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ORIGINAL ARTICLE

Serum Vitamin B12 in Relation to Anaemia and Neuropathy by MNSI among Type 2 Diabetes

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Abstract

Background: Hematological and neurological symptoms can be signs of B12 inadequacy. This work was intended to evaluate the severity of B12 deficiency and link it to peripheral neuropathy (PN) and anaemia in type 2 diabetes mellitus (T2DM) adults who were using metformin.

Methods: To assess PN, the Michigan Neuropathy Screening Instrument (MNSI) directed a hospital-based cross-sectional investigation of 245 adults with T2DM who were taking metformin. 155 subjects who fulfilled the insertion and rejection criteria provided fasting blood samples for measurement of vitamin B12, haemoglobin, HbA1c, and cell morphology using chemiluminescent enzyme immunoassay (C.L.I.A), high performance liquid chromatography (HPLC), and C.B.C respectively.

Results: Among adults with T2DM taking metformin, B12 deficiency (B12< 200pg/ml) was prevalent in 52% cases. O.R of vegetarian diet and B12 insufficiency was 2.33 (C.I. 1.22-4.47) ($p < 0.05$). Despite the fact that there were no cases of macrocytic anaemia, 40% of people had anaemia, which was not related to a B12 deficiency. According to ROC analysis, serum B12 has emerged as a fair test to predict PN scores of 2.25 ($p < 0.001$). 38% of people had PN (PN score ≥ 2.5)

Conclusions: The usage of metformin was linked to biochemical B12 insufficiency. B12 deficiency was likely to result in PN scores ≥ 2.5 on the MNSI. In T2DM individuals using metformin, serum B12 screening and the use of MNSI for PN are suggested.

Keywords: metformin, B12 deficiency, neuropathy, MNSI, anemia, type 2 diabetes mellitus

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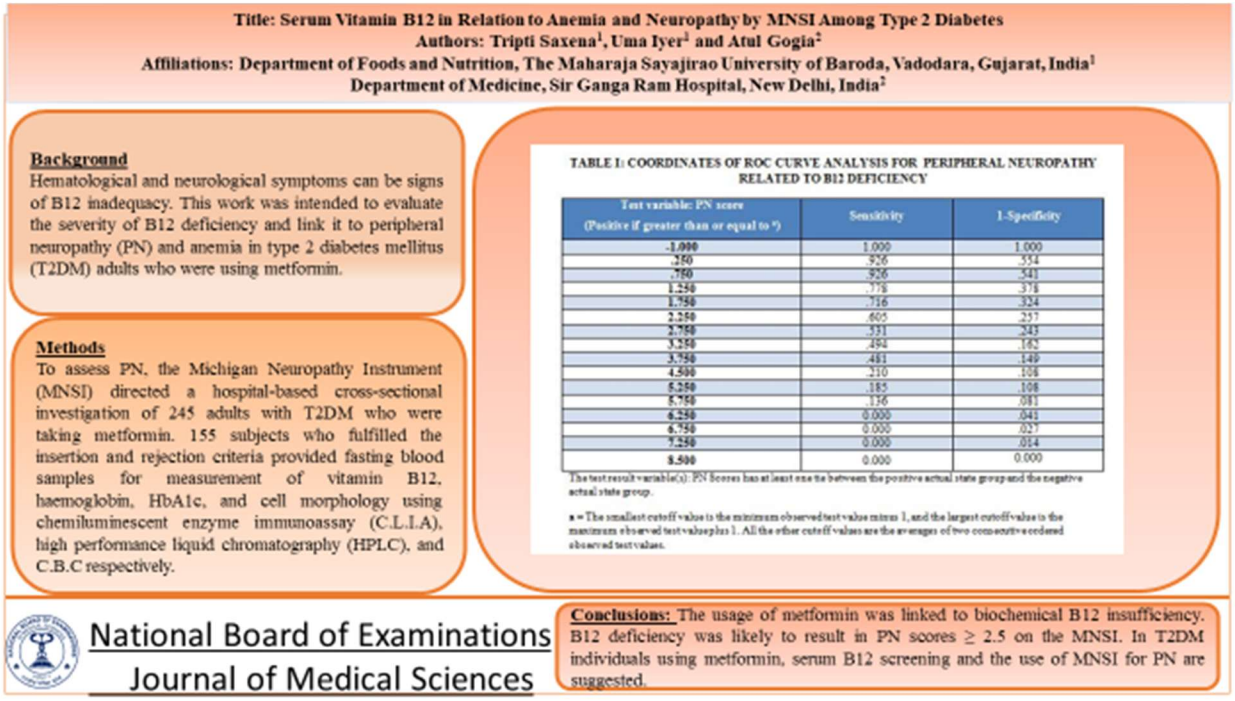
Graphical Abstract



