

## Original Research Article

# Vitamin B12 deficiency in patients of type 2 diabetes mellitus treated with metformin: a cross section observational study from a tertiary care centre in Sub-Himalayan region of North India

Laxmi Nand<sup>1\*</sup>, Sunita Mahto<sup>2</sup>, Dalip K. Garg<sup>1</sup>, Bhupender Kumar<sup>1</sup>

<sup>1</sup>Department of Medicine, <sup>2</sup>Department of Biochemistry, Indira Gandhi Medical College and Hospital Shimla, Himachal Pradesh, India

**Received:** 13 February 2020

**Accepted:** 19 February 2020

### \*Correspondence:

Dr. Laxmi Nand,

E-mail: [laxminand66@gmail.com](mailto:laxminand66@gmail.com)

**Copyright:** © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

## ABSTRACT

**Background:** Metformin is first line of treatment in type 2 diabetes mellitus (T2DM). It has been reported to be associated with vitamin B12 deficiency with variable results in various studies. The aim of our study is to correlate metformin use and vitamin B12 levels in patients of T2DM with high prevalence in Sub-Himalayan region of north India.

**Methods:** The study was conducted from August 2017 to July 2018 enrolling 124 patients, aged 18 years and above with T2DM taking metformin treatment for >4 months. Vitamin B12 levels were estimated and the levels <200, 200-300 and >300 pg/ml were defined as having definite deficiency, borderline deficiency and normal levels respectively.

**Results:** A total of 124 patients included 66 (53.2%) male and 58 (46.8%) female patients with T2DM on metformin treatment. The mean vitamin B12 level was found significantly low,  $176.23 \pm 60.96$  pg/ml and 18 (14.5%) patients were found to have significant vitamin B12 deficiency and 8(6.5%) patients, borderline deficiency in longer duration of metformin use for >10 years ( $p < 0.001$ ). Vitamin B12 deficiency was found significantly more in 14 (11.3%) patients taking lower doses  $\leq 1000$  mg/day of metformin compared to higher doses, a negative correlation. Peripheral neuropathy was significantly present in 15.3% of patients in metformin induced vitamin B12 deficiency.

**Conclusions:** Vitamin B12 deficiency was found to be significantly correlated to longer duration of metformin treatment and neuropathy in T2DM but negatively correlated to higher doses of metformin. Therefore, we recommend the assessment and supplementation of vitamin B12 in metformin use in T2DM, compromising financial burden but not the outcome of its deficiency.

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

## INTRODUCTION

Diabetes mellitus is one of the most common endocrine disorder and leading cause of death and disability in the world. The worldwide prevalence has risen dramatically over the past two decades and India is the global capital of diabetes.<sup>1</sup> Amongst the major complications of T2DM,

lower extremity amputation and diabetic kidney disease (DKD) are the most ominous complications. Metformin is first line and cornerstone amongst the recommended treatment for T2DM.<sup>2,3</sup> Folate and vitamin B12 deficiencies occur primarily as a result of insufficient dietary intake especially in elderly and poor absorption.<sup>4</sup> Metformin treatment in T2DM is associated with vitamin

B12 deficiency as one of its side effects.<sup>5,6</sup> Apart from the clinical benefits metformin has been reported with high prevalence of vitamin B12 deficiency in some studies.<sup>7,8</sup> Longer duration and higher doses of metformin use in treatment of T2DM were associated significantly with B12 deficiency.<sup>9-11</sup> 22% prevalence of metabolically confirmed vitamin B12 deficiency in T2DM population was found.<sup>12</sup> Metformin is associated with increase risk factors for vitamin B12 deficiency.<sup>13,14</sup> The mechanism suggested for metformin induced vitamin B12 deficiency includes, alteration in small bowel motility leading to bacterial overgrowth and inhibition of vitamin B12 absorption, alteration in intrinsic factor levels, interaction with the cubulin endocytic receptor and inhibition of the calcium dependent absorption of vitamin B12 and intrinsic factor (IF) complex at terminal ileum.<sup>8,15,16</sup> Metformin induced decrease in vitamin B12 starts as early as the 4<sup>th</sup> month, the clinical features of vitamin B12 deficiency become clinically evident by the 5 years due to delayed depletion of liver stores.<sup>15,16</sup> Vitamin B12 is involved in DNA synthesis, fatty acid metabolism and energy production and is essential for normal functioning of haemopoietic, neurological and vascular system.<sup>17,18</sup> It mediates its effects by methylation of homocysteine to methionine and donation of methyl group.<sup>18,19</sup> Vitamin B12 deficiency mainly leads to megaloblastic anemia, pancytopenia, subacute combined degeneration of spinal cord and peripheral neuropathy.<sup>19,20</sup> India has high prevalence of T2DM and also of vitamin B12 deficiency due to nutritional deficiency and other causes.<sup>21</sup> A study from India reported negative correlation between metformin use and vitamin B12 levels and that the supplementation of vitamin B12 was not necessary and reduced the financial burden.<sup>22</sup> Therefore, we are more interested and aim to further correlate metformin use in treatment of T2DM and vitamin B12 levels through this study in Sub-Himalayan region of north India.

