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Original Article

Internal Medicine Section

A Cross-sectional Study on Prevalence of Vitamin B12 Deficiency and Peripheral Neuropathy between Metformin Users and Non Users in Participants with Type 2 Diabetes Mellitus

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ABSTRACT

Introduction: Metformin is a first line drug for treatment of Type 2 Diabetes Mellitus (T2DM) which acts by decreasing insulin resistance. Metformin use can lead to vitamin B12 deficiency.

Aim: To evaluate the difference in proportion of vitamin B12 deficiency and clinical neuropathy between metformin users and metformin non users in patients with T2DM.

Materials and Methods: This cross-sectional study was conducted at Department of General Medicine, Vydehi Institute of Medical Sciences and Research Centre, Bengaluru, Karnataka, India, from Jul 2015 to June 2017. Hundred patients with T2DM were recruited in the study, and divided into two groups i.e., T2DM patients on metformin (n=50) and without metformin (n=50). One group were patients of diabetes who were on metformin for more than three years, and the other group were those who had not taken metformin in last three months. All participants were evaluated with serum vitamin B12. Toronto Clinical Neuropathy (TCN) score was used to diagnose peripheral neuropathy. Multivariable logistic regression was done to look for association between metformin use and vitamin B12 deficiency and peripheral neuropathy separately.

Results: Between the metformin users and non metformin user group, the proportion of males (72% vs 66%, p-value=0.66) and the age (56.02±9.19 vs 56.16±8.33 years, p-value=0.93) were similar in both groups. Median duration of diabetes among metformin users was 10.18 years (IQR=6.68-16.68 year) and non metformin user group was 7.68 years (IQR=5.68-12.68 year). There was a significantly higher prevalence of vitamin B12 deficiency in metformin users (38,76%) than non metformin users (21,42%). The prevalence of vitamin B12 deficiency was highest (11,100%) in those with metformin use for more than 15 years. Association of vitamin B12 deficiency in metformin users was significant {(OR=7.17 (2.46-20.92), p-value <0.001}. Peripheral neuropathy assessed by TCN scoring was significantly more common in metformin users (37,74% vs 23,46%; p-value=0.004).

Conclusion: This study reports high prevalence of vitamin B12 deficiency in T2DM patients with significantly higher prevalence among metformin users. Peripheral neuropathy was significantly more common in metformin users and was associated with vitamin B12 deficiency. Hence, metformin treated T2DM patients, especially those with peripheral neuropathy should be evaluated for vitamin B12 deficiency.

Keywords: Glycated haemoglobin, Hypovitaminosis, Toronto clinical neuropathy scale

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is the most common metabolic abnormality in a physician's practice. India, a developing country, is often called the "diabetic capital of the world" due to a rapid increase in the prevalence of T2DM. The global diabetes prevalence in 2019 was estimated to be 9.3% (463 million people), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045. The prevalence was found to be higher in urban (10.8%) than rural (7.2%) areas, and in high-income (10.4%) than low-income countries (4.0%). One in two (50.1%) people living with diabetes did not know that they have diabetes. The global prevalence of impaired glucose tolerance was estimated to be 7.5% (374 million) in 2019 and projected to reach 8.0% (454 million) by 2030, and 8.6% (548 million) by 2045. With an increasing incidence worldwide, T2DM is expected to be the leading cause of morbidity and mortality in the future [1].

Metformin is commonly used as the first-line drug in the treatment of T2DM at all levels of health system, from primary to tertiary care level, which is also endorsed by clinical practice guidelines including those from the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) [2,3]. Although the efficacy and safety of metformin for the treatment of T2DM have been well established by its long-term clinical use, the administration of this drug is often associated with adverse events [4]. Several studies have reported vitamin B12 deficiency as an adverse effect with frequent use of metformin, especially among older patients [5-8].

A substantial proportion of Asian Indians consume a vegetarian diet due to cultural and religious reasons. A strict vegetarian diet is associated with an increased risk of vitamin B12 deficiency. Also, since metformin is generally the first drug prescribed to newly diagnosed diabetes patients, it is the need of the hour to estimate burden of vitamin B12 deficiency among Indian T2DM patients, especially those who are on metformin [9].