Introduction

Metformin which forms 1st line therapies in treating type 2 diabetes mellitus (T2DM) across World [1] has been recognized to induce low serum B12 [2,3,4,5,6,7,8]. But there is sparse evidence in this context for Indian population. The symptoms of B12 deficiency are similar to those presented with peripheral neuropathy (PN), a common secondary complication of type 2 diabetes. Assessment of PN has been challenging due to various tools and varying study definitions. The magnitude of PN and its determinants in Indian population has limited evidence. Co-existence of PN along with metformin-induced low B12 levels can be detrimental in T2DM. Thus the present study was formulated with the objectives to map the magnitude of metformin-induced B12 deficiency and PN between T2DM patients in metformin and to study metformin induced B12 deficiency with connection to neuropathy and anaemia in T2DM.

Materials and Methods

A cross-sectional study of hospitals study on 245 T2DM adults on metformin for minimum of four months duration [3] was done from Medicine O.P.D of Sir Ganga Ram hospital, a tertiary care institute in Delhi, India from December 2013 to February 2019. The study's aim was to calculate the magnitude of serum B12 deficiency among T2DM adults on metformin and study its relation with nutritional anaemia and neuropathy. Those who were diagnosed type 2 diabetes by ADA 2013 [9] [2h plasma glucose≥200mg/dL during an OGTT or random blood sugar ≥ 200mg/dL in presence of classical diabetes symptoms or fasting (no

caloric intake for at least 8h) blood sugar ≥126 mg/dL or HbA1c ≥ 6.5%] from latest blood reports and had been receiving metformin therapy for at least four months [2] were enrolled in the study. The calculated sample size was 245 using the formula $N=t^2\chi p\ (1-p)/m^2$ where p is 33%, type 2 diabetes and estimated prevalence of B12 deficiency, t = 95% C.I. and m=margin of error at 5%. After excluding patients with confounders (pregnancy, alcoholism, pernicious anaemia, drugs like P.P.I, H2RAs/Hydrogen blockers, history of CRF, liver disease, CKD, Cardiopulmonary disease, bowel disease/surgery, cancer, acid-base disturbance, those on multivitamin or B12 supplement or B12 injections) that affect B12 absorption, 155 of 245 were screened for serum B12 by Chemiluminescence Immuno assay (C.L.I.A.), anaemia by haemoglobin from C.B.C and cell morphology and HbA1c by high performance liquid chromatography (HPLC) on fasting blood samples (not eating anything for at least 8 hours). The serum from fasting blood samples was kept at a temperature of 20 °C and used to calculate the serum total Vitamin B12 levels by competitive chemiluminescent enzyme immunoassay on Beckman Coulter Access 2 Immuno Assay system using commercial kits.

The study received ethical approval from the Institutional Ethics Committee of Maharaja Sayajirao University of Baroda (No. IECHR/2013/19) and written informed consents were received from each and every participant.