## METHODS

The study was conducted from August 2017 to July 2018 in Indira Gandhi Medical College and Hospital Shimla, a tertiary care centre in the state of Himachal Pradesh in Sub-Himalayan region of north India in Asian continent. A total of 124 patients admitted with T2DM were studied.

### Inclusion criteria

- Patients aged 18 year and above of both sexes,
- Patients fulfilling WHO criteria for diagnosis as having T2DM with fasting blood sugar  $\geq 126$  mg % or blood sugar  $> 200$  mg %, 2 hours after glucose challenge and
- With T2DM taking metformin for  $>4$  months were included in the study.

### Exclusion criteria

- Patients with aged  $<18$  years,

- Not consenting
- The patients of T2DM with preexisting vitamin B12 anemia, on vitamin B12 supplementation, celiac disease, kidney disease, liver disease, thyroid disease, gastro-intestinal surgeries and non consenting were excluded from the study.

Approval from ethical committee was accordingly obtained for the study. Details of patients including doses and duration of metformin use and investigations were determined. Vitamin B12 levels were estimated in fasting blood samples by Chemiluminescent microparticle immunoassay and defined as having definite deficiency ( $<200$  pg/ml), border line deficiency (200-300 pg/ml) and normal levels ( $>300$  pg/ml).

### Statistical analysis

All the data were entered in Excel Sheet and statistical analysis was performed using EPI info version 7. Data were expressed in frequency and percentages. Categorical variables were compared using Chi Square test ( $\chi^2$ ) and means compared using ANOVA. For all comparison p value  $<0.05$  was considered significant.

## RESULTS

A total of 124 patients with T2DM included 66 (53.2%) male and 58 (46.8%) female patients with age ranging from 36 to 83 years and mean age of  $57.75 \pm 10.48$ ,  $58.41 \pm 10.57$  and  $57 \pm 10.42$  years respectively. The patients revealed mean vitamin B12 level of  $365.02 \pm 190.46$  pg/ml,  $382.50 \pm 213.52$  pg/ml in males and  $345.14 \pm 159.79$  pg/ml in females (Table 1).

The patients were grouped into  $\leq 50$ , 51-65, and  $>65$  years of age groups and corresponding mean vitamin B12 levels were  $466 \pm 203.19$  pg/ml,  $379.01 \pm 178.22$  pg/ml and  $236.29 \pm 135.43$  pg/ml, and definite vitamin B12 deficiency ( $<200$  pg/ml) was found in 1(0.8%), 8 (6.5%) and 15 (12.1%) patients, respectively (Table 2). Vitamin B12 deficiency in metformin use was found significantly progressively increasing with increasing age, maximum in elderly  $>65$  years ( $p < 0.001$ ). Definite vitamin B12 deficiency ( $<200$  pg/ml) was found in 24 (19.4%), borderline (200-300 pg/ml) in 21(16.9%) and normal ( $>300$  pg/ml) in 79 (63.7%) patients of metformin use.

