Print ISSN: 2319-2003 | Online ISSN: 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

DOI: http://dx.doi.org/10.18203/2319-2003.ijbcp20180104

Original Research Article

Prevalence of vitamin B12 deficiency among individuals with type 2 diabetes mellitus in a South Indian rural community

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Received: 08 December 2017
Accepted: 02 January 2018

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ABSTRACT

Background: To estimate the prevalence of vitamin B12 deficiency in a rural south Indian community and to evaluate the association between metformin use and prevalent vitamin B12 deficiency in people with T2DM stratified by oral vitamin B12 supplementation.
Methods: Using a cross sectional study design, a random sample of people with T2DM (N=438) was recruited from a rural community. Vitamin B12 deficiency was defined as serum B12 ≤200pg/ml. Data on metformin dose, duration of use, oral vitamin B12 supplementation, and diet were collected. Laboratory measurements included complete blood count, tests for hepatic, renal, and thyroid function, as well as serum vitamin B12 levels and HbA1c.
Results: The prevalence of vitamin B12 deficiency in people with T2DM was 11.2% (95% Confidence Interval (CI) 8.2%-14.1%). The odds of vitamin B12 deficiency in patients receiving a metformin dose of 2 grams/day were 4 times higher compared to those receiving ≤1 gram/day, after adjusting for oral B12 supplementation (odds ratio 4.2;95% CI 1.5-11.8). The odds of vitamin B12 deficiency in those taking metformin and receiving oral vitamin B12 supplementation were lower compared to those on metformin and not receiving vitamin B12 supplementation (adjusted odds ratio 0.20; 95% CI 0.06-0.70).
Conclusions: Vitamin B12 deficiency affects 1 in 10 people with T2DM, is associated with higher dose metformin use, and oral vitamin B12 supplementation mitigates B12 deficiency in this group.

Keywords: Metformin, Oral B12 supplementation, Type 2 diabetes mellitus, Vitamin B12

INTRODUCTION

Metformin is a common initial drug used in the treatment of type 2 diabetes mellitus (T2DM).[1] Beyond improving glycaemic control, it has been associated with reduced long-term cardiovascular morbidity and mortality.[2,3] Vitamin B12 malabsorption due to metformin treatment has been reported as early as 1971.[4] Several studies have

assessed the relationship between metformin use and vitamin B12 deficiency, but results have been conflicting.[5-19] This may be partly due to the variability in dose of metformin used, duration of its use, and concomitant oral vitamin B12 supplementation.

Prevalence estimates from studies on vitamin B12 deficiency in the general Indian population range from 16% to more than 60%.[20-23] Furthermore, about 65 million people in India have diabetes.[24] Since the reported deficiency of vitamin B12 in the general population in India is high, people with diabetes receiving metformin therapy could be at a greater risk of developing vitamin B12 deficiency. However, the prevalence of vitamin B12 deficiency in individuals with diabetes in India has not been previously reported.

Given the high prevalence of both vitamin B12 deficiency and diabetes in India, authors aimed to estimate the prevalence of vitamin B12 deficiency in people with T2DM. In addition, there were planned to evaluate the association between metformin use characteristics, including dose and duration, with prevalent vitamin B12 deficiency. Lastly, it was sought to describe the influence of oral vitamin B12 supplementation on this relationship.

METHODS

Study population and sampling

Authors used a cross sectional study design. The source population originated from people residing in 40 villages with a cumulative population of about 46,000 in a South Indian district (Ranga Reddy district) adjoining Hyderabad, India. A list of people with T2DM was generated in a survey conducted by SHARE India-MediCiti Institute of Medical Sciences, Hyderabad in 2009. This list was subsequently updated in the second half of 2013, two months prior to the commencement of the study. Participants were selected by a simple random sampling technique.