Data Collection and analysis: A total of 245 T2DM adults on metformin was screened for PN by Michigan Neuropathy Screening Instrument (MNSI) and data was collected on medical history, drug history, diet history, life style factors, socio-demographic characteristics, B.P. and anthropometry by pre designed B12 screening proforma. Individuals consuming non-vegetarian foods like meat/fish/chicken at least once weekly were defined as non-vegetarians, those consuming eggs at least twice weekly but no other non veg foods were defined as ovo vegetarians and those not consuming eggs or non-vegetarian foods but consuming milk were considered vegetarians. Indian diets being rich in phytates provide low calcium for absorption [10] and major absorbable calcium is from milk. Individuals consuming < 200ml milk (~60% RDA for calcium) were defined to have low calcium intake. Body Mass Index (BMI) was calculated as kg/m^2 and to Indian population [11] 18.5–22.9 was normal, 23-24.9 was over weight and ≥25 were obese. Waist circumference (W.C.) and hip boundary was weighed and the waist-to-hip ratio (W.H.R.) calculated. Men with W.C. ≥90 cm (35in) / W.H.R. ≥ 0.90 cm and women with W.C. ≥80cm (31.5in)/W.H.R ≥ 0.85 were said to have abdominal obesity. JNC VIII cut offs were used for B.P. classification. WHO haemoglobin cut offs were used to define anaemia [12]. Serum B12 ≤ 200pg/ml was defined as B12 deficient [13]. Subjects with HbA1c >7% were said to have poor glycemic control by ADA 2020 criteria [1]. MNSI: is a simple, non invasive valid tool in both hospital and community set up [14,15,16]. The MNSI consists of two parts, first is history related to signs and symptoms of PN and second is

a set of physical examination including: 1) feet appearance, 2) Ulceration, 3) Ankle Reflex, 4) Great toe vibration perception and 5) monofilament test. MNSI history score was computed by a sum of 'yes' responses to 11 questions and a 'no' response on 2 items was scored as 1. Question on weakened circulation and all-around weakness were not included in MNSI history score. MNSI physical assessment score was computed by a sum of score for each foot for physical appearance, ulceration, ankle reflex by hammer, tunning fork by 128 Hz tunning fork, vibration perception at great toe by 128 Hz tunning fork and monofilament test by 10g monofilament and this was called as PN score.

The data was entered into SPSS 16.0 and evaluated in Microsoft Excel 2007 (SPSS, Chicago, IL, USA). Box plot was constructed in Excel 2016. Odds ratio (O.R) was calculated to estimate the strength of a relationship between an exposure and a result.

The usefulness of the Receiver Operating Characteristic (ROC) curve analysis was used to assess the utility of Serum B12 test in predicting PN. PN scores as assessed by MNSI physical assessment were taken as 'test variable' and B12 deficiency ≤ 200pg/ml was taken as 'state variable'/binary variable. The objective to construct ROC curve was used to calculate the reflection point (cut off) of PN score for B12 deficiency. Area under the ROC curve (AUC) is a measure of a test's effectiveness (Serum B12) in predicting the desired outcome (PN). A test does not perform any better than chance when the AUC value is equal to 0.5. The fair test performance is indicated by AUC values between 0.70 and 0.79.

Results

Of 245 subjects majority (76.8%) were ≥50y and the study subjects had mean age of 58.2y and mean duration of diabetes was 8y. Mean PCI was Rs. 21,700.82 (Rs. 4000 - 1,00,000). 90% did not drink alcohol nor did they consume tobacco/cigarette. 10% were ovo vegetarian, 43% were vegetarian and 47% were non vegetarian while majority (~ 67%) had low dietary calcium (~60% R.D.A) as studied by milk consumption. By BMI, 40.4% were obese, 23% were overweight and 20% were morbid obese. Also 93% had abnormal WHR.76% had abdominal obesity by WC. More females (83%) than males (64%) had abnormal WC ($p < 0.01$). Majority (62%) were pre hypertensive followed by hypertension stage I (28.2%) and stage II (7.3%). Majority (57.6%) were on 1000mg metformin dosage. Metformin dosage was associated with GI side effects ($p < 0.001$). Three-fourth (70%) reported metallic taste with the metformin dosage upto 2500 pg/ml.