The classical vitamin B12 deficiency is associated with megaloblastic anaemia with neurological symptoms and myelopathy. In a study by Pflipsen MC et al., about 30% of diabetic patients older than 40 years have impaired sensation in the feet. Unfortunately, the symptoms of diabetic neuropathy overlap with paresthesia, impaired vibration sense, and impaired proprioception associated with vitamin B12 deficiency. Vitamin B12 deficiency-induced nerve damage may contribute to diabetic peripheral neuropathy. Mere vitamin B12 replacement may reverse neurologic symptoms inappropriately attributed to hyperglycaemia [10].

Vitamin B12 deficiency is usually diagnosed by a laboratory finding of low serum vitamin B12 levels. Vitamin B12 is necessary for the synthesis of methionine from Homocysteine (Hcy); low levels of vitamin B12 lead to increases in total serum homocysteine and may be identified in patients with deficiency at an early reversible stage [11].

Vitamin B12 deficiency is a known adverse effect of metformin, and the literature reports variable prevalence (ranging from 9.5%-53.2%) and causes of vitamin B12 deficiency in Asian Indian patients with T2DM [12,13]. This study was conducted to determine the difference in proportion of vitamin B12 deficiency and clinical neuropathy between metformin users and metformin non users in patients with T2DM.

MATERIALS AND METHODS

This was a comparative cross-sectional study among 100 T2DM patients aged more than 40 years. All participants were recruited from the outpatient and inpatient services of the Department of General Medicine, Vydehi Institute of Medical Sciences and Research Centre, Bengaluru, Karnataka, India, from July 2015 to June 2017.. The study was approved by the Ethical Committee of the Institution (EC Reg No: VIMS&RC/IEC/029/2014-15 Dated 25/11/2014). Informed consent was taken from all the participants. A detailed history-taking and clinical examination were performed.

Sample size calculation: Estimated sample size was 49, rounded to 50 for each group, calculated for difference of proportion in vitamin B12 deficiency among both groups considering proportion of controls exposed as 28%, proportion of cases with exposure as 55%, alpha error of 0.05 and power of 0.80 [14].

Inclusion criteria: Patients on metformin for three years or more (n=50) were included in the metformin group (average dose of 1 gram/day), whereas those who had not received metformin at least in the last three months were included as a comparative group.

Exclusion criteria: Patients with type 1 diabetes mellitus, chronic alcoholism, chronic renal insufficiency, history of prior bariatric surgery, bowel resection, and malabsorption syndromes, vegetarians, those who had received vitamin B12 supplementation in the last three months, and those who had received metformin for less than three years were excluded from the study.

Study Procedure

Diagnosis of T2DM was based on the ADA criteria [15]. Neuropathy was diagnosed using the TCN scoring system [16]. Laboratory investigations including complete blood count, glycaemic profile {fasting plasma glucose, postprandial plasma glucose, Glycated Haemoglobin (HbA1c)}, renal function test, were done in all patients. Serum vitamin B12 was measured by chemiluminescent immunoassay using a Unicel Dxl 800 auto-analyser (Beckman Coulter, Inc., CA). Serum vitamin B12 level <200 pg/mL was considered deficient whereas B12 level >200 pg/mL was considered normal [17]. Hypovitaminosis D was defined as serum {(25OH) D} level of <20 ng/mL [18].

STATISTICAL ANALYSIS

The data were analysed using MedCalc Statistical Software version 19.2.6 (MedCalc Software bv, Ostend, Belgium; https://www.medcalc.org; 2020). Continuous variables were represented as mean±SD and categorical variables as absolute numbers and percentages as appropriate. Student's t-test or Wilcoxon ranksum test was used to compare continuous variables whereas Chi-square or Fisher-exact test were used to compare categorical variables between two groups. A two-tailed p-value of <0.05 was considered statistically significant. Trend of vitamin B12 deficiency with dose of metformin was calculated by Cochran-Armitage Chi-square test.

