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ORIGINAL ARTICLE

Prevalence of vitamin B_{12} deficiency in South Indians with different grades of glucose tolerance

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Abstract

Aims To determine the prevalence of vitamin B_{12} deficiency in an urban south Indian population in individuals with different grades of glucose tolerance.

Methods A total of 1500 individuals [900 normal glucose tolerance (NGT), 300 prediabetes and 300 type 2 diabetes (T2DM)] who were not on vitamin B_{12} supplementation were randomly selected from the Chennai Urban Rural Epidemiological Study (CURES) follow-up study. Anthropometric, clinical and biochemical investigations, which included vitamin B_{12}, insulin, homocysteine, HbA1c and serum lipids, were measured. Vitamin $B_{12} \leq 191$ pg/ml was defined as absolute vitamin B_{12} deficiency and vitamin $B_{12} > 191$ pg/ml and ≤ 350 pg/ml as borderline deficiency.

Results The mean levels of vitamin B_{12} significantly decreased with increasing degrees of glucose tolerance (NGT 444 ± 368; prediabetes 409 ± 246; T2DM 389 ± 211 pg/ml, $p = 0.021$). The prevalence of absolute vitamin B_{12} deficiency was 14.9% while 37.6% had borderline deficiency. The prevalence of absolute vitamin B_{12} deficiency was significantly higher among individuals with T2DM (18.7%) followed by prediabetes (15%) and NGT(13.7%) [p for trend = 0.05]. The prevalence of vitamin B_{12} significantly increased with age ($p < 0.05$) and in those with abdominal obesity ($p < 0.001$). Men and vegetarians had twice the risk of vitamin B_{12} deficiency compared to women and non-vegetarians, respectively. Among individuals with NGT, prediabetes and T2DM, vitamin B_{12} negatively correlated with homocysteine.

Conclusion This study reports that the levels of vitamin B_{12} decreased with increasing severity of glucose tolerance.

Keywords Vitamin B_{12} · Diabetes · Glucose tolerance · Prevalence · South Indians

Introduction

Vitamin B_{12}, one of the eight B vitamins is a water-soluble vitamin, plays a significant role in the normal functioning of the brain and nervous system [1]. It cannot be produced by human body and should be taken externally. It is also involved in the formation of red blood cells and helps to create, regulate DNA, in the fatty acid and amino acid metabolism and especially metabolism affecting DNA synthesis. Vitamin B_{12} deficiency presents with diverse clinical manifestations ranging from impaired memory, dementia, delirium, peripheral neuropathy, subacute combined degeneration of the spinal cord, megaloblastic anemia and pancytopenia [2]. Although vitamin B_{12} deficiency is a well-known health problem, it does not seem to have attracted the same level of attention as deficiency of other micronutrients such as iron or vitamin A. Vitamin B_{12} deficiency is closely connected to cell metabolism and plays an important role in methionine synthase, which is involved in the conversion of the homocysteine to methionine. Indeed increased plasma total homocysteine is a sensitive marker of vitamin B_{12} deficiency [3, 4].

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Though Indians are known to be more insulin resistant and more susceptible to diabetes and cardiovascular disease, there are very few population-based studies in India that have evaluated the levels of vitamin B_{12} in the general population [5, 6]. Earlier clinical and population-based studies have demonstrated that vitamin B_{12} deficiency is highly prevalent in adults with type 2 diabetes(T2DM) [7–9]. However, data regarding vitamin B_{12} levels in those with prediabetes are very limited.

Our earlier studies demonstrated that vitamin B_{12} deficiency is associated with adverse lipid profile in patients withT2DM [10]. Deficiency of vitamin B_{12} cause elevated serum homocysteine, which has been shown to be a risk factor for hypertension [11] and T2DM [12] diabetes-related complications [13] and coronary artery disease [14]. This underscores the importance of studying vitamin B_{12} levels especially among Asian Indians who have increased susceptibility to T2DMand premature coronary artery disease [15]. Therefore, in this paper, we aimed to measure the serum levels of vitamin B_{12} and to study their association among South Indians with different grades of glucose tolerance.

Materials and methods

The study subjects were recruited from the Chennai Urban Rural Epidemiology Study (CURES) cohort [16]. The methodology of CURES has been published elsewhere [16, 17]. In brief, CURES was performed between 2001 and 2003 on a representative sample of 26,001 adults ≥ 20 years of age from Chennai. In the baseline survey (Phase 1), of the 26,001 individuals screened, all the individuals with T2DM (Phase 2, $n = 1382$) and 1 in every 10 individuals in Phase 2 (Phase 3, $n = 2207$) underwent further detailed investigations, and these constituted the cohort for the follow-up study ($n = 3589$) which was conducted between 2012 and 2013. Of the 3589 individuals followed up, 645 individuals were lost to follow-up (18%). 1179 individuals were lost to follow-up due to migration, refusal and death. Verbal autopsy was done in 381/534 individuals who died. In addition, glycemic status was available in 29 individuals (15 with normal glucose tolerant (NGT) and 14 prediabetes at baseline) who died and were included in analysis for incidence data. Hence, a total of 2439 individuals were followed up, which included 44% individuals with NGT, 12.3% individuals with prediabetes and 43.6% individuals with T2DM. This study had considered the cases and controls based on the usual recommendation to include not more than five controls for each case [18]. Further when the sample size was calculated assuming an expected prevalence of vitamin B_{12} deficiency of 66% based on an earlier study from western India [19] and allowing an α error of 5% in a population of 2410, the sample size was estimated to be ~ 300 in each group of glucose tolerance. For every case of prediabetes and/or T2DM, three controls (NGT) were sufficient to match the power of sample size as well as to be cost effective for the secondary analyses of CURES follow-up study. Thus, among the 2410 individuals, 1500 individuals, which included 900 NGT, 300 prediabetic and 300 T2DM individuals were randomly selected for this study. Individuals were excluded from participation if they were known cases of type one diabetes, had diabetes secondary to other causes, e.g., chronic pancreatitis, if they were < 20 years or > 80 years of age or were taking vitamin B_{12} supplements.

