

# Association of vitamin B<sub>12</sub> deficiency in people with type 2 diabetes on metformin and without metformin: a multicenter study, Karachi, Pakistan

Zahid Miyan <sup>1</sup>, Nazish Waris,<sup>2,3</sup> MIBD

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<sup>1</sup>Department of Medicine, Baqai Institute of Diabetology and Endocrinology, Baqai Medical University, Karachi, Pakistan

<sup>2</sup>Clinical Biochemistry and Psychopharmacology Research Unit, Department of Biochemistry, University of Karachi, Karachi, Pakistan

<sup>3</sup>Department of Research, Baqai Institute of Diabetology and Endocrinology, Baqai Medical University, Karachi, Pakistan

**Correspondence to**  
Dr Zahid Miyan;  
drzahidmiyan@yahoo.com

## ABSTRACT

**Objective** To assess the prevalence of vitamin B<sub>12</sub> deficiency in people with type 2 diabetes mellitus (T2DM) on metformin and without metformin.

**Methodology** Between May 2018 and January 2019, this prospective multicenter observational study recruited participants from seven centers in four provinces of Pakistan (Sindh, Punjab, Baluchistan and Khyber Pakhtunkhwa). Participants with T2DM treated with metformin for >2 years and those not on metformin underwent assessment of hemoglobin, vitamin B<sub>12</sub>, homocysteine and diabetic neuropathy (vibration perception threshold (VPT) >15V) and painful diabetic neuropathy (Douleur Neuropathique 4 (DN4) ≥4) and Diabetic Neuropathy Symptom (DNS) score ≥1.

**Results** Of 932 subjects, 645 (69.2%) were treated with metformin, while 287 (30.8%) were not on metformin. Overall, B<sub>12</sub> deficiency (<200 pg/mL) was significantly higher in metformin users of 25 (3.9%), compared with non-metformin users of 6 (2.1%), while B<sub>12</sub> insufficiency (200–300 pg/mL) was significantly lower in metformin users of 117 (18.4%) compared with non-metformin users of 80 (27.9%). Subjects with B<sub>12</sub> deficiency and insufficiency with hyperhomocysteinemia (≥15) were found in 19 (76%) μmol/L and 69 (60.5%) μmol/L in metformin users compared with 6 (100%) μmol/L and 57 (73.1%) μmol/L in non-metformin users, respectively. VPT>25 and DN4 score ≥4 were significantly higher in B<sub>12</sub>-deficient metformin users compared with non-metformin users. Similarly, DNS score ≥1 was non-significantly higher in B<sub>12</sub>-deficient metformin users compared with non-metformin users.

**Conclusion** This study shows that vitamin B<sub>12</sub> insufficiency was frequently found in our population and may progress into B<sub>12</sub> deficiency. It is also associated with neuropathy in subjects on metformin. Further interventional studies to assess the benefit of B<sub>12</sub> treatment on painful neuropathy in patients on metformin may be warranted. B<sub>12</sub> levels may be checked in people with T2DM using metformin for >2 years.

## INTRODUCTION

Worldwide, type 2 diabetes mellitus (T2DM) has affected an estimated 463 million people in 2019 and projected to reach 700 million by 2045, reported by the International Diabetes

## Significance of this study

### What is already known about this subject?

► Accumulating evidence suggests that metformin as the first choice of therapy for glycemic control may lead to low levels of serum vitamin B<sub>12</sub>.

### What are the new findings?

► Vitamin B<sub>12</sub> insufficiency was frequently found in our population and may progress into B<sub>12</sub> deficiency that is also associated with neuropathy in subjects on metformin.

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

► In clinical practice, B<sub>12</sub> levels may be checked especially in people with type 2 diabetes mellitus using metformin for >2 years to confirm B<sub>12</sub> deficiency and/or B<sub>12</sub> insufficiency.

