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Association of vitamin B_{12} deficiency in people with type 2 diabetes on metformin and without metformin: a multicenter study, Karachi, Pakistan

Zahid Miyan ,[1] Nazish Waris,[2,3] MIBD

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[1]Department of Medicine, Baqai Institute of Diabetology and Endocrinology, Baqai Medical University, Karachi, Pakistan
[2]Clinical Biochemistry and Psychopharmacology Research Unit, Department of Biochemistry, University of Karachi, Karachi, Pakistan
[3]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_{12} 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_{12}, 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_{12} 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_{12} 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_{12} 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_{12}-deficient metformin users compared with non-metformin users. Similarly, DNS score ≥1 was non-significantly higher in B_{12}-deficient metformin users compared with non-metformin users.

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

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_{12}.

What are the new findings?

- Vitamin B_{12} insufficiency was frequently found in our population and may progress into B_{12} 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_{12} levels may be checked especially in people with type 2 diabetes mellitus using metformin for >2 years to confirm B_{12} deficiency and/or B_{12} insufficiency.

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 Federation (IDF).[1] In the recent second National Diabetes Survey of Pakistan 2016–2017, the prevalence of diabetes was 26.3%.[2] 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.[1 3] Accumulating evidence from both observational and interventional studies has revealed that vitamin B_{12} deficiency may occur with metformin treatment.[4] It has also been reported that vitamin B_{12} deficiency ranges from 9% to 52% in people with T2DM and has been partially attributed to long-term use of metformin.[5–7]

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

deficiency and peripheral neuropathy.[9–11] Furthermore, there are limited studies assessing metformin-induced vitamin B_{12} deficiency in people with T2DM[12–14] and no such study assessing the relationship to diabetic neuropathy in Pakistan.

This study was undertaken to establish the prevalence of B_{12} 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_{12} 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_{12}, 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_{12} 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_{12} assay employs a competitive test principle using intrinsic factor specific for vitamin B_{12}. In the sample, vitamin B_{12} competes with the added vitamin B_{12} labeled with biotin for the binding sites on the ruthenium-labeled intrinsic factor complex. Serum vitamin B_{12} >300 pg/mL was defined as normal, 200–300 pg/mL insufficient and <200 pg/mL as deficient.[15]

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^2)	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 χ^2 test were applied.
P<0.05 considered to be statistically significant.
BMI, body mass index; DM, diabetes mellitus.

Subjects with vitamin B_{12} deficiency and insufficiency underwent assessment of Hcy levels (<15 µmol/L normal, ≥15 µmol/L hyperhomocysteinemia).[16 17] 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.[18] VPT was considered normal (<15V), intermediate (16–25V), and abnormal (>25V).[19] The DN4 comprised 10 questions and a score ≥4 was used to define neuropathic pain.[20] A DNS score ≥1 was considered to be indicative of neuropathy.[21]

Statistical analysis

Data analysis was performed in Statistical Package for Social Sciences (SPSS V.20). Student's t-test, analysis of variance, χ^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_{12} 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_{12} 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_{12} 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_{12} 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_{12}-deficient metformin users compared with non-metformin users. Similarly, DNS score ≥1 was non-significantly higher in B_{12}-deficient metformin users compared with non-metformin users (table 3).

B_{12} 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_{12} levels were negatively correlated to duration of 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).

Table 2 Vitamin B_{12} and homocysteine in non-metformin and metformin users

Parameters	Metformin use		P value
	No	Yes	
Vitamin B_{12}			
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_{12} levels and homocysteine			
B_{12} deficient and homocysteine			
Normal	0 (0%)	6 (24%)	0.197
Hyper	6 (100%)	19 (76%)	
B_{12} 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 (%).
χ^2 test was applied.
P<0.05 considered to be statistically significant.

DISCUSSION

This is the largest multicenter study to date assessing the relationship between metformin use and B_{12} deficiency, and its association with diabetic neuropathy. In this study, significantly increased prevalence of B_{12} deficiency was observed in people with T2DM treated with metformin as compared with non-metformin users.[9 14 22] On contrary, B_{12} insufficiency was significantly higher in non-metformin users compared with metformin users. It indicates that B_{12} insufficiency was generally found in our population, and after initiation of metformin in people with diabetes, the B_{12} insufficiency may develop into B_{12} deficiency. Moreover, we observed that subjects with B_{12} 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.[23 24] Indeed, Zalaket *et al* showed reversal of neuropathy after B_{12} supplementation.[25]