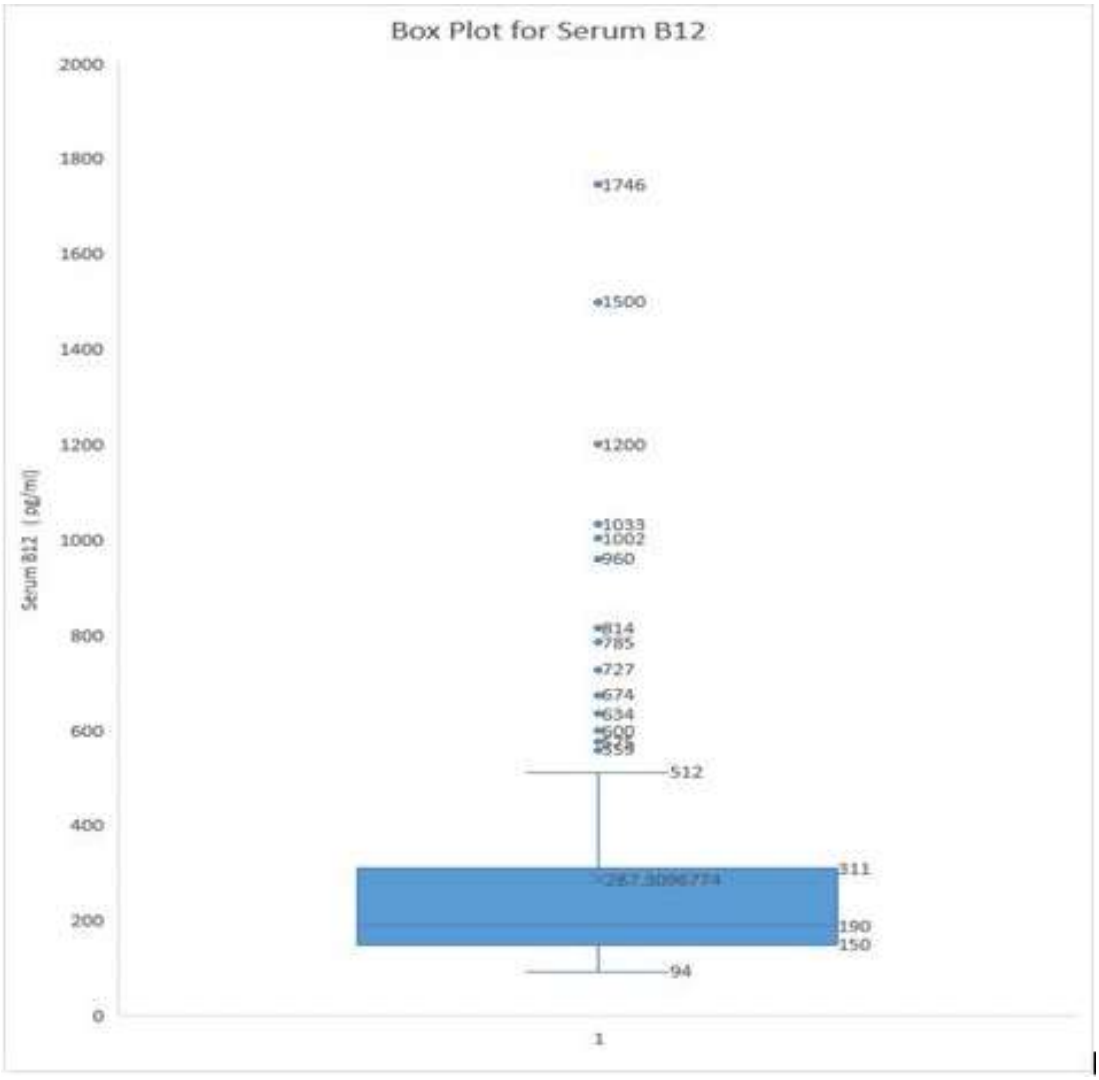


Figure 1. Box plot of Serum B12 distribution for T2DM adults on metformin.