Inclusion and exclusion criteria

The ethics committees of MediCiti (Hyderabad, India) and the Centre for Chronic Disease Control (New Delhi, India) approved the research project. Written informed consent was obtained from all participants prior to enrolment. Individuals>18 years of age with a physician diagnosis of T2DM were included in this study. Those fulfilling one or more of the following conditions were excluded: acute illness or hospitalization in the three months prior to recruitment, hepatic aspartate aminotransferase (AST) and alanine aminotransferase (ALT) elevated beyond the upper limit of normal, chronic alcoholism, thyroid stimulating hormone (TSH) levels suggestive of thyroid dysfunction, and estimated glomerular filtration rate (eGFR)<60 ml/min per 1.73m^2. Individuals being actively treated with one or more of the following medications that could affect vitamin B12 levels were not included in this study: proton pump inhibitors, H2 receptor blockers, thyroxine, anti-thyroid drugs, anti-malignancy drugs, oral contraceptive pills, acarbose, miglitol, voglibose, calcium preparations, antiepileptic drugs. Individuals with a history of treatment with multivitamin or vitamin B12 injections irrespective of the dose in the past 6 months or those who were using or had used >500 mcg of oral vitamin B12 in the past 6 months were also excluded.

Study participants were screened for eligibility according to the inclusion and exclusion criteria listed above using an interviewer administered structured questionnaire and /or clinical examinations as well as biochemical evaluation where appropriate.

Data collection

A pretested structured questionnaire was used to collect data on demographics, medications being used, and diet. Demographic and medication use data were collected from government issued identity cards and physician records respectively. Dietary data were based on self-report. Vegetarians were defined as those who consumed only plant derived food. Lacto-vegetarians were defined as those who consumed plant derived food plus milk and/or milk products. Lacto-ovo-vegetarians were defined as those who consumed plant derived food, milk and/or milk products, and egg products. Those who consumed plant derived food and egg but not milk was defined as Ovo-vegetarians. Non-vegetarians were defined as those who consumed fish or poultry or meat in addition to any or all of the foods listed for other diet categories.

Authors assessed the socioeconomic status (SES) using updated Prasad's scale, incorporating consumer price indices for the study period; SES category I being the highest and V being the lowest.[25] Authors used CAGE questionnaire to identify chronic alcoholism.[26] Estimated GFR (eGFR) was calculated using Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) calculator.[27,28] Serum samples for biochemical estimation of B12 were analysed using Chemiluminiscence Immunoassay (Siemens Advia Centaur XP). Biochemical vitamin B12 deficiency was defined as serum vitamin B12 levels ≤ 200pg/ml.[7] Blood samples for complete blood picture-including haemoglobin, red blood cell(RBC) count, white blood cell count, platelet count, indices of RBC i.e., mean corpuscular volume(MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were assessed on an auto analyser (Mindray BC 3000plus). Glycosylated haemoglobin (HbA1c) was estimated by high performance liquid chromatography (Biorad D10). Serum creatinine estimation was done by modified Jaffe Kinetic method on Siemens Dade Dimension machine. Thyroid stimulating hormone was analyzed by Chemiluminescence using Siemens Advia Centaur XP and liver function tests-aspartate amino transferase (AST) and alanine amino transferase (ALT) were done by Tris Buffer method (Randox).

Statistical analysis

Sample size

A pilot study conducted in the same geographical area 2 weeks prior to this study showed a prevalence of vitamin B12 deficiency in 11% of patients with T2DM. Based on this, the minimum sample size required was 418, with an absolute precision of 3% at a confidence level of 95%. We included 438 people with T2DM in this study. Statistical analysis was conducted using STATA (version 13, Stata Corp, College Station, Texas, USA). Data were assessed for normality using the Shapiro Wilk test. Normally distributed continuous data were summarized as means and standard deviations (mean±SD). Nonparametric data were summarized using medians and interquartile range (IQR). Prevalence of vitamin B12 deficiency was calculated as the proportion of people with vitamin B12 deficiency among the study sample. Comparison of vitamin B12 levels in patients with diabetes on metformin and those not on metformin was done using the Wilcoxon rank sum test. Comparison of means of normally distributed continuous variables was done using unpaired Student's t test assuming unequal variance. Chi-square ($\chi 2$) test and Fisher's exact test were used to compare the proportion of people with vitamin B12 deficiency between those people on metformin and those not on metformin. Comparison of proportion of people with vitamin B12 deficiency between those on metformin and those not on metformin was done after stratifying study participants into those receiving and not receiving oral vitamin B12 supplementation.