Federation (IDF).<sup>1</sup> In the recent second National Diabetes Survey of Pakistan 2016–2017, the prevalence of diabetes was 26.3%.<sup>2</sup> The American Diabetes Association (ADA), the European Association for the Study of Diabetes and the IDF recommend metformin as the first choice of therapy for glycemic control.<sup>1,3</sup> Accumulating evidence from both observational and interventional studies has revealed that vitamin B<sub>12</sub> deficiency may occur with metformin treatment.<sup>4</sup> It has also been reported that vitamin B<sub>12</sub> deficiency ranges from 9% to 52% in people with T2DM and has been partially attributed to long-term use of metformin.<sup>5–7</sup>

Vitamin B<sub>12</sub> is essential for remethylation of homocysteine (Hcy) to methionine and B<sub>12</sub> deficiency could lead to hyperhomocysteinemia, which has been associated with macrovascular complications in people with T2DM.<sup>8</sup> B<sub>12</sub> deficiency may also increase the severity of peripheral neuropathy in T2DM.<sup>9</sup> However, reports are contradictory on the association between metformin-induced vitamin B<sub>12</sub>

deficiency and peripheral neuropathy.<sup>9–11</sup> Furthermore, there are limited studies assessing metformin-induced vitamin B<sub>12</sub> deficiency in people with T2DM<sup>12–14</sup> and no such study assessing the relationship to diabetic neuropathy in Pakistan.

This study was undertaken to establish the prevalence of B<sub>12</sub> deficiency in people with T2DM treated with metformin and its relationship to diabetic peripheral neuropathy (DPN) in Pakistan.

## METHODOLOGY

This prospective multicenter observational study was conducted by Baqai Institute of Diabetology and Endocrinology (BIDE), Baqai Medical University (BMU), Karachi, Pakistan. Duration of study was between May 2018 and January 2019. An estimated sample size of 1000 subjects of which 750 have T2DM treated with metformin for >2 years and 250 non-diabetics without metformin was calculated. Subjects were selected from seven tertiary care centers across four provinces of Pakistan (Sindh, Punjab, Baluchistan and Khyber Pakhtunkhwa).

Subjects with a history of pernicious anemia, iron deficiency anemia, malabsorption (celiac disease, inflammatory bowel disease, gastrointestinal surgery), malnutrition (pure vegans, anorexia nervosa), history of thyroid disease and thyroxine treatment and/or a history of other organ-specific autoimmune conditions (vitiligo, Addison's, primary ovarian failure, hypoparathyroidism), peripheral arterial disease and history of

another cause of neuropathy were excluded. Subjects with previous gastric resection or bariatric surgery or on a vegetarian diet, who had received oral or intramuscular vitamin B<sub>12</sub> supplementation, vitamin D supplementation, multivitamins, calcium supplements and proton-pump inhibitors (PPI) within the last 3 months, pregnancy and hearing or visual impairment or dementia were also excluded.

Baseline demographic and anthropometric details including age, gender, duration of metformin use, daily dose of metformin, blood pressure and body mass index (BMI) were noted using predesigned questionnaire. Blood samples were collected into a dedicated evacuated tube for biochemical parameters including hemoglobin (Hb), serum vitamin B<sub>12</sub>, and Hcy levels. From all centers, blood samples were transported to the laboratory of BIDE-BMU. Equipment used throughout the study were standardized with measure of quality assurance.

Vitamin B<sub>12</sub> was analyzed using the Roche Diagnostic cobas e411 Immunoassay System—a fully automated, random access, software-controlled system for immunoassay analysis. The e411 vitamin B<sub>12</sub> assay employs a competitive test principle using intrinsic factor specific for vitamin B<sub>12</sub>. In the sample, vitamin B<sub>12</sub> competes with the added vitamin B<sub>12</sub> labeled with biotin for the binding sites on the ruthenium-labeled intrinsic factor complex. Serum vitamin B<sub>12</sub> >300 pg/mL was defined as normal, 200–300 pg/mL insufficient and <200 pg/mL as deficient.<sup>15</sup>

**Table 1** Comparison of demographic, anthropometric and clinical characteristics between non-metformin users and metformin users