As shown by box plot (Figure 1) the serum B12 of the studied population varied from 94-1746 pg/ml with a median of 190pg/ml and mean of 287.31 pg/ml. 50% of the subjects were in between 150-311pg/ml (Figure 1). Males had higher serum B12 levels than females (363.92±353.88 vs. 250.83±170.62) ($p<0.05$). More than half (52%, 81 of 245) were B12 deficient, more so in females (58%) than males (40%) ($p < 0.05$). Majority (30.3%) had mild B12 deficiency (150-200pg/ml). There was significantly higher proportion of vegetarians (54.3%) among those who were B12 deficient in comparison to those who had normal B12 ($p < 0.05$). The odds ratio for vegetarian diets and B12 deficiency was 2.33 (CI. 1.22-4.47) ($p<0.05$). Low dietary calcium (~60% R.D.A.) as assessed by milk consumption showed no significant association with B12 deficiency.

The mean haemoglobin in this research population was 12.25±1.41g/dl (7.6-14.6). Majority (60%) had normal haemoglobin and greater males (58%) than females (22.9%) were anemic ($p < 0.001$), due to the custom of recommending iron supplements to women (46) rather than men (29). Subjects given iron supplements had higher haemoglobin than non-supplemented group (12.99 vs.11.55g/dl) ($p < 0.001$). Anaemia was more in subjects supplemented with iron than those not on iron supplements (78.7% vs. 21.3%) ($p < 0.001$). Cell morphology and C.B.C showed no positive cases of macrocytic anaemia showing the absence of any clinical vitamin B12 deficiency. There was no significant difference in mean haemoglobin as well as the prevalence of anaemia among B12 deficient and those with normal B12 levels.

HbA1c varied from 6.4-12% and 71% had poor glycemia (HbA1c >7%). GI side effects of metformin were more in B12 deficient ($p < 0.05$). There was significant difference in the glycemic control of those who were B12 deficient than those who had normal B12 levels ($p < 0.05$). Of those suffering from PN (low or high) majority had B12 deficiency in comparison to those with no PN ($p<0.001$). Amongst the good glycemic control (HbA1c ≤7%) majority (53.8%) had no PN ($p<0.001$). As HbA1c increased the PN score also increased ($r = 0.381$, $p <0.001$).

ROC curve to define the performance of serum B12 in predicting PN is depicted in Figure 2 Among the several coordinates of the curve (Table 1) the PN cut-off of 1.75 would give a sensitivity ~72% both the specificity of ~67%. The PN cut-off of 2.25 gives a sensitivity of 60% and specificity of 74%. Ideally, one wants both sensitivity and specificity to be high, but typically for screening, specificity is given more importance, so we select the 2.25 cut-off for PN score to define PN as assessed by MNSI. AUC was calculated as 0.742 (95 C.I. 0.661–0.832, $p < 0.001$) indicating that the serum B12 test performed fairly well in predicting PN in T2DM adults on metformin ($p < 0.001$).

By using the MNSI physical assessment score of 2.5 cut off to define PN the prevalence of PN in T2DM adults on metformin was 38% and the mean PN scores were 2.14 ± 1.98 with no significant gender difference. However as regards various grades of PN it was found that most common grade of PN was low grade PN (39.2%) *(MNSI physical assessment score/PN score > 0 ≥ 2.5)* followed by high grade PN (34.3%) *(MNSI physical assessment score/PN score > than 2.5).*

In addition, MNSI history showed that majority showed numbness (67.8%), burning pain (53.5%) in their legs, feet too sensitive to touch (33%) while few (10%) had an open sore on their foot and amputation cases were very rare (0.4%). Only 6.1% answered 'Yes' for 'if their doctor has ever told you that you have diabetic neuropathy' depicting that PN was under diagnosed.

The PN scores (1.55±2.03 vs 3.1±1.86) calculated from MNSI Physical assessment as well as MNSI history scores (4.9±2.79 vs 2.80±2.48) were higher in those with B12 deficiency than those without B12 deficiency respectively ($p<0.001$).