To determine association between metformin usage with Vitamin B12 deficiency and neuropathy respectively, first, crude odds ratio was calculated by logistic regression. Later, a multivariable logistic regression was done separately for vitamin B12 deficiency and for categories of neuropathy, and both models were adjusted for duration of diabetes mellitus and HbA1c.

RESULTS

Between the metformin users and non metformin users, the proportion of males (72% vs 66%, p-value=0.66) and the age (56.02±9.19 vs 56.16±8.33 years, p-value=0.93) were similar in both groups. Median duration of diabetes among metformin users was 10.18 years (IQR=6.68-16.68 year) and non metformin users group was 7.68 years (IQR=5.68-12.68 year). Difference in duration of diabetes was not significant among both groups, as p-value (rank sum) was 0.1959. Fasting blood sugar and HbA1c were significantly higher in Metformin users [Table/Fig-1].

Laboratory parameters	Metformin users (n=50)	Non metformin users (n=50)	p-value
Fasting plasma glucose (mg/dL)	197.94±77.34	159.26±43.63	0.002*
Postprandial plasma glucose (mg/dL)	309.74±107.24	260.40±56.69	0.005*
Glycated haemoglobin (%)	9.49±2.08	8.23±1.40	<0.001*
Blood urea (mg/dL)	31.58±13.01	25.16±9.13	0.005*
Serum creatinine (mg/dL)	0.84±0.31	0.71±0.32	0.045*
Serum vitamin B12 (pg/mL)	175.82±73.81	251.18±204.40	0.016*
Vitamin B12 deficiency, n (%)	38 (76%)	21 (42%)	0.001#
Hypovitaminosis D, n (%)	35 (70%)	20 (40%)	0.003#

[Table/Fig-1]: Summary of laboratory parameters in both groups (mean±standard deviation).
*Unpaired students' t-test; #Chi-square test; p-value of <0.05 was considered statistically significant

The proportion of vitamin B12 deficiency was significantly more common in metformin users than non metformin users (76% vs 42%, p-value=0.001) and serum vitamin B12 levels were significantly lower in metformin users than non metformin users. Vitamin B12 deficiency was significantly associated with duration of metformin use with 100% prevalence of vitamin B12 deficiency among those who used metformin for >15 years [Table/Fig-2].

Frequency	Serum vitamin B12	
	<200 pg/mL	>200 pg/mL
<5 years (n=8)	6 (75%)	2 (25%)
5-10 years (n=22)	15 (73%)	7 (27%)
10-15 years (n=9)	8 (88.9%)	1 (11.1%)
>15 years (n=11)	9 (81.8%)	2 (18.2%)
p-value (Chi-square test)	0.62	

[Table/Fig-2]: Distribution of categories of duration of metformin use with serum vitamin B12 deficiency among cases (metformin users).

Based on the Toronto Clinical Neuropathy Scoring (TCNS), neuropathy was significantly higher in metformin users than non metformin users (74% vs 46%, p-value=0.004) [Table/Fig-3]. It is also important to note that among patients with low B12 level, 33.3% among metformin non users and 61.5% among metformin users had no neuropathy. Neuropathy

TCNS categories	Metformin users (cases)			Non metformin users (control)		
	Total	Serum vitamin B12 <200 pg/mL	Serum vitamin B12 >200 pg/mL	Total	Serum vitamin B12 <200 pg/mL	Serum vitamin B12 >200 pg/mL
No neuropathy (1-5)	13 (26%)	8 (61.5%)	5 (38.5%)	27 (54%)	9 (33.3%)	18 (66.6%)
Mild neuropathy (6-8)	23 (46%)	13 (56.5%)	10 (43.5%)	17 (34%)	10 (58.8%)	7 (41.2%)
Moderate neuropathy (9-11)	8 (16%)	8 (100%)	0 (0%)	6 (12%)	1 (16.7%)	5 (83.3%)
Severe neuropathy (12+)	6 (12%)	6 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
p-value (Chi-square)		0.035			0.130	