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

Table 3 Association of VPT, DNS and DN4 scores with B_{12} deficiency

	Non-metformin users					Metformin users				
	B_{12}					B_{12}				
Parameters	<200	200–300	>300	P value	Overall	<200	200–300	>300	P value	Overall
VPT										
<15	4 (66.6%)	33 (44.6%)	65 (33.5%)	<0.0001	102 (37.2%)	7 (29.2%)	69 (68.3%)	53 (16.8%)	<0.0001	129 (29.3%)
15–25	1 (16.7%)	12 (16.2%)	21 (10.8%)		34 (12.4%)	0 (0%)	14 (13.9%)	45 (14.3%)		59 (13.4%)
>25	1 (16.7%)	29 (39.2%)	108 (55.7%)		138 (50.4%)	17 (70.8%)	18 (17.8%)	217 (68.9%)		252 (57.3%)
DNS										
<1	5 (83.3%)	61 (79.2%)	116 (62.7%)	0.001	182 (64.2%)	4 (16%)	31 (27%)	76 (18.8%)	0.137	111 (20.4%)
≥1	1 (16.7%)	16 (20.8%)	69 (37.3%)		86 (35.8%)	21 (84%)	84 (73%)	328 (81.2%)		433 (79.6%)
DN4										
<4	6 (100%)	70 (92.1%)	146 (80.2%)	0.01	222 (84.1%)	7 (29.2%)	70 (60.9%)	175 (43.3%)	<0.0001	252 (46.4%)
≥4	0 (0%)	6 (7.9%)	36 (19.8%)		42 (15.9%)	17 (70.8%)	45 (39.1%)	229 (56.7%)		291 (53.6+%)

Data presented as n (%).
χ^2 test was applied.
P<0.05 considered to be statistically significant.
DN4, Douleur Neuropathique 4; DNS, Diabetic Neuropathy Symptom; VPT, vibration perception threshold.

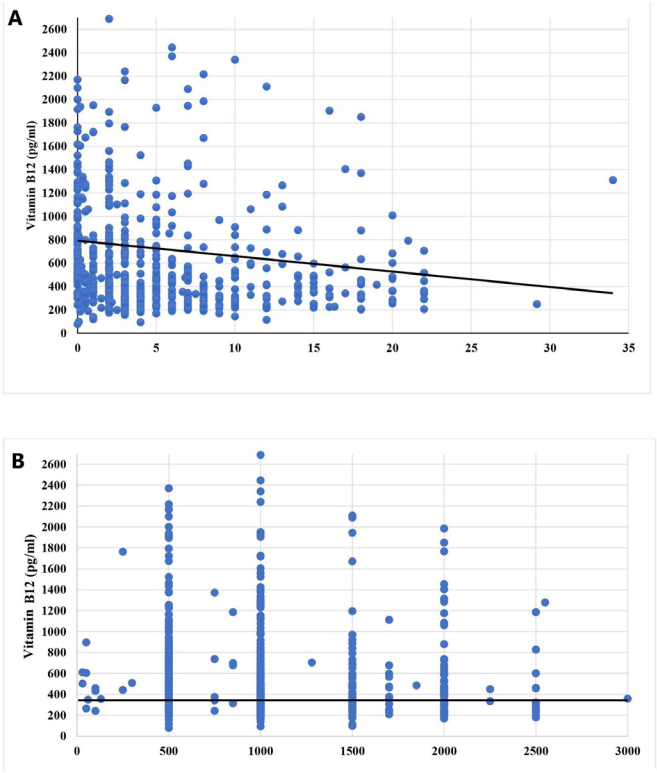

Figure 1 Correlation of vitamin B_{12} level with (A) duration and (B) dose of metformin in metformin users.

deficiency, such as holotranscobalamin, methylmalonic acid, red cell-B_{12}, and plasma concentrations of methylation indices, is beyond the scope of our study.

Vitamin B_{12} deficiency is a multifactorial condition caused by insufficient intake (nutritional deficiency) as well as acquired or inherited defects that disrupt B_{12} absorption and processing pathways. Similarly, metformin-induced B_{12} deficiency is also thought to occur due to vitamin B_{12} 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_{12} intrinsic factor absorption in the terminal ileum.[28] The use of PPIs is also thought to contribute to B_{12} 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_{12} deficiency and neuropathy.[11 29 30] A recent study from Qatar, however, showed no association between metformin use and B_{12} deficiency or diabetic neuropathy.[20] de Groot-Kamphuis *et al* have shown a lower prevalence of DPN in people with T2DM on metformin compared with those not on metformin.[31] Our study confirms a weak but significant correlation between B_{12} 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_{12} levels in metformin users.[29] In the present study, the metformin users were significantly older, but no such association between age and B_{12} levels exists in related studies.[11 32]