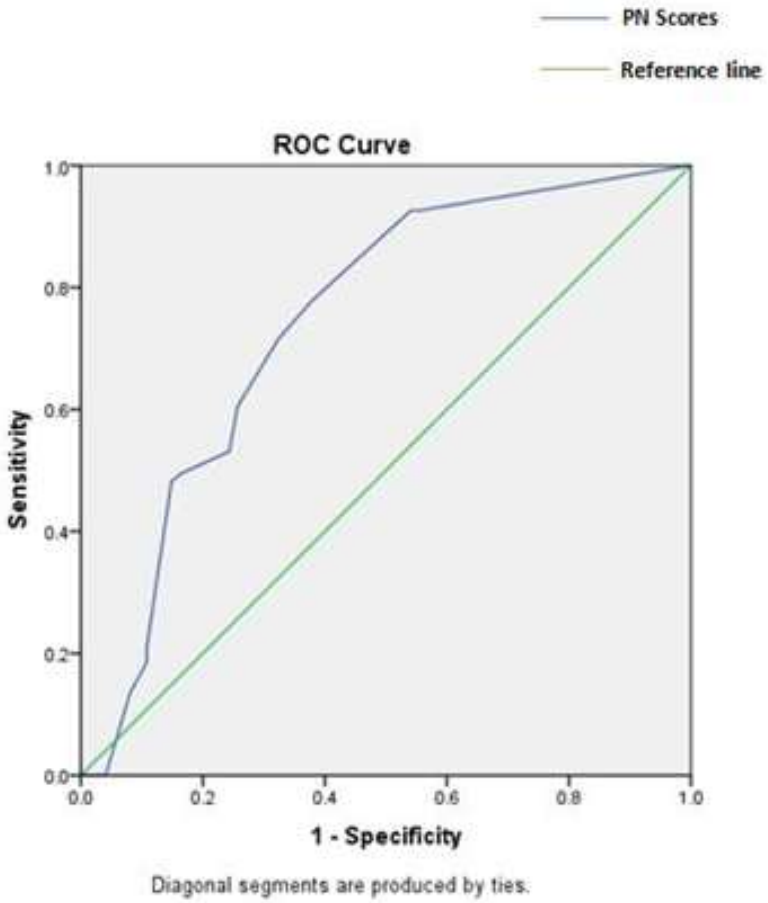


Figure 2. Receiver operating characteristic (ROC) curve for peripheral neuropahy score (PN score) related to vitamin B12 deficiency.

It was seen that of those suffering from PN whether low or high majority had B12 deficiency (58.2% and 70.5% respectively) in comparison to those with no PN where B12 deficient population was only 15.4%. A significant association between PN and B12 deficiency have been found ($p < 0.001$) (Table 2). Odds ratio between B12 deficiency and PN was 10.0 (C.I. 3.89–26) suggesting that those who are B12 deficient are ten times more likely to have PN in comparison to those who are not B12 deficient.

Table 1. Coordinates of ROC curve analysis for peripheral neuropathy related to B12 deficiency

Test variable: PN score (Positive if greater than or equal to [a])	Sensitivity	1-Specificity
-1.000	1.000	1.000
.250	.926	.554
.750	.926	.541
1.250	.778	.378
1.750	.716	.324
2.250	.605	.257
2.750	.531	.243
3.250	.494	.162
3.750	.481	.149
4.500	.210	.108
5.250	.185	.108
5.750	.136	.081
6.250	0.000	.041
6.750	0.000	.027
7.250	0.000	.014
8.500	0.000	0.000

The test result variable(s): PN Scores has at least one tie between the positive actual state group and the negative actual state group.

a = The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Table 2. Association of serum B12 status and PN grades of the T2DM adults on metformin

PN Scores	Total	Serum B12 >200 pg/ml (N=74)		Serum B12 ≤200 pg/ml (N= 81)	
	N	n	%	n	%
No PN (Scores =0)	39	33	84.6	6	15.4
Low grade PN (Score≤2.5)	55	23	41.8	32	58.2
High grade PN (Score>2.5)	61	18	29.5	43	70.5
p value	0.000***				

Table 3. Risk factor association for peripheral neuropathy (PN Scores ≥ 2.5) among T2DM adults of metformin