A written informed consent was obtained from all the study participants and the Madras Diabetes Research Foundation (MDRF) Institutional Ethics committee approved the study. A questionnaire was administered to all the participants to elicit details pertaining to demography, socioeconomic status, medical history, family history diabetes and dietary pattern. Anthropometric details such as height, weight and waist were measured using standardized techniques [20]. The blood pressure was recorded in the sitting position in the right arm with a mercury sphygmomanometer. Two readings were taken 5 min apart and the mean of the two was recorded as the blood pressure.

A fasting venous blood sample was collected after an overnight fast of at least 10 h for biochemical investigations, which included vitamin B_{12}, homocysteine, insulin, HbA1c and lipids. Biochemical analyses were performed in a laboratory certified by the National Accreditation Board for Testing and Calibration Laboratories (NABL) and the College of American Pathologists (CAP). Serum insulin and serum vitamin B_{12} concentration was estimated using the electrochemiluminescence using a Roche e601Cobas immunoassay analyzer (Roche Diagnostics, Indianapolis, Indiana, USA). The intra- and inter-assay coefficients of variation for vitamin B_{12} assay were 0.95% and 4.08%, respectively. Serum homocysteine was measured using enzymatic assay using the Beckman Coulter AU2700 (Fullerton, CA, USA) Biochemistry analyser. Urine samples were collected after an overnight fast. Microalbumin concentration was measured using an immuno-turbidometric assay the Beckman Coulter AU2700 (Fullerton, CA, USA) Biochemistry analyser.

Screening of diabetes complications including diabetic retinopathy (DR), neuropathy and nephropathy, coronary artery disease (CAD) and peripheral vascular disease (PVD) was also undertaken. Screening for retinopathy was done using four-field stereo color retinal photography (Zeiss FF 450 plus camera) by trained and certified photographers. Neuropathy was assessed using Biothesiometer (Biomedical Instrument Co., Newbury, OH, USA). Vibratory perception threshold (VPT) of the great toes was measured in a standardized fashion by a single observer. To assess CAD, a resting 12-lead ECG was performed using the Myocard R electrocardiograph (Marks Electronics, Chennai, India).

Doppler studies were performed to screen for PVD, which included recording of pressure tracings using the KODY Vaslab Machine (Kody Medical Electronics, Ltd., Chennai, India). The ankle/brachial pressure index ratio was calculated in every subject.

Dietary intake was assessed in a subsample ($n = 835$) using a validated Food Frequency Questionnaire (FFQ). The development and validation of the same has been published elsewhere [21]. The FFQ included both the frequency as well as the quantity of food items consumed by the participants, which include frequently consumed non-vegetarian food items by the trained dietitians. The food and nutrient intake of the participants was calculated using in-house software 'EpiNu'. We used the residual method to adjust dietary vitamin B_{12} for total energy by performing the regression model [22].

Definitions

Diabetes (T2DM) Fasting plasma glucose ≥ 126 mg/dl or 2-h post-load glucose (2hPG) level ≥ 200 mg/dl, or a past medical history (self-reported diabetes under treatment by a physician), or on oral drug treatment for diabetes [23].

Impaired fasting glucose (IFG) Fasting plasma glucose ≥ 110 mg/dl and < 126 mg/dl and 2-h post-glucose value < 140 mg/dl [23].

Impaired glucose tolerance (IGT) 2-h post-glucose ≥ 140 mg/dl but < 200 mg/dl and fasting value < 126 mg/dl [23].

Prediabetes Individuals with IFG or IGT or both.

Normal glucose tolerance (NGT) 2hPGwas < 140 mg/dl and fasting plasma glucose < 110 mg/dl [23].

Vitamin B_{12} deficiency Vitamin B_{12} levels ≤ 191 pg/ml [24], whereas borderline deficiency was considered at levels > 191 pg/ml and ≤ 350 pg/ml [25, 26].

Hyperhomocysteinemia Homocysteine levels > 15 µmol/l [27].

Body mass index (BMI) was calculated using the formula: weight (in kg) divided by height (in meter squared).

Overweight Defined as a BMI ≥ 23 kg/m^2 but < 25 kg/m^2 for both genders (based on the World Health Organization Asia Pacific Guidelines) [28].

Generalized obesity Defined as a BMI ≥ 25 kg/m^2 for both genders (based on the World Health Organization Asia Pacific Guidelines) [28].

Abdominal obesity Defined as a waist circumference (WC) ≥ 90 cm for men and ≥ 80 cm for women [28].