In order to identify determinants of serum vitamin B12 in those on metformin, simple linear regression analysis was performed. Serum vitamin B12 values were log transformed for performing linear regression. Subsequently, the independent variables affecting vitamin B12 levels were analysed using a logistic regression model. A logistic regression model with vitamin B12 deficiency as the dependent variable and metformin dose and oral vitamin B12 supplementation as categorical (independent) variables was constructed (Table 4). Metformin dose was categorized into three categories: category 1: metformin dose ≤1g/day, category 2: metformin dosage between 1-2g/day, category 3: metformin dose: ≥2g/day. Oral vitamin B12 supplementation was dichotomously categorized as, category 1: receiving supplementation, and category 2: not receiving supplementation.

RESULTS

438 people with T2DM participated in this study, 316 were taking metformin and 122 were not. Vitamin B12 deficiency was observed in 11.2% of the study people (95% CI 8.2%-14.1%). There were no significant differences between metformin users and nonusers with respect to age, sex, diet, socio-economic status, and oral vitamin B12 supplementation (Table 1). Further, no differences were found with respect to mean daily dose of oral vitamin B12 supplementation, glycosylated haemoglobin (HbA1c) levels, average blood haemoglobin, and average red blood cell mean corpuscular volume (Table 1). The median daily metformin dose was 1g (IQR 0.5-1g). The minimum and maximum daily doses of metformin were 0.5g and 2g, respectively and none of the metformin users had a dose change in the past three months. Only 7% of patients on metformin were on a daily dose of 2gram, of which 35% received oral vitamin B12 supplementation and 65% did not. The median duration of metformin use was 4 years (IQR 2-7years).

Table 1: Characteristics of the study participants.

Variable	Not on metformin (N=122)	On metformin (N=316)	P value
Age, years (Mean±SD)	54.58±11.9	55.19±10.5	0.60
% Male	57.8	50.3	0.15
Distribution of participants by diet			0.80
Vegetarian (%)	0	0	
Lacto-vegetarian (%)	0	0	
Ovo-lacto-vegetarian (%)	26.2	26.2	
Non-vegetarian (%)	73.8	73.9	
% of Individuals by Socio-Economic Status (SES) category *			0.82
I	2.0	3.3	
II	9.8	8.4	
III	84.9	83.5	
IV	3.3	I4.8	
V	0	0	
Median duration of medication use (years)	3.5	4.0	0.14
Proportion receiving daily oral B12 supplementation (%)	15.6	18.0	0.54
Daily oral B12 supplementation (mcg) (Mean±SD)	11.1 ±3.9	12.8±3.5	0.09
HbA1c (%) (Mean±SD)	8.3 ±2.0	8.3±1.9	0.80
MCV(femtoliters) (Mean±SD)	87.2±8.7	87.0±6.2	0.81
Hb (g/dl) (Mean±SD)	12.8±2.1	12.8±1.7	0.90

*SES categories according to monthly per-capita income in Indian New Rupees (INR): category I ≥5455, category II ≥ 2728 but ≤ 5454, category III ≥1637 but ≤2727, category IV ≥818 but ≤1636 category V≤ 817.

The duration of metformin use ranged from 6 months to 20 years. Therefore, metformin users group comprised those with a minimum exposure to metformin for a period of six months. The median duration of medication use in those

not on metformin was 3.5 years (IQR 1.5-6 years). 80% of metformin users were receiving mono-therapy and the remaining 20% additionally received sulfonylurea and/or insulin. In the non-metformin group, 10% received insulin alone and the remaining 90% received various combination therapy, comprising sulfonylureas, pioglitazone, and insulin. The non-metformin group comprised never users of metformin.