[Table/Fig-3]: Distribution of Toronto Clinical Neuropathy Score (TCNS) categories with metformin use and vitamin B12 deficiency.
*p-value (Chi-square) for distribution of categories of neuropathy among total cohorts of metformin users and non metformin users was 0.003

and B12 deficiency were significantly associated with metformin usage with crude odds ratio 4.37 (95% CI: 1.85-10.31, p<0.001) [Table/Fig-4]. Even after adjustment for duration of disease and glycaemic status, association with Vitamin B12 deficiency remained significant. Odds of having Vitamin B12 deficiency was 7.17 (95% CI: 2.46-20.92) times higher among metformin group compared to non metformin group after adjusting for HbA1c levels. The association between TCNS categories and metformin usage was not significant after adjustment for HbA1c (proxy for glycaemic control) and duration of disease.

Variable (n=100)	Crude odds ratio (95% CI)	p-value	Adjusted odds ratio* (95% CI)	p-value
Vitamin B12 deficiency	4.37 (1.85-10.31)	<0.001	7.17 (2.46-20.92)	<0.001
Toronto Clinical Neuropathy Score (TCNS) categories				
No neuropathy	Reference			
Mild neuropathy	2.81 (1.12-6.99)	0.026	2.71 (0.97-7.53)	0.056
Moderate neuropathy	3.32 (0.90-12.17)	0.070	3.21 (0.77-13.40)	0.109
Severe neuropathy	12.46 (1.35-114.51)	0.026	10.46 (0.81-134.47)	0.072

[Table/Fig-4]: Association of metformin intake with vitamin B12 deficiency and neuropathy (according to TCNS categories).
*Both variables separately adjusted for HbA1c and duration of disease

DISCUSSION

In the present study, the prevalence of Vitamin B12 deficiency was high among T2DM and was significantly higher in metformin users than non metformin users, and metformin usage significantly correlated with the presence of neuropathy.

In this study, the prevalence of vitamin B12 deficiency among T2DM was high (59%). Several previous studies have reported high prevalence rates (22-54%) of vitamin B12 deficiency among T2DM patients [10,14,19,20]. However, a few studies have reported low prevalence rates of vitamin B12 deficiency among T2DM which are comparable to that of non diabetics [21]. The high prevalence of vitamin B12 deficiency among the study is most probably due to no or less frequent intake of non vegetarian diet.

The prevalence of vitamin B12 deficiency was 74% in metformin users and 46% in non metformin users and serum vitamin B12 levels were significantly lower among metformin users. Studies by Calvo Romero JM et al. (393.5 vs 509 pg/mL) and Sparre Hermann L et al., (289 vs 395 picomols/L) have reported significantly lower serum vitamin B12 levels in metformin users than non metformin users [22,23]. In Andres E et al., study, the prevalence of definite (29% vs 5%) and probable (52% vs 27%) vitamin B12 deficiency were significantly higher among metformin users [24]. Similarly, serum vitamin B12 levels were significantly lower in metformin users of our study than non metformin users (207.28 vs 329.85 pg/mL) which established the association of metformin use with vitamin B12 deficiency in T2DM patients. However, a few studies have refuted the association of vitamin B12 deficiency with metformin, probably due to the overall low prevalence of vitamin B12 deficiency among the cohort [25]. The present study also found increasing trend of vitamin B12 deficiency with a duration of metformin use. A similar observation was also reported in a study by Marar O et al., where serum vitamin B12 was significantly lower in those who had used metformin for >5 years than those who had used metformin for <5 years [26]. One study from north India had reported statistically significant increase in proportion of patients with vitamin B12 deficiency as duration of diabetes increases, but it was not seen in our research [12].