Hypertension Diagnosed in subjects who were on antihypertensive medications or had a systolic BP ≥ 140 mmHg and/or a diastolic BP ≥ 90 mmHg [29].

Diabetic Retinopathy Retinal photographs were graded using the modified version of the Early Treatment Diabetic Retinopathy Study (ETDRS) grading system. The minimum criteria on for diagnosis of DR were the presence of at least one definite microaneurysm in any field photographed. Briefly, level 10 represented no retinopathy, level ≥ 20 non-proliferative DR (NPDR) and level ≥ 60, proliferative DR (PDR) [30].

Neuropathy Diagnosed if vibratory perception threshold (VPT) of the great toe was ≥ 20 V [31].

Nephropathy Moderately elevated albuminuria was defined as 30–299 µg/mg of creatinine. Overt nephropathy (severely elevated albuminuria) diagnosed if albumin excretion was ≥ 300 µg/mg of creatinine [32].

Coronary artery disease (CAD) CAD was diagnosed based on a past history of documented myocardial infarction and/ or medical therapy (nitrates) or revascularization for CAD and/or electrocardiographic (ECG) changes suggestive of Q wave changes (Minnesota codes 1–1–1 to 1–1–7) and/or ST segment depression (Minnesota codes 4–1 to 4–2) [33].

Peripheral vascular disease (PVD) Diagnosed if ankle–brachial index (ABI) was < 0.9 [34].

Statistical analysis

Statistical analyses were performed with SPSS statistical package (version 22.0, SPSS Inc, Chicago, IL). Continuous variables are reported as mean ± standard deviation (SD). Categorical variables are reported in percentages. Receiver operating characteristic (ROC) curves were plotted for vitamin B_{12} and sensitivity and specificity for identifying hyperhomocysteinemia were calculated for various vitamin B_{12} cut-off points. Sensitivity against 100% minus specificity was plotted at each cutoff threshold, and the area under the curve (AUC) values that reflect the probability of correctly identifying hyperhomocysteinemia were computed. The cut-point was derived using Youden index which is defined as the optimal cut-point as the point maximizing the product of sensitivity and specificity ($J = \max[\text{sen} + \text{spe}]$) [35]. One-way ANOVA with post hocTukey HSD or Student's "*t*" test were used to compare groups for continuous variables and Chi-square test was used to compare proportions between two groups. Pearson's correlation analysis was carried out to determine the correlation between vitamin B_{12} and homocysteine levels. Univariate logistic regression analysis was performed to look at the association of glucose tolerance, age and gender with vitamin B_{12} deficiency in our population. Multiple logistic regression was also performed to look at the association of T2DMwith vitamin B_{12} deficiency adjusting for various confounding variables. *p* values of < 0.05 were considered as statistically significant.

Results

The characteristics of the study population are shown in Table 1. Forty five percent of the study population was male. Diabetic individuals were older, had a greater systolic blood pressure, BMI and waist circumference ($p < 0.001$) compared to NGT subjects. Mean duration of diabetes was 7.2 ± 5.9 years and 65% of those with T2DM had a family history of diabetes. There was an increasing trend in fasting plasma glucose, glycosylated hemoglobin, fasting insulin and triglycerides levels ($p < 0.001$) with decreasing glucose tolerance. The overall prevalence of hyperhomocysteinemia was 21.2% with increase in increasing glucose tolerance with 19.9% among NGT followed by prediabetes (22.3%) and diabetic individuals (24.0%), respectively. Among individuals with NGT, prediabetes and T2DM, vitamin B_{12} negatively correlated with homocysteine ($r = -0.428$, -0.406 and -0.484, $p < 0.001$ respectively).

Figure 1 shows the mean serum levels of vitamin B_{12} in participants with different grades of glucose tolerance.