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_{12}deficiency and low Hb concentration was also noted.[33 34] Metformin-induced B_{12} deficiency has been attributed to alterations in small bowel motility and enhanced bacterial overgrowth or interference of metformin with calcium-dependent intrinsic factor release.[35] To date, there are no guidelines recommending routine screening for B_{12} deficiency in T2DM subjects on metformin, although the recent ADA-ADA consensus guidelines recommended the assessment of B_{12} in subjects with DPN being treated with metformin.[36]

Table 4 Correlation between B_{12} 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.

Strengths and limitations

This is a cross-sectional multicenter study and therefore a true cause effect between metformin use and B_{12} 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_{12} insufficiency was frequently found in our population and may progress into B_{12} deficiency. It is also associated with neuropathy in subjects on metformin. Further interventional studies to assess the benefit of B_{12} treatment on painful neuropathy in patients on metformin may be warranted. B_{12} levels

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, Nishter 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) Khursheed 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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ORCID iD

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

REFERENCES

1 IDF diabetes atlas 9th edition, 2019. Available: https://www.diabetesatlas.org. [Accessed 27 Jan 2020].
2 Basit A, Fawwad A, Qureshi H, *et al*. Prevalence of diabetes, pre-diabetes and associated risk factors: second national diabetes survey of Pakistan (NDSP), 2016–2017. *BMJ Open* 2018;8:020961.
3 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;2017:1–6.
4 de Jager J, Kooy A, Lehert P, *et al*. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. *BMJ* 2010;340:c2181.
5 Nervo M, Lubini A, Raimundo FV, *et al*. Vitamin B12 in metformin-treated diabetic patients: a cross-sectional study in Brazil. *Rev Assoc Med Bras* 2011;57:46–9.
6 Olt S, Oznas O. Investigation of the vitamin B12 deficiency with peripheral neuropathy in patients with type 2 diabetes mellitus treated using metformin. *North Clin Istanb* 2017;4:233.
7 Pflipsen MC, Oh RC, Saguil A, *et al*. The prevalence of vitamin B(12) deficiency in patients with type 2 diabetes: a cross-sectional study. *J Am Board Fam Med* 2009;22:528–34.
8 Russo GT, Di Benedetto A, Magazzù D, *et al*. Mild hyperhomocysteinemia, C677T polymorphism on methylenetetrahydrofolate reductase gene and the risk of macroangiopathy in type 2 diabetes: a prospective study. *Acta Diabetol* 2011;48:95–101.
9 Liu Q, Li S, Quan H, *et al*. Vitamin B12 status in metformin treated patients: systematic review. *PLoS One* 2014;9:e100379.
10 Roy RP, Ghosh K, Ghosh M, *et al*. Study of Vitamin B_{12} deficiency and peripheral neuropathy in metformin-treated early Type 2 diabetes mellitus. *Indian J Endocrinol Metab* 2016;20:631–7.
11 Alharbi TJ, Tourkmani AM, Abdelhay O, *et al*. The association of metformin use with vitamin B12 deficiency and peripheral neuropathy in Saudi individuals with type 2 diabetes mellitus. *PLoS One* 2018;13:e0204420.
12 Iftikhar R, Kamran SM, Qadir A, Iqbal Z, *et al*. Prevalence of vitamin B12 deficiency in patients of type 2 diabetes mellitus on metformin: a case control study from Pakistan. *Pan Afr Med J* 2013;16:67.
13 Channa AA, Shah M, Shah NA, *et al*. Type 2 Diabetes Mellitus:(The vitamin B12 deficiency during metformin therapy). *Indo American J Pharm Sci* 2017;4:533–7.
14 Rauf A, Khan M, Arshad A. Frequency of vitamin B12 deficiency in patients with type 2 diabetes mellitus taking metformin. *Pak J Medical & Health Sci* 2015;9:1316–8.
15 Tas Kilic D, Akdogan A, Kilic L, *et al*. Evaluation of vitamin B12 deficiency and associated factors in patients with systemic sclerosis. *J Clin Rheumatol* 2018;24:250–4.
16 Price BR, Wilcock DM, Weekman EM. Hyperhomocysteinemia as a risk factor for vascular contributions to cognitive impairment and dementia. *Front Aging Neurosci* 2018;10:350.
17 Moretti R, Caruso P. The controversial role of homocysteine in neurology: from Labs to clinical practice. *Int J Mol Sci* 2019;20:231.
18 Elhadd T, Ponirakis G, Dabbous Z, *et al*. Metformin use is not associated with B12 deficiency or neuropathy in patients with type 2 diabetes mellitus in Qatar. *Front Endocrinol* 2018;9.
19 Shen J, Liu F, Zeng H, *et al*. Vibrating perception threshold and body mass index are associated with abnormal foot plantar pressure in type 2 diabetes outpatients. *Diabetes Technol Ther* 2012;14:1053–9.
20 Spallone V, Morganti R, D'Amato C, D'amato C, *et al*. Validation of DN4 as a screening tool for neuropathic pain in painful diabetic polyneuropathy. *Diabet Med* 2012;29:578–85.
21 Gogia S, Rao CR. Prevalence and risk factors for peripheral neuropathy among type 2 diabetes mellitus patients at a tertiary care hospital in coastal Karnataka. *Indian J Endocrinol Metab* 2017;21:665.
22 Khan A, Shafiq I, Hassan Shah M, Shah MH. Prevalence of vitamin B12 deficiency in patients with type II diabetes mellitus on metformin: a study from Khyber Pakhtunkhwa. *Cureus* 2017;9:e1577.
23 Algeffari MA. Painful diabetic peripheral neuropathy among Saudi diabetic patients is common but under-recognized: multicenter cross-sectional study at primary health care setting. *J Family Community Med* 2018;25:43.
24 Singh AK, Kumar A, Karmakar D, *et al*. Association of B12 deficiency and clinical neuropathy with metformin use in type 2 diabetes patients. *J Postgrad Med* 2013;59:253–7.
25 Zalaket J, Wehbe T, Abou Jaoude E. Vitamin B12 deficiency in diabetic subjects taking metformin: a cross sectional study in a Lebanese cohort. *J Nutr Intermed Metab* 2018;11:9–13.
26 Hannibal L, Lysne V, Bjørke-Monsen A-L, *et al*. Corrigendum: Biomarkers and Algorithms for the Diagnosis of Vitamin B_{12} Deficiency. *Front Mol Biosci* 2017;4:53.
27 Esmaeilzadeh S, Gholinezhad-Chari M, Ghadimi R. The effect of metformin treatment on the serum levels of homocysteine, folic acid, and vitamin B12 in patients with polycystic ovary syndrome. *J Hum Reprod Sci* 2017;10:95.
28 Aroda VR, Edelstein SL, Goldberg RB, *et al*. Long-Term metformin use and vitamin B12 deficiency in the diabetes prevention program outcomes study. *J Clin Endocrinol Metab* 2016;101:1754–61.
29 Ting RZ-W, Szeto CC, Chan MH-M, *et al*. Risk factors of vitamin B(12) deficiency in patients receiving metformin. *Arch Intern Med* 2006;166:1975–9.
30 Nareddy VA, Boddikuri IP, Ubedullah SK, *et al*. Correlation of Vit B12 levels with metformin usage among type 2 diabetic patients in a tertiary care hospital. *Int J Adv Med* 2018;5:1128–32.