Variable	Non-metformin users	Metformin users	P value	Overall
n	287	645	–	932
Age (years)	39.77±14.95	51.16±14.64	<0.0001	47.66±15.64
Gender				
Male	157 (54.7%)	280 (43.4%)	0.001	437 (46.9%)
Female	130 (45.3%)	365 (56.6%)		495 (53.1%)
Marital status				
Single	68 (23.7%)	12 (1.9%)	<0.0001	80 (8.6%)
Married	219 (76.3%)	633 (98.1%)		852 (91.4%)
Duration of DM (years)	–	8.03±5.4	–	8.03±5.4
BMI (kg/m <sup>2</sup> )	26.03±5.42	27.91±5.12	<0.0001	27.36±5.28
Systolic blood pressure (mm Hg)	126.94±18.06	134.41±18.58	<0.0001	132.2±18.73
Diastolic blood pressure (mm Hg)	78±14.6	81.61±13.62	0.001	80.54±14.01
Sulfonylureas	–	119 (18.44%)		119 (18.44%)
Thiazide diuretics	–	39 (6.06%)		39 (6.06%)
Dipeptidyl peptidase-4 (DPP4) inhibitor	–	164 (25.4%)		164 (25.4%)
Hb (g/dL)	14.05±2.3	13.41±2.32	<0.0001	13.61±2.33

Data presented as n (%) or mean±SD.

Student's t-test and  $\chi^2$  test were applied.

P<0.05 considered to be statistically significant.

BMI, body mass index; DM, diabetes mellitus.

Subjects with vitamin B<sub>12</sub> deficiency and insufficiency underwent assessment of Hcy levels (<15 µmol/L normal, ≥15 µmol/L hyperhomocysteinemia).<sup>16 17</sup> Subjects underwent assessment of vibration perception threshold (VPT), Douleur Neuropathique 4 (DN4) score and Diabetic Neuropathy Symptom (DNS) score. VPT was measured on the pulp of the large toe on both right and left legs with a neurothesiometer.<sup>18</sup> VPT was considered normal (<15V), intermediate (16–25V), and abnormal (>25V).<sup>19</sup> The DN4 comprised 10 questions and a score ≥4 was used to define neuropathic pain.<sup>20</sup> A DNS score ≥1 was considered to be indicative of neuropathy.<sup>21</sup>

### Statistical analysis

Data analysis was performed in Statistical Package for Social Sciences (SPSS V.20). Student's t-test, analysis of variance,  $\chi^2$  test, and Fisher's exact test were applied to check the significant difference between groups. Pearson's correlation analysis was used to examine the relationship between vitamin B<sub>12</sub> and other parameters. A two-tailed p value <0.05 was considered statistically significant.

### RESULTS

Out of 1000 sample size, 932 subjects were recruited of whom 287 (30.8%) were not on metformin supplementation and 645 (69.2%) were on metformin supplementation. The mean age of non-metformin users was 39.77±14.95 years and metformin users were 51.16±14.64 years. Metformin users had a higher BMI (27.91±5.12 vs 26.03±5.42, p<0.0001), systolic blood pressure (134.41±18.58 vs 126.94±18.06, p<0.0001) and diastolic blood pressure (81.61±13.62 vs 78±14.6, p=0.001). Hb was significantly lower in metformin users (13.41±2.32) compared with non-metformin users (14.05±2.3) (table 1).

Overall, B<sub>12</sub> deficiency (<200 pg/mL) was significantly higher in metformin users of 25 (3.9%) compared with non-metformin users of 6 (2.1%), while B<sub>12</sub> insufficiency (200–300 pg/mL) was significantly lower in metformin users of 117 (18.4%) compared with non-metformin users of 80 (27.9%). Subjects with B<sub>12</sub> deficiency and insufficiency with hyperhomocysteinemia (≥15) were found in 19 (76%) µmol/L and 69 (60.5%) µmol/L in metformin users compared with 6 (100%) µmol/L and 57 (73.1%) µmol/L in non-metformin users, respectively (table 2).

Either the VPT>25 or DN4 score ≥4 was significantly higher in B<sub>12</sub>-deficient metformin users compared with non-metformin users. Similarly, DNS score ≥1 was non-significantly higher in B<sub>12</sub>-deficient metformin users compared with non-metformin users (table 3).