Table 1 Characteristics of the study subjects

Variables	Participants with NGT ($n = 900$)	Participants with prediabetes ($n = 300$)	Participants with diabetes ($n = 300$)	p value
Age (years)	43 ± 11	48 ± 12*	54 ± 11*#	< 0.001
Male gender n (%)	397 (44.1)	130 (43.3)	141 (47.0)	0.467
Height (cms)	158 ± 10	157 ± 9**	157 ± 10	0.008
Weight (Kg)	64.6 ± 13.3	67.2 ± 13.6	66.1 ± 11.5##	0.007
Systolic BP (mm/Hg)	123 ± 18	129 ± 20*	132 ± 19*#	< 0.001
Diastolic BP (mm/Hg)	78 ± 12	80 ± 12**	79 ± 11	0.001
Waist circumference (cms)	86 ± 11	90 ± 11*	90 ± 10*	< 0.001
Body mass index (kg/m^2)	25.7 ± 5.0	27.4 ± 5.1*	26.8 ± 4.6*	< 0.001
Fasting plasma glucose (mg/dl)	89.4 ± 6.8	103.3 ± 17.8*	154.4 ± 61.0* #	< 0.001
HbA1c				
%	5.5 ± 0.5	5.9 ± 0.6*	8.1 ± 1.9* #	< 0.001
mmol/mol	37 ± 5.5	41 ± 7*	65 ± 21* #	
Fasting insulin (μIU/ml)	7.6 ± 5.1	8.1 ± 5.6	11.1 ± 7.0* #	< 0.001
Serum cholesterol (mg/dl)	177 ± 35	188 ± 37*	184 ± 41**	< 0.001
Serum triglycerides (mg/dl)	130 ± 79	154 ± 99*	169 ± 140*	< 0.001
HDL cholesterol (mg/dl)	40.8 ± 8.9	39.6 ± 8.3	40.1 ± 8.1	0.108
LDL cholesterol (mg/dl)	100 ± 30	117 ± 32**	110 ± 38##	0.005
Cholesterol /HDL ratio	4.5 ± 1.1	4.9 ± 1.1*	4.7 ± 1.1**	< 0.001
Serum homocysteine(μmol/l)	13.2 ± 6.0	13.3 ± 7.3	14.8 ± 11.7##	0.006
Hyperhomocysteinemia n (%)	179 (19.9)	67 (22.3)	72 (24.0)	0.111
Household income (INR/month)				
< 5000 (USD < 71) n (%)	162 (18.0)	65 (21.7)	100 (33.3)*	< 0.001
5000–10,000 (USD 71–141) n (%)	490 (54.4)	153 (51.0)	128 (42.7)*	< 0.001
10,000 (USD > 141) n (%)	248 (27.6)	82 (27.3)	72 (24.0)	0.268
Family history of diabetes [yes] n (%)	299 (35.9)	129 (43.7)*	195 (65.0)*#	< 0.001
Duration of diabetes (years)	–	–	7.2 ± 5.9	–
Hypertension n (%)	205 (22.8)	88 (29.3)**	220 (73.3)*#	< 0.001
Generalized obesity n (%)	639 (71.2)	240 (80.0)**	244 (81.6)*	< 0.001
Abdominal obesity n (%)	452 (50.3)	188 (62.7)*	193 (64.8)**	< 0.001
Dietary pattern (non-veg)	856 (95.1)	279 (93.0)	272 (90.7)**	0.005
Energy (Kcals)@	2785 ± 796	2695 ± 929	2557 ± 806*	0.004
Energy adjusted vitamin B_{12} (μg) @	2.2 ± 2.7	2.0 ± 2.0	2.0 ± 3.7	0.72
Metformin use n (%)	–	–	227 (75.7)	–
Duration of metformin use (years)^	–	–	3.0 (0.10–8.5)	–

BP blood pressure, *HDL* high density lipoprotein, *LDL* low density lipoprotein

*$p < 0.001$ and **$p < 0.05$ compared to participants with normal glucose tolerance (NGT); #$p < 0.001$ and ##$p < 0.05$ compared to participants with prediabetes; @$n = 835$; ^Median (Interquartile range)

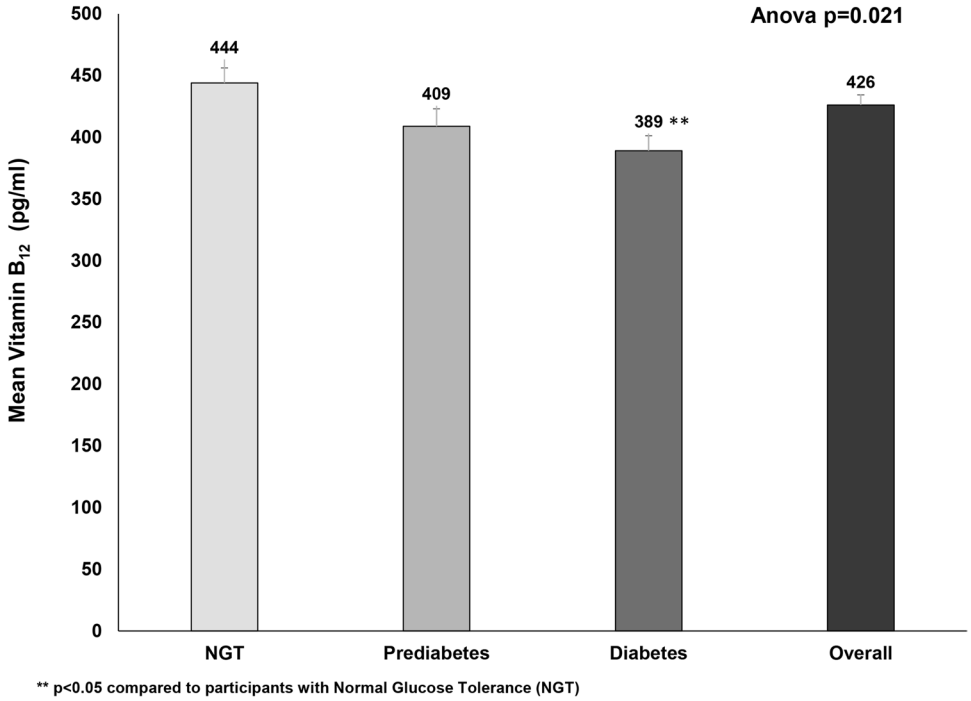


Fig. 1 Mean serum levels of vitamin B_{12} in different degrees of glucose tolerance

A significant decreasing trend ($p = 0.021$) was observed in mean vitamin B_{12} levels with increasing glucose tolerance, viz. vitamin B_{12} levels was 444.0 pg/ml, 409 pg/ml and 389 pg/mlin individuals with NGT, prediabetes and T2DM respectively.