Factors	Total N	PN Positive (PN score≥ 2.5)	No PN (PN score<2.5)	O.R	95% CI	P value
Hypertension Present(Includes Prehypertensives)	240	92	148	2.48	0.27-22.59	0.403
Hypertension Absent	5	1	4			
Sex (F)	162	65	97	0.759	0.437-1.32	0.329
(M)	83	28	55			
Age≤50 (26-50)	65	20	45	1.53	0.83-2.81	0.163
Age>50 (>50-96)	180	73	107			
BMI≥23	206	71	135	0.406	0.203-0.814	0.009**
BMI<23	39	22	17			
Glycated Hb>7	110	59	51	4.62	2.03-10.51	0.000***
Glycated Hb≤7	45	9	36			
B12 deficiency (≤200pg/ml)	81	49	32	4.43	2.23-8.80	0.000***
Normal B12 (>200pg/ml)	74	19	55			
Metformin dosage>1000mg	50	15	35	0.64	0.32-1.25	0.193
Metformin dosage≤1000mg	195	78	117			
Duration of DM>5yrs	122	52	70	1.48	0.88-2.49	0.134
Duration of DM≤10yrs	123	41	82			

**p<0.01, ***p<0.001

Further risk factors of PN (MNSI physical assessment score/PN score ≥2.5) were associated by O.R. (Table III). Among the several risk factors of PN, hypertension, age, glycemic control (HbA1c), B12 deficiency and duration of T2DM were associated (O.R >1).Nevertheless, glucose regulation (HbA1c ≤7) and B12 deficiency were the only significant risk factors for the occurrence of PN among T2DM adults on metformin (p<0.001).The odds of PN (PN scores≥2.5) was 4.43 times higher (C.I. 2.23-8.80) among T2DM adults on metformin if they were B12 deficient in comparison to those who had normal B12 (p < 0.001). The odds of PN (PN scores ≥ 2.5) was 4.62 times higher (CI-2.03-10.51) among T2DM adults on metformin if they had poor glycemic control (HbA1c > 7) in comparison to those who had good glycemic control (HbA1c ≤ 7) (p < 0.001).

Discussion

The wide range of serum B12 seen here in T2DM adults taking metformin and the presence of outliers can be speculated due to the use of B12 supplements/B12 injections in past as the liver is where vitamin B12 is kept, and it may take years for the stores to run out and cause biochemical B12 deficiency [17]. Although a concerted effort was made to exclude patients who had received vitamin B12-containing supplements for any reason (a review of the available medical prescriptions was made, and patients were questioned about the use of Vitamin B12-containing supplements), it cannot be conclusively said that patients had never taken these preparations before because they are available over the counter.

Present study exhibited decreased amounts of vitamin B12 after taking metformin which confirms comparable results from other research published earlier [2,3,4,5,6,7,8]. However, our study indicated that vitamin B12 insufficiency was more common among T2DM persons taking metformin than was previously documented in other nations 14%, 22%, 33%, 6.9%, 8.6% and 10.5% of patients with T2DM on metformin [3,6,7,5,8,18]. Due to the increased incidence of vitamin B12 deficiency in India's general population, the higher prevalence of B12 deficiency in our study (52%) is not shocking which is reported to be as high as 33.3%–67% [19,20]. In comparison to earlier Indian research on metformin, our study found a higher rate of B12 insufficiency in T2DM [21,22,23]. It really is likely that the increased frequency of B12 insufficiency in our study is due to significantly higher proportion of vegetarians (54.3%) among those who were B12 deficient in comparison to those who had normal B12 ($p < 0.05$).

A lack of vitamin B12 caused by metformin as explained by the fact that metformin competes with calcium ions and the ileal uptake of vitamin B12 is dependent on calcium and can therefore be harmed by metformin [24]. Furthermore, it has been noted that Indian diets are low in calcium [10]. The present study, however, found no correlation between B12 deficiency and dietary calcium of less than ~60% R.D.A among Indians was found.

This study demonstrated that despite metformin's ability to diminish serum B12 levels, the haematological parameters (haemoglobin and cell morphology) that were evaluated in our investigation were unaffected and it seems to be iron deficiency anaemia and not the macrocytic anaemia due to B12 deficiency. Similar results depicting that anaemia is associated with studies from various countries and an Indian study [22] have both indicated that metformin use has not been linked to B12 insufficiency in country [25]. One recent study indicates risk of anaemia with metformin use in T2DM but its mechanism is unknown and study is limited to the fact that it has no mention of B12 deficiency with metformin [26].