Metformin is the first-line anti-diabetic agent commonly used for the treatment of T2DM in primary care [15]. It is most often initiated at the diagnosis and continued until estimated Glomerular Filtration Rate (eGFR) <30 mL/min/1.73 m^2 or the development of intolerance. Hence, demonstration of the association of vitamin B12 deficiency with long-term metformin use in T2DM patients in several studies emphasises the need for caution while using metformin [21,27-29]. Vitamin B12 deficiency manifestations may start three to six months after starting the use of metformin medication [30]. Metformin has been reported to lead to malabsorption of vitamin B12, thereby decreasing the concentration of serum vitamin B12 by 10-30% [31,32]. Two mechanisms have been proposed to explain metformin-induced vitamin B12 deficiency among patients with T2DM. First, alteration in small bowel motility which leads to bacterial overgrowth and alterations in Intrinsic Factor (IF) levels causing vitamin B12 deficiency. Second, the current and most accepted explanation for metformin-induced vitamin B12 malabsorption and deficiency is the disturbance of calcium-dependent membrane action in the ileum. Absorption of the vitamin B12 IF complex is calcium-dependent and metformin interferes with this absorption [32].

Vitamin B12 deficiency is associated with macrocytic-megaloblastic anaemia; anaemia often precedes neuropathy. Clinically, the earliest manifestations of vitamin B12 deficiency are numbness and paresthesia in the feet, followed by weakness, ataxia, sphincter disturbance, and changes in mental status. Studies have reported conflicting findings regarding the consequences of vitamin B12 deficiency in T2DM patients. Aroda VR et al., found a higher prevalence of neuropathy in the metformin group than the placebo group in a large cohort study of 2,155 participants [28]. In contrast, Alharbi TJ et al., study found no significant differences in the prevalence of peripheral neuropathy between metformin users and non metformin users [27]. In our study, peripheral neuropathy was significantly more frequent in metformin users who were on metformin for ≥3 years. Hendrawati YD et al., have also reported higher proportions of neuropathy symptoms among patients who took metformin for >3 years [33].

Vitamin B12 is a vitamin essential to the proper functioning and development of the central and peripheral nervous systems, ensuring effective nerve-impulse transmission. Vitamin B12 deficiency can lead to demyelination and nerve damage with neurological manifestations and peripheral neuropathy with the associated symptoms of pain, tingling, numbness, paresthesia, and loss of sensation. Studies have shown the improvement of neurological symptoms after vitamin B12 supplements and may be a clear indication of this relationship [34-37].

It is alarming to see that 42% of metformin non users and 76% of metformin users in the study sample of diabetic patients had vitamin B12 deficiency. Neuropathy too was present in 46% metformin non users and 74% metformin users. Indian standard treatment guidelines [38] do not lay emphasis on routine screening for Vitamin B12 deficiency in T2DM subjects on metformin, although the recent ADA guidelines recommended the assessment of Vitamin B12 in subjects with peripheral neuropathy [15]. Based on our study results, we suggest that patients with T2DM receiving metformin should be screened for vitamin B12 deficiency and peripheral neuropathy. Since Vitamin B12 is a water-soluble vitamin with low risk for toxicity and long storage capacity, in settings where Vitamin B12 assay is not present, a routine supplementation of all metformin treated T2DM, at least those with metformin use of >5 years or severe neuropathy may be a useful approach.

Limitation(s)

First, diet of the participants was not taken into consideration. Secondly, the biomarkers of metabolic vitamin B12 deficiency such as methyl-malonic acid, and homocysteine, which may have resulted in a better understanding of the true prevalence rate, were not measured. Also, baseline imbalance in glycaemic status was seen (higher HbA1c and fasting plasma glucose) among metformin users. This can have confounding effect on peripheral neuropathy, but not for vitamin B12 deficiency. We have tried to adjust for the status of glycaemic control by regression during data analysis. However, residual confounding may be present. Lastly, controls were defined as not having used metformin in past three months, but could have used metformin in past before switching to other drugs. This would lead to underestimation of association of B12 deficiency and metformin use.

CONCLUSION(S)

In the present study, vitamin B12 deficiency was more frequent in metformin users than non metformin users and was positively

associated with duration of metformin use suggesting metformin as a potential cause of vitamin B12 deficiency among T2DM patients. Vitamin B12 deficiency was also associated with peripheral neuropathy. Hence, metformin-treated T2DM patients, especially those with peripheral neuropathy should be evaluated for vitamin B12 deficiency. However, larger studies are warranted to confirm the study findings and evaluate the benefit of vitamin B12 replacement on neuropathy in those with vitamin B12 deficiency.