The prevalence and association of vitamin B_{12} deficiency with different degrees of glucose tolerance, age, gender and dietary patterns are shown in Table 2. The prevalence of absolute vitamin B_{12} deficiency in the study population was 14.9%, while borderline vitamin B_{12} deficiency was observed in 37.6%. The corresponding homocysteine levels were 27.2 ± 11.3 and 12.5 ± 2.9 µmol/l, respectively. The highest prevalence of vitamin B_{12} deficiency was observed among diabetic individuals (18.7%) followed by prediabetes

Table 2 Prevalence and association of vitamin B_{12} deficiency with different degrees of glucose tolerance, age, gender and dietary pattern

Subgroups	Prevalence of vitamin B_{12} deficiency (%)	Unadjusted odds ratio (95% CI) p value	Adjusted odds ratio (95% CI) p value[$]
Glucose tolerance			
Normal	13.7	1.00	1.00
Prediabetes	15.0	1.2 (0.77–1.61) $p = 0.56$	0.973 (0.660–1.433) $p = 0.888$
Diabetes	18.7** [##]	1.5 (1.03–2.05) $p = 0.04$	3.2 (1.499–6.764) $p = 0.003$
Age			
≤40 years	11.5	1.00	1.00
41–50	16.1	1.5 (1.04–2.12) $p = 0.03$	1.4 (0.96–2.02) $p = 0.078$
≥51 years	17.7* [##]	1.7 (1.17–2.37) $p = 0.001$	1.6 (1.1–2.5) $p = 0.016$
Gender			
Women	10.3	1.00	1.00
Men	20.7[@]	2.3 (1.7–3.0) $p < 0.001$	2.3 (1.7–3.21) $p < 0.001$
Diet pattern			
Non-vegetarians	14.1	1.00	1.00
Vegetarians	28.0[@]	2.4 (1.5–3.0) $p < 0.001$	2.3 (1.41–3.81) $p = 0.001$

*$p < 0.001$ and **$p < 0.05$ compared to participants with normal glucose tolerance; [#]Anova $p < 0.001$; [##]Anova $p < 0.05$; [@]$p < 0.001$; [@@]$p < 0.05$

[$]Adjusted for each other and further adjusted for BMI, systolic blood pressure, duration of diabetes, diabetes medication, house hold income and triglycerides

(15.0%) and NGT (13.7%). Individuals with T2DM had higher risk for vitamin B_{12} deficiency compared to subjects without T2DM [odds ratio (OR) 3.2] even after adjusting for age, gender, BMI, systolic blood pressure, house hold income, serum triglyceride, dietary pattern and diabetes duration and medication. Vitamin B_{12} deficiency increased significantly with increasing age. Data show that high risk for vitamin B_{12} deficiency was observed in the age group of 41–50 years and ≥ 51 years compared to the younger age group (≤ 40 years). It was also observed that with respect to gender, men had higher risk for vitamin B_{12} deficiency when compared to women. The mean serum vitamin B_{12} levels were lower in men compared to women (men vs. women 392.2 ± 294 vs. 453.5 ± 338, $p < 0.001$).

Regarding the dietary pattern, 93.8% of the study population were non-vegetarians. However, vegetarians had significantly higher vitamin B_{12} deficiency compared to non-vegetarians (28.0% vs. 14.1%, respectively, $p < 0.001$) [Table 2]. The prevalence of vitamin B_{12} deficiency significantly decreased with increasing tertiles of dietary energy adjusted vitamin B_{12} [1st tertile (< 1.2 µg),17.3%; 2nd tertile (1.2–2.2), 16.8%; 3rd tertile (> 2.2 µg), 10.8%, $p = 0.032$). The risk for vitamin B_{12} deficiency was 1.7 times higher (CI 1.03–2.87, $p = 0.037$) in those individuals having lesser dietary intake of vitamin B_{12} (1st tertile) compared to those having higher intake of vitamin B_{12} (3rd tertile) even after adjusting for confounding factors such as age, gender, BMI, systolic and diastolic blood pressure, fasting blood sugar, cholesterol, triglycerides, diabetes medication, income and energy.

Figure 2A presents the prevalence of vitamin B_{12} deficiency in relation to generalized and abdominal obesity in the study population. The prevalence of vitamin B_{12} deficiency was higher among those with generalized obesity and abdominal obesity. However, a significant increase was observed in those with abdominal obesity ($p < 0.001$).

Mean vitamin B_{12} levels and vitamin B_{12} deficiency in relation to metformin drug use among diabetic individuals are presented in Fig. 2B. Of the 300 T2DM individuals, 75.7% were either on metformin alone or in combination. The mean vitamin B_{12} level was lower among T2DM individuals on metformin compared with those who were