In our study the vitamin B12 deficiency was associated with peripheral neuropathy (PN score) in T2DM adults on metformin which is similar to the finding of a multi centric study from an Asian country Pakistan [30] and several studies from other countries [7,23]. On the contrary there are some studies which have shown no association of B12 deficiency with

neuropathy in T2DM patients on metformin [22,25]. The studies on association of B12 deficiency and neuropathy has shown conflicting results as there were differences in designs and settings of various studies stated above. Neuropathy was assessed by different tools with various degrees of subjectivity and most of the studies had relatively small sample sizes.

In present study the prevalence of PN by MNSI physical assessment score ≥2.5 was 38% which was similar to the prevalence of PN reported in one study from Turkey where neuropathy defined by MNSI physical assessment score ≥2.5 was found in 32.1% in type 2 diabetes patients [28]. The prevalence of various grades of PN in our study was higher than that studied by Rani et al. 2010 [29] where diabetic neuropathy was considered as present if the VPT value was >20 V, mild neuropathy (VPT score, 20-24.99 V), moderate neuropathy (VPT score, 25-38.99 V), and severe neuropathy (VPT score, >39 V).The study showed the prevalence of diabetic neuropathy was 18.84% (95% CI: 16.79-20.88); the prevalence of mild diabetic neuropathy was 5.9% (95% CI: 4.68-7.15), moderate diabetic neuropathy was 7.9% (95% CI: 6.50-9.33), and severe diabetic neuropathy was 5% (95% CI: 3.86-6.14). In our study the PN prevalence as defined by MNSI PN scores ≥2.5 was 38% which was similar to 39.3% shown by one study in 2019 [30] where PN prevalence was studied by a predesigned semi-structured questionnaire, Semmes Weinstein 10g monofilament test, ankle reflexes, and vibration perception threshold. In our study among the several risk factors for PN studied by O.R the significant risk factors were B12 deficiency and Glycemic control. However one study in 2019 [30] found significant association between age, sex, BMI, duration of diabetes, and hypertension and the odds of PN. We would like to mention the fact that our population was T2DM patients on metformin while the study population in other studies was T2DM patients.

It can be said from our study that vitamin B12 test has fair chance of predicting PN among T2DM patients on metformin and taking a decision regarding which patients should be referred to a neurologist for electrophysiological studies.

Surrogate biomarkers of vitamin B12 deficiency like serum homocysteine and serum methyl malonic acid levels which are widely utilized in clinical medicine to improve diagnostic sensitivity, despite poor specificity [17] have not been estimated in our study which may seem as limitation to some [22]. However elevated levels of MMA do not necessarily co-relate with clinically evident cobalamin deficiency as evident from published literature [17].

Strength of our study is that the data on serum B12 is without the confounders (pregnancy, alcoholism, pernicious anemia, drugs like P.P.I,H2RAs/Hydrogen blockers, history of CRF, liver disease, CKD, Cardiopulmonary disease, bowel disease/surgery, cancer, acid-base disturbance, those on multivitamin or B12 supplement or B12 injections) which may affect B12 absorption. Secondly the PN has been studied with MNSI: a comprehensive and valid tool for measurement of neuropathy. However, the PN assessment was limited to the MNSI and these results

were not compared by Nerve conduction velocity or Neurothesiometer or Biothesiometer.

Conclusions

The prevalence of B12 deficiency in tertiary care T2DM patients on metformin was estimated at 52%. Metformin use was not associated with clinical B12 deficiency as there were no cases of macrocytic anemia. T2DM patients on metformin with B12 deficiency are probable to have PN scores of 2.25 by physical assessment of feet. To prevent neuropathy in T2DM, MNSI is a useful screening tool in an Indian setting.

Acknowledgement

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Conflicts of interest

The authors declares that they do not conflict of interest.

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