In longer duration of metformin use  $>10$  years, all the 26 (21%) patients were found to have vitamin B12 deficiency in 18 (14.5%) patients and borderline deficiency in 8 (6.5%) patients. In the groups of  $\leq 5$ , 5-10 and  $>10$  years of duration of metformin use, the corresponding mean vitamin B12 levels were  $439.69 \pm 184.21$ ,  $383.67 \pm 175.15$  and  $176.23 \pm 60.96$  pg/ml and vitamin B12 deficiency was found in 2 (1.6%), 4 (3.2%) and 18 (14.5%) patients respectively (Table 3). The mean levels of vitamin B12 were found decreasing leading to significantly increasing definite B12 deficiency with increasing duration of metformin use ( $p < 0.001$ ).

**Table 1: Characteristics of study patients.**

Characteristics	Sex		Total (n=124)	p value	
	Female (%)	Male (%)			
FBS (mg/dl)	157.84±62.67	152.0±70.20	154.73±66.58	0.628	
HbA <sub>1c</sub> (%)	9.26±1.95	8.82±2.27	9.0±2.14	0.251	
Hemoglobin (gm/dl)	13.51±2.57	13.45±2.65	13.47±2.55	0.956	
Age groups (years)	≤50	12(9.6)	14(11.2)	26(21.0)	0.99
	51-65	33(26.6)	37(29.8)	70(56.4)	
	>65	13(10.4)	15.(12.2)	28(22.6)	
	Total	58(46.8)	66(53.2)	124(100)	
	Mean (years)	57±10.42	58.41 ± 10.57	57.75 ± 10.48	0.45
Mean vitamin B12 (pg/ml)	345.14±159.79	382.50±213.52	365.02±190.46	0.278	
Duration of metformin intake (years)	≤5	26(21.0)	29(23.4)	55(44.4)	0.866
	5.1-10	21 (16.9)	22 (17.7)	43 (34.7)	
	>10	11 (8.9)	15 (12.1)	26 (21.0)	
Metformin dose (mg/day)	≤1000	23 (18.5)	18 (14.5)	41 (33.1)	0.006*
	1001 - 1500	5 (4.0)	21 (16.9)	26 (21.0)	
	1501 - 2000	30 (24.2)	27 (21.8)	57.(46.0)	

**Table 2: Correlation of serum levels of vitamin B12 and age groups.**

Vitamin B12 (pg/ml)	Age groups			Total	p value
	≤50 years	51-65 years	>65 years		
<200	1 (0.8%)	8 (6.5%)	15 (12.1%)	24 (19.4%)	<0.001*
200-300	4 (3.2%)	12 (9.7%)	5 (4.0%)	21 (16.9%)	
>300	21 (16.9%)	50 (40.3%)	8 (6.5%)	79 (63.7%)	
Total	26 (21.0%)	70 (56.5%)	28 (22.6%)	124 (100%)	
Mean vitamin B12	466±203.19	379.01±178.22	236.29±135.43	365.02±190.466	<0.001*

**Table 3: Correlation between serum levels of vitamin B12 and duration of metformin use.**

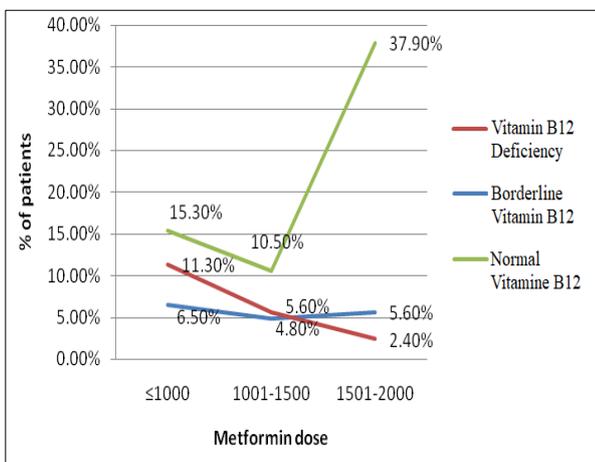
Vitamin B12 (pg/ml)	Duration of metformin use			Total	p value
	≤5 years	5-10 years	>10 years		
<200	2 (1.6%)	4 (3.2%)	18 (14.5%)	24 (19.4%)	<0.001*
200-300	6 (4.8%)	7 (5.6%)	8 (6.5%)	21 (16.9%)	
>300	47 (37.9%)	32 (25.8%)	0 (0.0%)	79 (63.7%)	
Total	55 (44.4%)	43 (34.7%)	26 (21.0%)	124 (100.0%)	
Mean vitamin B12	439.69±184.21	383.67±175.15	176.23±60.96	365.02±190.466	<0.001*