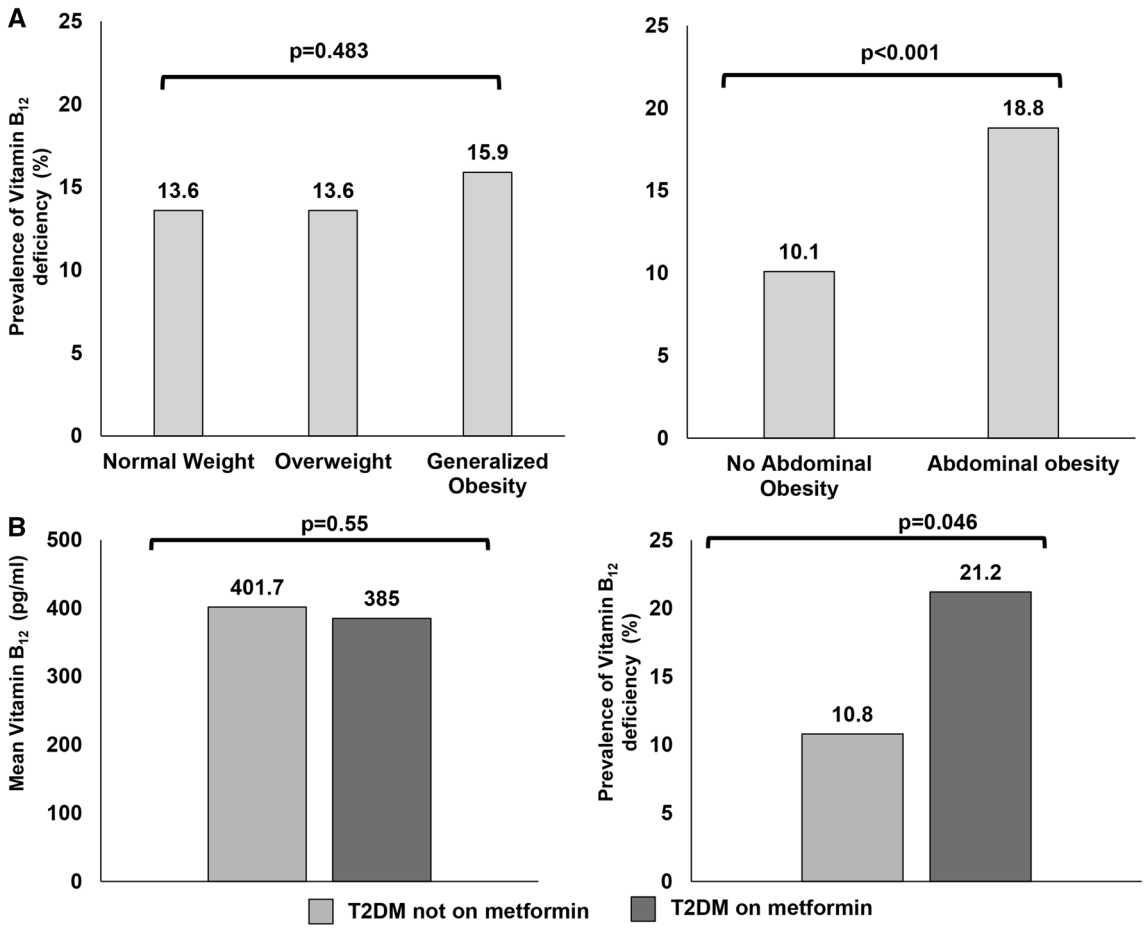
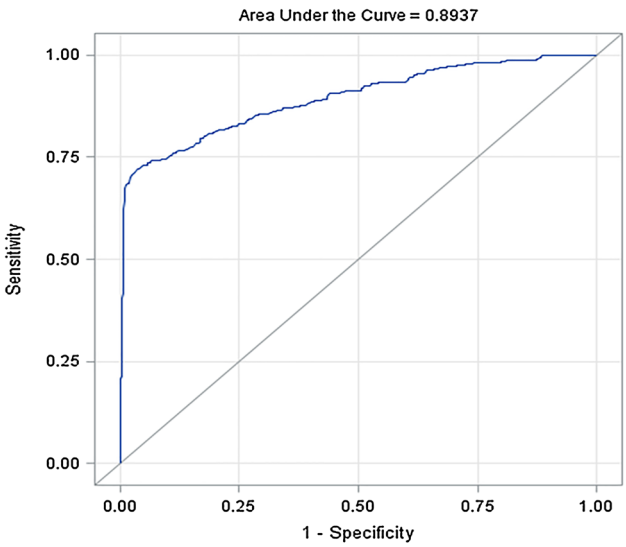


Fig. 2 **A** Prevalence of vitamin B_{12} deficiency in relation to generalized and abdominal obesity. **B** Mean vitamin B_{12} levels and vitamin B_{12} deficiency relation to metformin use among individuals with diabetes

not on metformin (385.0 ± 222.0 vs. 401.7 ± 175.0 pg/ml, $p = 0.55$). Also, the prevalence of vitamin B_{12} deficiency was significantly higher among T2DM individuals using metformin (21.2%) compared to those who were not on metformin (10.8%) ($p = 0.046$, $\chi^2 = 3.99$). Vitamin B_{12} negatively correlated with homocysteine, among T2DM individuals not on metformin use($r = -0.470$, $p < 0.001$) as well as in those on metformin use ($r = -0.488$, $p < 0.001$).

The prevalence of secondary complication in T2DM was as follows: microvascular complications including DR was 36.4% ($n = 79/217$), neuropathy was 45% ($n = 130/289$), moderately elevated albuminuria was 21.3% ($n = 64/300$) and severely elevated albuminuria was 4.0% ($n = 12/300$). Macro vascular complications such as CAD was present in 17.3% ($n = 277/300$) and PVD in 4.4% ($n = 13/294$). However, there was no association between vitamin B_{12} deficiency and secondary complications of diabetes among T2DM (data not presented).