B<sub>12</sub> levels were not associated with age (r=0.172, p<0.0001), BMI (r=-0.089, p=0.013), duration of diabetes (r=0.017, p=0.706), VPT (r=0.262, p<0.0001), DNS score (r=0.128, p<0.0001) and DN4 score (r=0.318, p<0.0001), while B<sub>12</sub> levels were negatively correlated to duration of

**Table 2** Vitamin B<sub>12</sub> and homocysteine in non-metformin and metformin users

Parameters	Metformin use		P value
	No	Yes	
Vitamin B <sub>12</sub>			
Deficiency (<200)	6 (2.1%)	25 (3.9%)	0.002
Insufficiency (200–300)	80 (27.9%)	117 (18.4%)	
Normal (>300)	200 (70%)	494 (77.7%)	
B <sub>12</sub> levels and homocysteine			
B <sub>12</sub> deficient and homocysteine			
Normal	0 (0%)	6 (24%)	0.197
Hyper	6 (100%)	19 (76%)	
B <sub>12</sub> insufficient and homocysteine			
Normal	21 (26.9%)	45 (39.4%)	0.05
Hyper	57 (73.1%)	69 (60.5%)	
Overall homocysteine			
Normal	21 (25%)	51 (36.7%)	0.047
Hyper	63 (75%)	88 (63.3%)	

Data presented as n (%).

$\chi^2$  test was applied.

P<0.05 considered to be statistically significant.

metformin use (r=-0.24; p=0.0001) (figure 1A), dose of metformin use (r=-0.21; p=0.0001) (figure 1B), HbA1c (r=-0.09, p=0.378) and Hcy levels (r=-0.147, p=0.038) (table 4).

### DISCUSSION

This is the largest multicenter study to date assessing the relationship between metformin use and B<sub>12</sub> deficiency, and its association with diabetic neuropathy. In this study, significantly increased prevalence of B<sub>12</sub> deficiency was observed in people with T2DM treated with metformin as compared with non-metformin users.<sup>9 14 22</sup>

On contrary, B<sub>12</sub> insufficiency was significantly higher in non-metformin users compared with metformin users. It indicates that B<sub>12</sub> insufficiency was generally found in our population, and after initiation of metformin in people with diabetes, the B<sub>12</sub> insufficiency may develop into B<sub>12</sub> deficiency. Moreover, we observed that subjects with B<sub>12</sub> deficiency have high VPT (>25), DNS score (≥1) and DN4 score (≥4) as compared with non-metformin users, similar to Algeffari and Singh *et al*'s studies.<sup>23 24</sup> Indeed, Zalaket *et al* showed reversal of neuropathy after B<sub>12</sub> supplementation.<sup>25</sup>

Regarding the clinical significance of biochemical vitamin B<sub>12</sub> deficiency versus true tissue deficiency, a significant debate already exists. Up to now, the most commonly surrogate markers used for detection of vitamin B<sub>12</sub> deficiency are plasma Hcy and methylmalonic acid.<sup>26</sup> In our population, concurrently elevated Hcy levels were also observed in people with B<sub>12</sub> insufficiency and B<sub>12</sub> deficiency.<sup>27</sup> However, measurement of additional biomarkers for more comprehensive assessment of B<sub>12</sub>

**Table 3** Association of VPT, DNS and DN4 scores with B<sub>12</sub> deficiency

Parameters	Non-metformin users				Metformin users			
	B <sub>12</sub>				B <sub>12</sub>			
	<200	200–300	>300	P value	Overall	<200	200–300	>300
<b>VPT</b>								
<15	4 (66.6%)	33 (44.6%)	65 (33.5%)	<0.0001	102 (37.2%)	7 (29.2%)	69 (68.3%)	53 (16.8%)
15–25	1 (16.7%)	12 (16.2%)	21 (10.8%)		34 (12.4%)	0 (0%)	14 (13.9%)	45 (14.3%)
>25	1 (16.7%)	29 (39.2%)	108 (55.7%)		138 (50.4%)	17 (70.8%)	18 (17.8%)	217 (68.9%)
<b>DNS</b>								
<1	5 (83.3%)	61 (79.2%)	116 (62.7%)	0.001	182 (64.2%)	4 (16%)	31 (27%)	76 (18.8%)
≥1	1 (16.7%)	16 (20.8%)	69 (37.3%)		86 (35.8%)	21 (84%)	84 (73%)	328 (81.2%)
<b>DN4</b>								
<4	6 (100%)	70 (92.1%)	146 (80.2%)	0.01	222 (84.1%)	7 (29.2%)	70 (60.9%)	175 (43.3%)
≥4	0 (0%)	6 (7.9%)	36 (19.8%)		42 (15.9%)	17 (70.8%)	45 (39.1%)	229 (56.7%)