In the groups of metformin doses of ≤1000, 1001-1500 and 1501-2000 mg/day, the corresponding mean B12 levels were 339.49±218.95, 316.42±160.07 and 405.56±175.35 pg/ml and the vitamin B12 deficiency was found in 14 (11.3%), 7 (5.6%) and 3 (2.4%) patients respectively. Higher mean vitamin B12 level was found in higher doses of 1501 - 2000 mg/day (Table 4). There was significant vitamin B12 deficiency associated with lower doses of ≤1000 mg/day compared to higher doses (p<0.001). A negative correlation between mean B12 levels, definite and borderline vitamin B12 deficiency

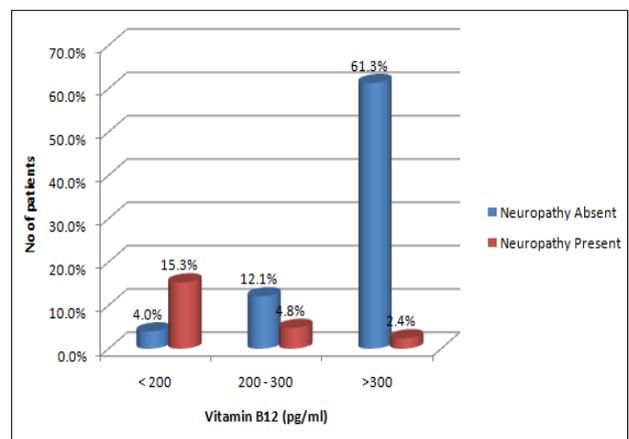
and higher doses of metformin was observed as depicted graphically (Figure 1 and 2). Non significant dip of mean vitamin B12 level (316.42±160.07 pg/ml) seen in slightly higher doses of 1001-1500 mg/day but again rising to mean vitamin B12 level (405.56±175.35 pg/ml) in further higher doses of 1501-2000 mg/day and almost straight lines of mean vitamin B12 deficit and borderline levels revealed negative correlation to higher doses (Figure 2). The patients with significant vitamin B12 deficiency were found in elderly >65 years, longer duration >10 years and lower doses of ≤1000 mg/day of metformin use (Table 2, 3 and 4).

**Table 4: Correlation between serum levels of vitamin B12 and metformin dose.**

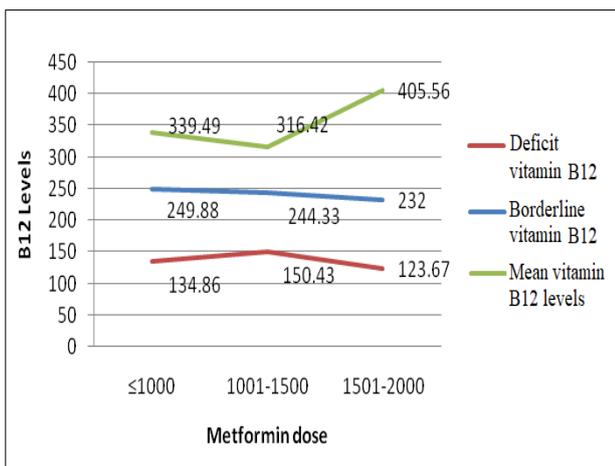
Characteristics	Metformin dose			Total (n=124)	p value	
	≤1000 mg/day	1001-1500 mg/day	1501-2000 mg/day			
Age (years)	61.29±11.92	58.77±6.79	54.74±10.02	57.75±10.05	0.007*	
HbA <sub>1c</sub> (%)	8.61±1.56	9.03±2.34	9.31±2.36	9.02±2.14	0.276	
Metformin duration	>10 years	16 (13%)	8 (6.5%)	2 (1.6%)	26 (21%)	<0.001*
	<10 years	25 (20.2%)	18 (14.5%)	55 (44.4%)	98 (79%)	
Vitamin B12 (pg/ml)	<200	14 (11.3%)	7 (5.6%)	3 (2.4%)	24 (19.4%)	0.001*
	200-300	8 (6.5%)	6 (4.8%)	7 (5.6%)	21 (16.9%)	
	>300	19 (15.3%)	13 (10.5%)	47 (37.9%)	79 (63.7%)	
	Total	41 (33.1%)	26 (21.0%)	57 (46.0%)	124 (100.0%)	
Mean	339.49±218.95	316.42±160.07	405.566±175.35	365.02±190.46	0.081	