ROC curve was plotted to derive the cut-off point of vitamin B_{12} with the best sensitivity and specificity for the prediction of hyperhomocysteinemia in the study population [Fig. 3]. The area under the curve was 0.89 (95% CI 0.87–0.92, $p < 0.001$) and a vitamin B_{12} cut-off point of 198 pg/ml was the optimum cut off with a sensitivity 71.1% and specificity of 97% for identifying the predicting hyperhomocysteinemia.



Marker	Cut point	Sensitivity	Specificity
Vitamin B $_{12}$	198 pg/ml	71.1%	97.0%

Fig. 3 Receiver operating characteristic (ROC) curve of vitamin B_{12} with homocysteine in the study population

Discussion

This is the first study from southern India to determine the levels of vitamin B_{12} among south Indian individuals with NGT, prediabetes and T2DM. Our study reports the following significant findings: First, mean levels of vitamin B_{12} decreased with increasing severity of glucose tolerance, i.e., individuals with T2DM had the lowest values followed by those with prediabetes and NGT. Second, the prevalence of vitamin B_{12} deficiency was higher in prediabetes and T2DM. Third, serum vitamin B_{12} levels were lower in men compared to women and men had 2.3 times higher risk for vitamin B_{12} deficiency. Finally, the prevalence of vitamin B_{12} deficiency significantly increased with age ($p < 0.05$) beginning from 40 years of age itself and also in those with abdominal obesity ($p < 0.001$).

Vitamin B_{12} is an enzyme co-factor plays an important role in the regeneration of methionine from homocysteine in the cytoplasm, and mediates the conversion of methylmalonic acid (MMA)-coenzyme A (CoA) to succinyl-CoA in the mitochondria. These reactions support DNA synthesis and lipid metabolism, and also detoxify the substrates homocysteine and MMA. Low-serum vitamin B_{12} levels have been associated with T2DM [36]. A study by Ko et al. [37] found that vitamin B_{12} was independently associated with T2DM patients. Although many studies have been carried out in western population [7, 38, 39] to document the increased prevalence of vitamin B_{12} deficiency in diabetic patients, there are no data either in the west or in the Indian population which reports on the prevalence of B_{12} deficiency in individuals with prediabetes. As vitamin B_{12} is known to be vital for maintaining the overall integrity of the vascular system [40], data on prevalence of low vitamin B_{12} among different grades of glucose tolerance are important to help guide formulation of guidelines in diabetes clinical care. Studies have shown that low vitamin B_{12} to be associated with sensory and peripheral motor nerve dysfunction in healthy individuals [41], individuals with diabetes [42]. Studies have also shown that vitamin-B_{12} deficiency could be an independent risk factor for diabetic retinopathy [43, 44]. Thus, early detection of vitamin B_{12} deficiency is indispensable to maintain normal neural functions.

In our study, we identified the overall prevalence of vitamin B_{12} deficiency to be 14.9% with 18.7% among T2DM individuals followed by prediabetes (15%) and NGT (13.7%). Borderline vitamin B_{12} deficiency was observed in 37.6%. Refsum et al. [45] have reported that 47% of the 204 subjects studied in western Indian had cobalamin deficiency (< 150 pmol/L; equivalent to 203 pg/ml) and concluded that about 75% of the subjects had metabolic signs of cobalamin deficiency. In another

study conducted in Pune, India, the prevalence of low vitamin B_{12} was reported to be 66% [19]. In this south Indian urban population, vitamin B_{12} deficiency is much lower than that reported in the west. Another community-based cross-sectional study conducted in urban south India among 630 apparently healthy adults [46] also reported a lower prevalence of vitamin B_{12} deficiency (35%) compared to western India. Earlier studies in Indian population have reported vitamin B_{12} rates ranging from 16% in apparently normal urban south Indian elderly [47] to 67% in middle-aged healthy men in western India [5] and 54% in individuals with diabetes [45]. Thus, our findings are in line with previous observations, and extend the knowledge on the association of vitamin B_{12} among individuals with prediabetes and T2DM. Currently, there are no guidelines advocating for routine screening for vitamin B_{12} deficiency among individuals with T2DM. Our study emphasizes the need of screening of vitamin B_{12} even in prediabetes stage.

Earlier studies have reported that the prevalence of vitamin B_{12} deficiency to range between 16 and 30% in individuals with >60 years of age [46, 47]. In our study, we observed a similar prevalence of vitamin B_{12} deficiency among individuals with 40 years of age. The prevalence of vitamin B_{12} deficiency among individuals <40 years of age is lower in our study (11.5%) compared to the study done in urban areas of Hyderabad in south India (44%) [46]. This assumes significance as Asian Indians are prone to develop T2DM and cardiovascular diseases at younger age. In our study, serum vitamin B_{12} levels were lower in men compared to women and men had 2.3 times higher risk for vitamin B_{12} deficiency. This finding is contrary to a reported in an African American population [48]. However, Moore et al. [49] have reported that serum vitamin B_{12} levels were lower in men than women, which is similar to our findings. Earlier studies conducted in Iranian and Indian population [46, 50] have reported a higher prevalence of vitamin B_{12} deficiency among men.