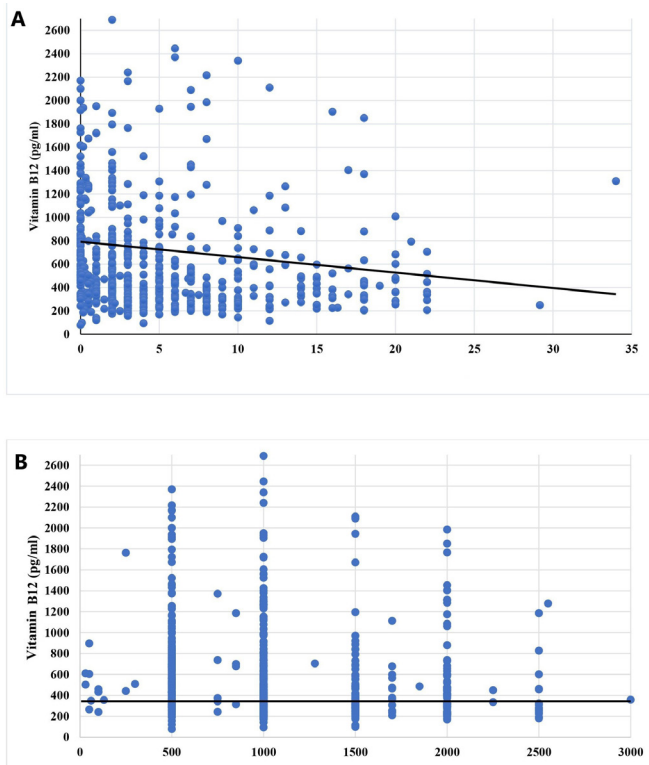
Data presented as n (%).

 $\chi^2$  test was applied.

P&lt;0.05 considered to be statistically significant.

DN4, Douleur Neuropathique 4; DNS, Diabetic Neuropathy Symptom; VPT, vibration perception threshold.





**Figure 1** Correlation of vitamin B<sub>12</sub> level with (A) duration and (B) dose of metformin in metformin users.

deficiency, such as holotranscobalamin, methylmalonic acid, red cell-B<sub>12</sub>, and plasma concentrations of methylation indices, is beyond the scope of our study.

Vitamin B<sub>12</sub> deficiency is a multifactorial condition caused by insufficient intake (nutritional deficiency) as well as acquired or inherited defects that disrupt B<sub>12</sub> absorption and processing pathways. Similarly, metformin-induced B<sub>12</sub> deficiency is also thought to occur due to vitamin B<sub>12</sub> malabsorption such as alteration of bile acid metabolism, small intestinal bacterial

overgrowth, or effects on intrinsic factor secretion, but a more currently accepted explanation is the interference by metformin on calcium-dependent membrane action responsible for vitamin B<sub>12</sub> intrinsic factor absorption in the terminal ileum.<sup>28</sup> The use of PPIs is also thought to contribute to B<sub>12</sub> deficiency, although this does not appear to be a factor in our study. Both observational and interventional studies have shown that the duration and dose of metformin are also associated with B<sub>12</sub> deficiency and neuropathy.<sup>11 29 30</sup> A recent study from Qatar, however, showed no association between metformin use and B<sub>12</sub> deficiency or diabetic neuropathy.<sup>20</sup> de Groot-Kamphuis *et al* have shown a lower prevalence of DPN in people with T2DM on metformin compared with those not on metformin.<sup>31</sup> Our study confirms a weak but significant correlation between B<sub>12</sub> levels and duration and dose of metformin. A significant association has also been found with age, gender, married individuals, BMI and blood pressure with B<sub>12</sub> levels in metformin users.<sup>29</sup> In the present study, the metformin users were significantly older, but no such association between age and B<sub>12</sub> levels exists in related studies.<sup>11 32</sup>