**Figure 1: Correlation between metformin dose and B12 deficiency.**



**Figure 3: Correlation between serum levels of vitamin B12 and periphera neuropathy.**



**Figure 2: Correlation between metformin dose and B12 levels.**

Peripheral neuropathy was observed in 28 (22.6%) in metformin use. It was present in 19 (15.3%) patients and absent in 5 (4%) patients in metformin induced vitamin B12 deficiency (Figure 3).

**DISCUSSION**

Metformin induces vitamin B12 deficiency which, in turn, leads to number of clinical conditions mainly memory impairment, peripheral neuropathy, subacute combined degeneration of spinal cord, megaloblastic anemia and pancytopenia.<sup>19,20</sup> A significant correlation was found between metformin use in treatment of T2DM and low vitamin B12 levels in various studies and recommended determination and supplementation of vitamin B12.<sup>23-25</sup> The mean level of vitamin B12 in 124 patients was 365.02±190.6 pg/ml. Definite deficiency of vitamin B12 (<200 pg/ml) in 19.4% patients in this study was showing higher prevalence compared to 5.8%, 8.6% and 6.3% in other studies.<sup>6,14,26</sup> Authors observation was not surprising in view of higher prevalence of vitamin B12 deficiency in apparently healthy population in India reported as high as 33.3% to 67%.<sup>21</sup> The mean vitamin B12 level, 176.23±60.96 pg/ml was significantly low and deficiency was significantly correlated to longer duration of the metformin use for >10 years duration (p <0.001). Authors observation was close to studies by De-Jager et al, and, KA Akinlade, et al.<sup>9,10</sup> Correlation is explained on the basis of depletion of vitamin B12 liver stores which are not quickly depleted.<sup>15,16</sup> However, lower levels of

vitamin B12 was observed in patients taking lower doses of metformin  $\leq 1000$  mg/day with definite deficiency in 11.3% patients as compared to 5.6% and 2.4% patients taking higher doses of 1001-1500 and 1501-2000 mg/day of metformin respectively, a significant negative correlation. Authors attributed it to use of higher doses of metformin in younger age group with shorter duration for strict diabetic control which is evident by higher level of HbA<sub>1c</sub>  $9.31 \pm 2.36\%$  vs lower level of  $8.61 \pm 1.56\%$  and lesser mean age of patients of  $54.74 \pm 10.02$  years vs. higher mean age of  $61.29 \pm 11.92$  years in higher doses of 1501 to 2000 mg/day vs. lower doses  $\leq 1000$  mg/day of metformin respectively. It was mainly attributed it to use of lower doses in long term metformin use in elderly age group of patients in view of decreasing glomerular filtration rate, add on anti-diabetic drugs and insulin therapy.<sup>26-28</sup> Conclusively the patients with vitamin B12 deficiency in longer duration of metformin use in elderly age group were found frequently distributed to lower doses. As the vitamin B12 deficiency was found significantly higher (12.1%) in advanced age group  $>65$  years and (14.5%) in longer duration  $>10$  years, therefore, we observed significant vitamin B12 deficiency (11.3%) in lower dose  $\leq 1000$  mg/day of metformin use. Mean vitamin B12 levels were also found significantly low  $236.29 \pm 135.43$  pg/ml in advanced age group  $>65$  years,  $176.23 \pm 60.96$  pg/ml in longer duration  $>10$  years and  $339.49 \pm 218.95$  pg/ml in lower doses  $\leq 1000$  mg/day of metformin use. The negative correlation is explained by the fact that liver stores of vitamin B12 are not quickly depleted and that mechanism for higher doses of metformin causing vitamin B12 deficiency is also not well understood.<sup>10,15,16</sup> The use of sulfonylurea, an independent factor for vitamin B12 deficiency, initially for strict diabetic control may be contributing factor.<sup>29</sup> However it has been shown that each gm daily intake of metformin caused a ratio of 2.88 increase in the risk of developing vitamin B12 deficiency.<sup>9,13</sup> Authors observation relating to negative correlation, although, contrary to, but they do not deny the significant correlation of lower levels of vitamin B12 to higher doses of metformin in other studies.<sup>9,10,13</sup> Apart from above explanation, this is also because, duration of metformin use is definitely ascertained compared to fluctuating doses and add on therapy, which might be a confounding factor also contributing to negative correlation in this study. The patients revealed mean BMI of  $23.55 \pm 3.0$  and  $23.64 \pm 3.50$  and HbA<sub>1c</sub> of  $8.90 \pm 1.54$  and  $8.92 \pm 0.76$  in male and females patients respectively in  $>10$  years of metformin use compared to the obese patients and higher HbA<sub>1c</sub> in some studies.<sup>14,30</sup> Vitamin B12 deficiency in 15.3% patients due to metformin use was found to be significantly associated with peripheral neuropathy in our study ( $p < 0.001$ ). Metformin indirectly via vitamin B12 deficiency was found to increase the neuropathy burden in T2DM.<sup>31,32</sup> Authors observations do not support the findings and recommendation of one of the studies from India which reported that the diabetic patients on metformin were not having any vitamin B12 deficiency and that the supplementation of vitamin B12 in long term