31 de Groot-Kamphuis DM, van Dijk PR, Groenier KH, *et al*. Vitamin B12 deficiency and the lack of its consequences in type 2 diabetes patients using metformin. *Neth J Med* 2013;71:386–90.
32 Allin KH, Friedrich N, Pietzner M, *et al*. Genetic determinants of serum vitamin B12 and their relation to body mass index. *Eur J Epidemiol* 2017;32:125–34.
33 Kwok T, Cheng G, Woo J, *et al*. Independent effect of vitamin B12 deficiency on hematological status in older Chinese vegetarian women. *Am J Hematol* 2002;70:186–90.
34 Soofi S, Khan GN, Sadiq K, *et al*. Prevalence and possible factors associated with anaemia, and vitamin B_{12} and folate deficiencies in women of reproductive age in Pakistan: analysis of national-level secondary survey data. *BMJ Open* 2017;7:e018007.
35 Damião CP, Rodrigues AO, Pinheiro MFMC, *et al*. Prevalence of vitamin B12 deficiency in type 2 diabetic patients using metformin: a cross-sectional study. *Sao Paulo Med J* 2016;134:473–9.
36 Pop-Busui R, Boulton AJM, Feldman EL, *et al*. Diabetic neuropathy: a position statement by the American diabetes association. *Diabetes Care* 2017;40:136–54.