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REFERENCES

[1] Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019;157:107843.
[2] Davies MJ, D'Alessio DA, Fradkin J, Kernan WN, Mathieu C, Mingrone G, et al. Management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia. 2018;61(12):2461-98.
[3] American Diabetes Association. 9. Pharmacologic Approaches to glycemic treatment: standards of medical care in diabetes-2021. Diabetes Care. 2021;44(Supplement 1):S111-24.
[4] Sugawara K, Okada Y, Hirota Y, Sakaguchi K, Ogawa W. Relationship between metformin use and vitamin B12 status in patients with type 2 diabetes in Japan. J Diabetes Investig. 2020;11(4):917-22.
[5] Wong CW, Leung CS, Leung CP, Cheng JN. Association of metformin use with vitamin B12 deficiency in the institutionalized elderly. Archives of Gerontology and Geriatrics. 2018;79:57-62.
[6] Al-Hamdi A, Al-Gahhafi M, Al-Roshdi S, Jaju S, Al-Mamari A, Al Mahrezi AM. Vitamin B12 Deficiency in Diabetic Patients on Metformin Therapy: A cross-sectional study from Oman. Sultan Qaboos Univ Med J. 2020;20(1):e90-94.
[7] Mazokopakis EE, Starakis IK. Recommendations for diagnosis and management of metformin-induced vitamin B12 (Cbl) deficiency. Diabetes Res Clin Pract. 2012;97(3):359-67.
[8] Ko SH, Ko SH, Ahn YB, Song KH, Han KD, Park YM, et al. Association of vitamin B12 deficiency and metformin use in patients with type 2 diabetes. J Korean Med Sci. 2014;29(7):965-72.
[9] Kim J, Ahn CW, Fang S, Lee HS, Park JS. Association between metformin dose and vitamin B12 deficiency in patients with type 2 diabetes. Medicine (Baltimore) 2019;98(46):e17918.
[10] Pflipsen MC, Oh RC, Saguil A, Seehusen DA, Seaquist D, Topolski R. The prevalence of vitamin B12 deficiency in patients with type 2 diabetes: A cross-sectional study. J Am Board Fam Med. 2009;22(5):528-34.
[11] Chan JCN, Malik V, Jia W, Kadowaki T, Yajnik CS, Yoon KH, et al. Diabetes in Asia: Epidemiology, risk factors, and pathophysiology. JAMA. 2009;301(20):2129-40.
[12] Raizada N, Jyotsna VP, Sreenivas V, Tandon N. Serum vitamin B12 levels in Type 2 diabetes patients on metformin compared to those never on metformin: A cross-sectional study. Indian J Endocrinol Metab. 2017;21(3):424-28.
[13] Refsum H, Yajnik CS, Gadkari M, Schneede J, Vollset SE, Orning L, et al. Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. Am J Clin Nutr. 2001;74(2):233-41.
[14] Roy RP, Ghosh K, Ghosh M, Acharyya A, Bhattacharya A, Pal M, et al. Study of Vitamin B12 deficiency and peripheral neuropathy in metformin-treated early Type 2 diabetes mellitus. Indian J Endocrinol Metab. 2016;20(5):631-37.
[15] Standards of Medical Care in Diabetes-2019 Abridged for primary care providers. Clin Diabetes. 2019;37(1):11-34.
[16] Bril V, Tomioka S, Buchanan RA, Perkins BA. Reliability and validity of the modified Toronto Clinical Neuropathy Score in diabetic sensorimotor polyneuropathy. Diabet Med. 2009;26(3):240-46.
[17] de Benoist B. Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies. Food Nutr Bull. 2008;29(2 Suppl):S238-44.
[18] Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911-30.
[19] Liu Q, Li S, Quan H, Li J. Vitamin B12 status in metformin treated patients: Systematic review. PLoS One. 2014;9(6):e100379.
[20] Khan A, Shafiq I, Hassan Shah M. Prevalence of Vitamin B12 deficiency in patients with type ii diabetes mellitus on metformin: A study from Khyber Pakhtunkhwa. Cureus. 2017;9(8):e1577.