metformin treatment was not necessary and reduced the financial burden.<sup>22</sup>

## CONCLUSION

Long term metformin treatment in T2DM is found to be significantly correlated to vitamin B12 deficiency and peripheral neuropathy, and negatively correlated to higher doses, attributed to lower doses in long term metformin treatment in advanced age group and add on anti-diabetic drugs and insulin therapy. Since our study has shown significant correlation of long term metformin use and peripheral neuropathy to vitamin B12 deficiency, therefore, vitamin B12 levels should be assessed and supplemented in use of metformin in T2DM, compromising financial burden but not the outcome of vitamin B12 deficiency. However, further long term study with larger number of subjects exclusively designed to ascertain correlation between doses of metformin and vitamin B12 levels, needs to be conducted.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee*

## REFERENCES

1. American diabetes association. Standards of Medical Care in Diabetes-2018. Diab Care. 2018;41(Supplement1):S1-S2.
2. American Diabetes Association. Standards of medical care in diabetes: 2013. Diab Care. 2013; 36:11-66.
3. Scarpello JH, Howlett HC. Metformin therapy and clinical uses. Diab Vasc Dis Res. 2008;5(3):157-67.
4. de Benoist B. Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies. Food Nutr Bull. 2008 Jun;29(2\_suppl1):S238-44.
5. Kumthekar AA, Gidwani HV, Kumthekar AB. Metformin Associated B12 Deficiency. J Assoc Physicians India. 2012;60:58-9.
6. Reinstatler L, Qi YP, Williamson RS, Garn JV, Oakley GP. Association of biochemical B12 deficiency with metformin therapy and vitamin B12 supplements: the National Health and Nutrition Examination Survey, 1999-2006. Diab Care. 2012 Feb 1;35(2):327-33.
7. Iftikhar R, Kamran SM, Qadir A, Alqbal Z, Bin Usman H. 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.
8. Andre's E, Noel E, Goichot B. Metformin-associated vitamin B12 deficiency. Arch Intern Med. 2002;162:2251-2.
9. De-Jager J, Kooy A, Lehert P, Wulffele 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.
10. Akinlade KS, Agbebaku SO, Rahamon SK, Balogun WO. Vitamin B12 levels in patients with type 2 diabetes mellitus on metformin. *Ann Ibd Pg Med.* 2015;13:79-83.
  11. Hermann LS, Nilsson BO, Wettre S. Vitamin B12 status of patients treated with metformin: a cross-sectional cohort study. *Br J Diab Vasc Dis.* 2004;4:401-6.
  12. 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;22:528-34.
  13. Ting RZ, Szeto CC, Chan MH, Ma KK, Chow KM. Risk Factors of Vitamin B12 Deficiency in Patients Receiving Metformin. *Arch Intern Med.* 2006;166:1975-9.
  14. Nervo M, Lubini A, Raimundo FV, Faulhaber GA, Leite C, Fischer LM, et al. Vitamin B12 in metformin treated diabetic patients, a cross sectional study in Brazil. *Revista DA Assoc Med Bras.* 2011;57(1):46-9.
  15. Wulffele MG, Kooy A, Lehert P, Bets D, Ogterop J C, Borger van der 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;254:455-63.
  16. Bauman WA, Shaw S, Jayatilleke E, Spungen AM, Herbert V. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. *Diab Care.* 2000; 23:1227-31.