Serum vitamin B12 levels were not different among metformin users and non-users, independent of oral vitamin B12 supplementation. Similarly, there was no difference in the proportion of people with vitamin B12 deficiency among metformin users and non-users independent of oral vitamin B12 supplementation (Table 2).

Table 2: Comparison of vitamin B12 status of study participants.

Variable	On metformin (N=316)	Not on metformin (N=122)	P value
Serum Vitamin B12 (pg/ml), Median (IQR)	324 (244-397)	359 (262-456)	0.06
Serum Vitamin B12 levels -not taking supplementation (pg/ml) Median (IQR)	321 (241- 399)	356 (256-456)	0.05
Proportion of total people with B12 deficiency (%)	11.7 (37/216)	9.8 (12/122)	0.58
Proportion of people with B12 deficiency - not taking supplementation (%)	12.7 (33/259) (95% CI=9.1-16.4)	10.7 (11/103) (95% CI=5.2-16.2)	0.66
Serum Vitamin B12 levels - taking supplementation(pg/ml), Median (IQR)	351 (275- 391)	387 (311- 541)	0.23
Proportion of people with B12 deficiency - taking supplementation (%)	7.0 (4/57) (95% CI=0.4-13.6)	5.3 (1/19) (95% CI= -4.8-15.3)	1.0

Age, sex, socioeconomic status, diet, and duration of metformin use were not associated with serum vitamin B12 levels. However, metformin dose was negatively associated with serum vitamin B12 and, oral vitamin B12 supplementation dose was positively associated with serum vitamin B12 (Table 3).

In subgroup analysis, authors found that the odds of B12 deficiency in patients receiving metformin at a dose of 2g/day were fourfold higher compared to those receiving less than1 g/day after adjusting for oral B12 supplementation (Table 4). However, vitamin B12 deficiency (adjusted for vitamin B12 intake) in those receiving at least 1 g/day (but less than 2 g/day) of metformin was not significantly different from those receiving metformin less than 1 g/day. Further, after adjusting for metformin dose, the odds ratio of vitamin B12 deficiency in patients receiving oral B12 supplementation was 0.21 compared to those not receiving supplementation (Table 4).

Table 3: Results of simple linear regression analysis with step wise backward elimination to identify determinants of serum vitamin B12 in people receiving metformin.

Variable	β coefficient	P value
Age	-0.0.0	0.52
Sex (reference- male)	-12.4	0.34
Socioeconomic status (SES)#		
SESII	155.4	0.35
SESIII	137.9	0.31
SESIV	114.7	0.41
Metformin dose (g)	-30.3	0.01
Metformin duration (years)	-1.9	0.41
Oral B12 supplementation dose (mcg)	2.9	0.02
HbA1c	-0.9	0.54
Diet category## Non-vegetarian	10.7	0.43

(Reference, SES=1)
(Reference, ovo-lacto-vegetarian)

Table 4: Odds of developing B12 deficiency by metformin dose and oral vitamin B12 supplementation.

Metformin dose	Odds ratio	95%CI for Odds ratio	P value
Metformin dose category 2 (n= 201)	1.9	0.6 - 5.8	0.29
Metformin dose category 3 (n=23)	4.2	1.5 - 11.8	0.01
Metformin dose category1 (n=214)	1.0	-----	-----
Oral B12 supplementation category 1 (n=93)	0.2	0.1 - 0.7	0.01
Oral B12 supplementation category 2(n=345)	1.0	------	------

DISCUSSION

Authors have observed vitamin B12 deficiency in over one-tenth of individuals with T2DM in this community-based study in a rural population in South India. While studying it was found that metformin at a daily dose of 2g was associated with vitamin B12 deficiency in those who

did not receive oral vitamin B12 supplementation, but not in those who received oral vitamin B12 supplementation.