In current study, significantly increased but low Hb levels were observed in metformin users compared with non-metformin users. In prior studies, the significant association between B<sub>12</sub> deficiency and low Hb concentration was also noted.<sup>33 34</sup> Metformin-induced B<sub>12</sub> deficiency has been attributed to alterations in small bowel motility and enhanced bacterial overgrowth or interference of metformin with calcium-dependent intrinsic factor release.<sup>35</sup> To date, there are no guidelines recommending routine screening for B<sub>12</sub> deficiency in T2DM subjects on metformin, although the recent ADA-ADA consensus guidelines recommended the assessment of B<sub>12</sub> in subjects with DPN being treated with metformin.<sup>36</sup>

### Strengths and limitations

This is a cross-sectional multicenter study and therefore a true cause effect between metformin use and B<sub>12</sub> deficiency cannot be established. We have attempted to exclude other confounding factors, although the patients on metformin were older. We lack complete data regarding VPT, DNS score and DN4 score from all centers is our limitation. Glycemic control not being assessed is also a limitation of this study. All laboratory assessments were undertaken in a central lab and exactly the same protocols were used to assess for diabetic neuropathy and painful diabetic neuropathy.

### CONCLUSION

This study shows that vitamin B<sub>12</sub> insufficiency was frequently found in our population and may progress into B<sub>12</sub> deficiency. It is also associated with neuropathy in subjects on metformin. Further interventional studies to assess the benefit of B<sub>12</sub> treatment on painful neuropathy in patients on metformin may be warranted. B<sub>12</sub> levels

**Table 4** Correlation between B<sub>12</sub> and various parameters

Parameters	Correlation	P value
Age	0.172	<0.0001
BMI	-0.089	0.013
Duration of DM	0.017	0.706
Duration of metformin	-0.236	<0.0001
Daily dose of metformin	-0.21	<0.0001
VPT	0.262	<0.0001
DNS score	0.128	<0.0001
DN4 score	0.318	<0.0001
HbA1c	-0.09	0.378
Homocysteine	-0.147	0.038

P<0.05 considered to be statistically significant.

BMI, body mass index; DM, diabetes mellitus; DN4, Douleur Neuropathique 4; DNS, Diabetic Neuropathy Symptom; VPT, vibration perception threshold.

may be checked in people with T2DM using metformin for >2 years.

**Correction notice** This article has been corrected since it was published. Name and affiliation of MIBD member has been corrected.

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**Collaborators** MIBD members: (1) Sobia Sabir Ali, Assistant Professor, Department of Diabetes and Endocrinology, Lady Reading Hospital, Peshawar, Pakistan; (2) Khalid Usman, MRCP (UK), Associate Professor, Endocrinology, Post Graduate Medical Institute, Hayatabad Medical Complex, Peshawar, Pakistan; (3) Salma Tanveer, FCPS, Professor of Medicine, In-charge Diabetes and Endocrinology, Nishtar Medical University, Multan, Pakistan; (4) Qazi Masroor, Professor of Medicine, Head of Department, Quaid-e-Azam Medical College, Bahawalpur, Pakistan; (5) Jamal Zafar, Professor of Medicine, Pakistan Institute of Medical Sciences, Islamabad, Pakistan; (6) Khurshed Ahmed, Consultant Physician, Zahid Medical Centre, Baluchistan, Pakistan; (7) Bilal Tahir, DDE, Research Coordinator and Diabetes Educator, Research Department, Baqai Institute of Diabetology and Endocrinology, Baqai Medical University, Karachi, Pakistan; (8) Asher Fawwad, PhD, Chairman and Professor of Biochemistry, Department of Biochemistry, Research Director, Baqai Institute of Diabetology and Endocrinology, Baqai Medical University, Karachi, Pakistan; (9) Rayaz A Malik, Professor of Medicine, Weill Cornell Medicine-Qatar, Qatar Foundation, Education City, Doha, Qatar.

**Contributors** ZM: concept, design, interpretation of data; wrote, edited and approved the final manuscript. NW: literature search, interpretation of data, wrote the manuscript. MIBD members: responsible for the supervision of the survey, concept, design, involved in the quality control and data management in their respective areas. All members approved the final submitted version.

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**Data availability statement** All data relevant to the study are included in the article.

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#### ORCID iD

Zahid Miyan <http://orcid.org/0000-0002-1592-0980>

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