4th month of treatment, but the clinical consequences of B12 deficiency become manifested about 5 years later after depletion of the liver stores [26]. So, the metformin duration in this study may not be long enough to cause significant vitamin B12 deficiency and PN. Moreover, PN showed no significant association with age of patients and duration of diabetes. This disagrees with *Elhadd and colleagues* (2018) study who reported that neuropathy increased with advanced age and long disease duration. The mean age was nearly similar in both studies while the mean disease duration was shorter than 10 years in our study, unlike *Elhadd and colleagues* (2018) study that was more than 10 years [13]. The incidence of diabetic PN increases by 50% after 10 years of diabetes disease duration [8].

Conclusions

Vitamin B12 level was lower in type 2 diabetic patients on metformin compared to those on other oral anti-diabetic drugs, but without significant deficiency. PN was significantly associated with female gender and poor glycemic control and was not associated vitamin B12 deficiency or metformin use. Vitamin B12 level was higher in females and patients with poor glycemic control. However, these raising points need further assessment in cohort studies with large sample size.

Abbreviations

ADA: American Diabetes Association; DM: Diabetes mellitus; ESHA: Elizabeth Stewart hands and associates; IDF: International diabetes federation; NCDs: Non-communicable diseases; PN: Peripheral neuropathy; SPSS: Statistical package for social sciences; T2DM: Type 2 diabetes mellitus; TCSS: Toronto clinical scoring system; TINIA: Turbidimetric inhibition immunoassay.

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Author contributions

All authors (NFHF, MG, DM, MMF, MMA and MFA) had participated in the study design, data management, data analysis, decision-making on content and paper write-up and revision of final draft. All authors read and approved the final manuscript.

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Availability of data and materials

Data may be made available upon reasonable request.

Declarations

Ethics approval and consent to participate

Informed written consent to participate in the study was obtained from all the study participants. The study protocol was reviewed and approved from the Research Ethical Committee of Faculty of Medicine, Ain Shams University (FMASU M D 264/ 2018).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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