This is the first community-based study to report the prevalence of vitamin B12 deficiency among those with T2DM in India. The prevalence of vitamin B12 deficiency in a clinic-based study in North India, with a relatively smaller sample size (N=136), was 19.9%.[16] In health facility-based studies conducted in patients with type 2 diabetes mellitus in different parts of the world, the prevalence of vitamin B12 deficiency ranged from 6.5% to 27.2%.[13-15] Part of this variation is related to the differences in study setting, sample size, biochemical markers used for assessing vitamin B12 deficiency, and variability in the definition used for vitamin B12 deficiency.

The observation that higher doses of metformin therapy are associated with lower serum vitamin B12 levels is consistent with other previous reports. For example, malabsorption of vitamin B12 was observed in 30% of patients receiving metformin therapy for more than 2 years only in those who were on a daily dose of 1.97g/day, but not in those who were on 1.75g/day.[4] Similarly, in a randomized controlled trial comparing insulin with metformin administered at a daily dose of 2.55g/day, the absolute risk of vitamin B12 deficiency was 7.2% higher in the metformin group than in the placebo group at the end of 4.3 years.[7,8] Our observation that vitamin B12 supplementation reduced the odds of its deficiency in patients with diabetes on metformin is similar to that reported in a prior cross sectional study.[6] Similarly, another study that analysed the National Health and Nutrition Examination Survey, USA (NHANES) data of adults aged ≥50 years reported a beneficial role for multivitamin supplementation containing vitamin B12 in a dose >6 mcg/day in individuals receiving metformin therapy.[7]

Only a small proportion (<10%) of our study participants were receiving metformin at a daily dose of 2g, of which approximately 35% were receiving oral vitamin B12 supplementation. Therefore, it is possible that authors could not detect a difference in overall median serum vitamin B12 levels between those on metformin and those not on metformin. Similarly, it is possible that due to the same reason authors could not detect a difference in proportion of people with vitamin B12 deficiency between those on metformin and those not on metformin. Stratification of study participants treated with metformin by daily dose of metformin and vitamin B12 supplementation revealed the association between higher doses of metformin and vitamin B12 deficiency; and the influence of oral vitamin B12 supplementation on the relationship between metformin use and vitamin B12 deficiency.

The strengths of this study are the use of random sampling of study participants and exclusion of those with known confounders that affect serum vitamin B12 levels. Limitations include a cross sectional study design and a relatively small sample size.

In this study population vitamin B12 deficiency was observed in about 10% of individuals with T2DM, which is mostly associated with metformin therapy of ≥2 g/day. Oral vitamin B12 supplementation protects against vitamin B12 deficiency in patients with T2DM on metformin. Studies with larger sample size, across multiple locations are needed to better estimate the prevalence of vitamin B12 deficiency in individuals with T2DM, particularly in a large and diverse country such as India, in order to determine the potential need for screening and monitoring B12 levels in such patients. Future research should additionally evaluate the role for prophylactic vitamin B12 supplementation in individuals with T2DM treated with metformin.

ACKNOWLEDGEMENTS

Dr. Shailendra D. was a trainee in the Fogarty International Center of the National Institutes of Health, USA training programs under Award Number D43 TW 009078 and 1D43HD05249.

Funding: Funding sources from the D43 Training Program (Award No. 1D43HD05249 of the Eunice Kennedy Shriver National Institute of Child Health and Human Development and Fogarty International Center [FIC]) of the National Institutes of Health
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee of MediCiti (Hyderabad, India) and the Centre for Chronic Disease Control (New Delhi, India)

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Cite this article as: Shailendra D, Kalani R, Narayan KMV, Prabhakaran S, Tandon N, Reddy PS. Prevalence of vitamin B12 deficiency among individuals with type 2 diabetes mellitus in a South Indian rural community. Int J Basic Clin Pharmacol 2018;7:309-14.