  17. Yajnik, CS, Deshpande SS, Lubree HG, Naik SS, Bhat DS, Uradev BS, et al. Vitamin B12 deficiency and hyperhomocysteinemia in rural and urban Indians. *J Assoc Physicians India.* 2006;54:775-82.
  18. Allen LH. How common is vitamin B12 deficiency? *Am J Clin Nutr.* 2009;89:693S-6S.
  19. Green R, Kinsella LJ. Current concepts in the diagnosis of cobalamin deficiency. *Neurol.* 1995;45:1435-40.
  20. Green, R. Vitamin B12 deficiency from the perspective of a practicing hematologist. A recent and authoritative up-to-date summary of the clinical aspects of B12 deficiency with emphasis on the haematological perspective. *Blood.* 2017;129:2603-11.
  21. Ingole JR, Patel RD, Ingole SJ, Pandave HT. Opportunistic screening of Vitamin B12 deficiency in IT professionals presenting for routine health check-up. *J Clin Diagn Res.* 2015;9:01-2.
  22. Nereddy VA, Boddikuri IP, Ubedullah SK, Papareddy A, Miriyam MK. 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.
  23. Pongchaidecha M, Srikusalanukul V, Chattananon A, Tanjariyaporn S. Effect of metformin on plasma homocysteine, vitamin B12 and folic acid: a cross-sectional study in patients with type 2 diabetes mellitus. *J Med Assoc Thai.* 2004; 87:780-7.
  24. Qureshi S, Ainsworth A, Winocour P. Metformin therapy and assessment for vitamin B12 deficiency: is it necessary? *Pract Diab.* 2011;28:302-4.
  25. Agarwal P, Mital P, Meena VK, Mital P, Nawal CL, Goyal LK. A comparative study of levels of vitamin B12 in patients of type 2 diabetes mellitus on metformin and not on metformin at tertiary care center. *Int J Adv Med.* 2016;3:759-63.
  26. American Diabetes Association. Pharmacologic approaches to glycemic treatment. *Diab Care.* 2017;40(Supplement 1):S64-S74.
  27. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet.* 1998; 352:854-65.
  28. Nagrebetsky A, Griffin S, Kinmonth AL, Sutton S, Craven A, Farmer A. Predictors of suboptimal glycaemic control in type 2 diabetes patients: the role of medication adherence and body mass index in the relationship between glycaemia and age. *Diabetes Res Clin Pract.* 2012;96(2):119-28.
  29. Kang D, Yun JS, Ko SH, Lim TS, Ahn YB, Park YM, et al. Higher prevalence of metformin-induced vitamin B12 deficiency in sulfonylurea combination compared with insulin combination in patients with type 2 diabetes: a cross-sectional study. *PLoS One* 2014;9:e109878.
  30. Malin SK, Kashyap SR. Effects of metformin on weight loss: potential mechanisms. *Curr Opin Endocrinol Diabetes Obes.* 2014;21:323-9.
  31. Rees JA, Dunstan DF, Chen S, Lansdown JA, Moat SJ, Ellis R, et al. An observational study of the effect of metformin on B12 status and peripheral neuropathy. *Br J Diabetes Vasc Dis.* 2012;12:189-93.
  32. Singh AK, Kumar A, Karmakar D, Jha RK. Association of B12 deficiency and clinical neuropathy with metformin use in type 2 diabetes patients. *J Postgrad Med.* 2013;59(4):253-7.

**Cite this article as:** Nand L, Mahto S, Garg DK, Kumar B. Vitamin B12 deficiency in patients of type 2 diabetes mellitus treated with metformin: a cross section observational study from a tertiary care centre in Sub-Himalayan region of North India. *Int J Res Med Sci* 2020;8:1248-53.