Studies have shown that vegetarians are more prone to develop vitamin B_{12} deficiency compared to non-vegetarians [51, 52]. Our study also shows that vegetarians are twice at risk for developing vitamin B_{12} deficiency compared to non-vegetarians. Another study done in western India, reported that vegetarians had four times risk for developing vitamin B_{12} deficiency [5]. The most common explanations for poor vitamin B_{12} status are a low dietary intake of the vitamin (i.e., a low intake of animal-source foods) and malabsorption [53]. Though in our data 93.8% of the population were non-vegetarians, their mean intake was only 32 g/day (data not presented), this included fish, chicken, meat and egg, which was very low compared to western population [54]. The earlier literature shows that studies conducted on small population have confirmed that both serum vitamin B_{12} levels and vitamin B_{12} intake increase gradually from vegans to lacto-ovo vegetarians, to those who take fish, chicken, lamb or pork, etc., to omnivores [55–57]. It has been reported that asymptomatic Indian lacto vegetarians have distinctly low vitamin B_{12} levels compared to non-vegetarians [58, 59]. In our study, also we have observed that inadequate dietary intake is significantly associated with vitamin B_{12} deficiency.

Several studies have reported associations between low vitamin B_{12} levels and adverse metabolic health profiles, including adiposity [60–62]. A study conducted in Turkey among 976 individuals with various degrees of obesity reported that vitamin B_{12} deficiency was significantly higher in individuals with obesity (40.1%) and overweight (37.7%) compared to healthy individuals (17.1%) [60]. In a study conducted in north India, waist circumference correlated negatively with serum vitamin B_{12} [62]. In our study, there was a higher prevalence of vitamin B_{12} deficiency among those with generalized and abdominal obesity, however, a significant increase was observed in those with abdominal obesity. These findings underscore the need for further assessment of vitamin B_{12} inadequacies among individuals who are obese.

Metformin use among individuals with T2DM has been shown to be associated with vitamin B_{12} deficiency [36, 37, 63, 64]. DeFronzo et al. [64], in a randomized controlled trial conducted in 1995, reported that metformin decreased the serum vitamin B_{12} levels by 22% and 29% compared to placebo and glyburide, respectively. The National Health and Nutrition Examination Survey showed that vitamin B_{12} deficiency was observed in 5.8% of T2DM individuals using metformin compared with 2.4% of those not using metformin [63]. In this study, vitamin B_{12} deficiency was present in 21.6% of T2DM individuals using metformin. This is similar to a recent study conducted in 1007 individuals with T2DM, which reported vitamin B_{12} deficiency to be present in 24.7% of individuals treated with metformin compared to 15.8% in those not treated with metformin [65]. In our study. vitamin B_{12} levels were negatively associated with homocysteine amongst both metformin treated T2DM as well as those who did not receive the drug. Although the clinical significance of vitamin B_{12} deficiency remains ambiguous, our data suggest the need for routine vitamin B_{12} screening and supplementation in individuals with T2DM, especially in metformin users.

Homocysteine is an important intermediate in amino acid methylation and transsulfuration pathways that require vitamin B_{12} as key coenzymes. The prevalence of hyperhomocysteinemia in our study population is lower (21.2%) than the rates reported by Yajnik et al. [5] (58.0%). A meta-analysis study conducted on 4011 cases and 4303 controls provided a strong support for a causal association of elevated homocysteine levels and T2DM [66]. Hyperhomocysteinemia, a sensitive marker of vitamin B_{12} deficiency [67], has been identified as a risk factor

for insulin resistance and T2DM [68]. In this context, a significant negative correlation between the serum levels of vitamin B_{12} with the serum level of homocysteine in our study population is an important finding. Another interesting observation in this study is that vitamin B_{12} showed a significant correlation with homocysteine even when subjects were categorized as prediabetes and NGT. Our findings may have implications for prevention. In addition to regular physical exercise, diets or dietary supplements rich in vitamin B_{12} may be beneficial in lowering plasma homocysteine levels, improving insulin sensitivity, and reducing risk for development of T2DM or cardiovascular disease.

There is evidence that vitamin B_{12} deficiency is associated with peripheral neuropathy [69], diabetic retinopathy [70] cardiovascular disease [71], nephropathy [72] and PVD [10]. It has also been reported that anemia is one of an independent risk factor for diabetic peripheral neuropathy in T2DM patients [73]. However, in our study, we did not find any significant association between vitamin B12 and micro and macrovascular complications of diabetes. This may be related to the small sample size of the participants with complications. Alternatively, it may be because complications of diabetes involve multiple etiopathological processes and low vitamin B_{12} by itself may not be a major factor contributing to complications of diabetes.

The strengths of the study are that study participants were recruited from a population-based study in an ethnic group with high a prevalence of T2DM. Moreover, careful inclusion and exclusion criteria were used. Individuals were classified into different grades of glucose tolerance, viz. NGT, prediabetes and T2DM using oral glucose tolerance test. One of the limitations of the study is that being a cross-sectional design, we are unable to demonstrate a cause and effect relation between vitamin B_{12} with prediabetes or T2DM.

In conclusion, this study suggests that the levels of vitamin B_{12} decreased with increasing severity of glucose tolerance. Prevalence of vitamin B_{12} deficiency is higher even in individuals with prediabetes. In addition, this study emphasizes the need for screening for vitamin B_{12} deficiency in this population.

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Compliance with ethical standards

Conflict of interest All authors have no relevant conflict of interest to disclose.

Ethical standard statement All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all patients for being included in the study which has done according to the ethical standards and in keeping with Helsinki Declaration of 2008 (ICH GCP).

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