[21] Reinstatler L, Qi YP, Williamson RS, Garn JV, Oakley GP. Association of biochemical B12 deficiency with metformin therapy and vitamin B12 supplements. Diabetes Care. 2012;35(2):327-33.
[22] Calvo Romero JM, Ramiro Lozano JM. Vitamin B(12) in type 2 diabetic patients treated with metformin. Endocrinol Nutr. 2012;59(8):487-90.
[23] Sparre Hermann L, Nilsson B, Wettre S. Vitamin B12 status of patients treated with metformin: A cross-sectional cohort study. Diabetes & Vascular Disease. 2004;4(6):401-06.
[24] Andrès E, Noel E, Goichot B. Metformin-associated vitamin B12 deficiency. Arch Intern Med. 2002;162(19):2251-52.
[25] de Jager J, Kooy A, Lehert P, Wulffelé MG, van der Kolk J, Bets D, 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.
[26] Marar O, Sentruk S, Agha A, Thompson C, Smith D. The prevalence of vitamin B12 deficiency in patients with type 2 diabetes mellitus on metformin. Royal College of Surgeons in Ireland Student Medical Journal. 2011;4(1):16-20.
[27] Alharbi TJ, Tourkmani AM, Abdelhay O, Alkhashan HI, Al-Asmari AK, Bin Rsheed AM, 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(10):e0204420.
[28] Aroda VR, Edelstein SL, Goldberg RB, Knowler WC, Marcovina SM, Orchard TJ, et al. Long-term metformin use and vitamin B12 deficiency in the diabetes prevention program outcomes study. J Clin Endocrinol Metab. 2016;101(4):1754-61.
[29] Wulffelé MG, Kooy A, Lehert P, Bets D, Ogterop JC, Borger van der Burg B, et al. Effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus: A randomized, placebo-controlled trial. J Intern Med. 2003;254(5):455-63.
[30] Chapman LE, Darling AL, Brown JE. Association between metformin and vitamin B12 deficiency in patients with type 2 diabetes: A systematic review and meta-analysis. Diabetes Metab. 2016;42(5):316-27.
[31] Tomkin GH. Malabsorption of vitamin B12 in diabetic patients treated with phenformin: A comparison with metformin. Br Med J. 1973;3(5882):673-75.
[32] Bauman WA, Shaw S, Jayatilleke E, Spungen AM, Herbert V. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. Diabetes Care. 2000;23(9):1227-31.
[33] Hendrawati YD, Andrajati R, Supardi S, Ariyani A. The risk of Cobalamin deficiency symptoms related to long-term metformin use in T2DM patients. Acta Endocrinol (Buchar). 2018;14(1):49-54.
[34] Stracke H, Lindemann A, Federlin K. A benfotiamine-vitamin B combination in treatment of diabetic polyneuropathy. Exp Clin Endocrinol Diabetes. 1996;104(4):311-16.
[35] Farvid MS, Homayouni F, Amiri Z, Adelmanesh F. Improving neuropathy scores in type 2 diabetic patients using micronutrients supplementation. Diabetes Res Clin Pract. 2011;93(1):86-94.
[36] Yaqub BA, Siddique A, Sulimani R. Effects of methylcobalamin on diabetic neuropathy. Clin Neurol Neurosurg. 1992;94(2):105-11.
[37] Fonseca VA, Lavery LA, Thethi TK, Daoud Y, DeSouza C, Ovalle F, et al. Metanx in type 2 diabetes with peripheral neuropathy: A randomized trial. Am J Med. 2013;126(2):141-49.
[38] Indian Council of Medical Research. ICMR guidelines for management of type 2 diabetes- 2018 [Internet]. Indian Council of Medical Research; 2018 [cited 2021 Jan 5]. Available from: https://main.icmr.nic.in/sites/default/files/guidelines/ICMR_GuidelinesType2diabetes2018_0.pdf.

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