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Correlation Of Vitamin B12 Levels And Peripheral Neuropathy With Metformin Therapy In Type 2 Diabetes Mellitus

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Abstract

Objectives : To evaluate the prevalence and severity of Vitamin B12 deficiency in T2DM patients who were on metformin therapy and to evaluate Vitamin B12 deficiency in T2DM patients on metformin therapy and its relation to peripheral neuropathy.

Material and methods : A total of 117 patients were enrolled in this retrospective observational study.Detailed history and physical examination was done in patients with type 2 diabetes mellitus who were on metformin therapy for at least 6 months.Diabetic neuropathy was evaluated using the Michigan Neuropathy Screening Instrument questionnaire and nerve conduction studies. Serum Vitamin B12 levels were measured by chemiluminescence immune assay (CLIA).

Results : In our study, the overall prevalence of vitamin B12 deficiency is 18.8%. The prevalence of altered vitamin B12 levels(low or borderline) in type 2 diabetes mellitus on metformin therapy is 25.64%.In patients who were having diabetic neuropathy, low level or vitamin B12 deficiency is observed in 54.5% patients and those who did not have diabetic neuropathy low level of vitamin B12 was seen 45.5% patients.

Conclusion : Dose of metformin therapy is significantly inversely associated with Vitamin B12 levels($F=4.271$, $P<0.05$).Duration of metformin therapy is significantly inversely associated with Vitamin B12 levels($F=5.344$, $P<0.05$).Diagnosis of diabetic neuropathy in T2DM patients on metformin therapy is associated with lower levels of vitamin B12 and it is statistically significant($X=7.355$,$p<0.05$).

Keywords: NIL

Introduction

T2DM is a metabolic disease that is increasingly becoming a public health concern. There are a number of systemic macrovascular and microvascular problems linked to the syndrome. Up to 50% of people may develop diabetic peripheral neuropathy (DPN), the most frequent complication (1). The most important drug that is used in almost all patients as first line treatment for type 2 diabetes mellitus (DM) in the world is metformin. Vitamin B12 deficiency has been associated with chronic metformin use; this association was initially observed by Berchtold et al.(2) in 1969 and has also been observed in various other studies(3–6). Vitamin B12 deficiency in patients on metformin therapy can range from 5 to 40% (3).

It is believed that metformin blocks calcium-dependent ileal channels, hindering absorb vitamin

B12 in the ileum. It is generally recognized that long duration of metformin therapy causes a deficiency of vitamin B12 via this mechanism. According to European and American guidelines, metformin should be used as the initial medication in the pharmacological treatment of type 2 diabetes. Results from observational and interventional research have confirmed the growing evidence that long-term metformin use is linked to low vitamin B12 levels (3–6). Since vitamin B12 is necessary in the conversion of homocysteine to methionine, vitamin B12 deficiency caused by metformin may result in hyperhomocysteinemia, which may have a questionable detrimental impact on macrovascular disease in T2DM patients. Metformin primarily affects the liver by lowering the amount of glucose produced and, as a result, increasing the amount of glucose that is absorbed by peripheral tissues, especially muscle. Liver kinase B1 (LKB-1) activation, which controls the downstream enzyme adenosine monophosphatase protein kinase, is responsible for these effects (AMPK). A transcriptional co-activator and transducer of regulated CREB protein 2 (TORC2) is phosphorylated by AMPK, which renders it inactive. As a result, transcriptional processes that increase the synthesis of gluconeogenic enzymes are downregulated. (7). Another method for reducing gluconeogenesis is to inhibit mitochondrial respiration because it lowers the amount of energy needed for this process(8). Hematological and neurological symptoms are frequently seen in the clinical presentation of vitamin B12 deficiency. Without a haematologic presentation, the only symptom of the shortage may be neuropathy(9). It is well-recognized that a lack of Vitamin B12 results in megaloblastic anemia. However, it should be noted that mild neurological manifestations first arise before hematological changes(10). Although there are several choices with varying sensitivity and specificity, it is unknown which diagnostic technique is best for detecting diabetic neuropathy in these patients. One of the best-known methods used in research in diabetic neuropathy screening is the Michigan Neuropathy Screening Instrument(MNSI) (11).

There is a lack of studies in north India on the prevalence of metformin related vitamin B12 deficiency in patients with type 2 diabetes mellitus. Moreover there are no guidelines to screen diabetic patients on metformin therapy for vitamin B12 deficiency risk, and if necessary prescribe vitamin B12 supplements. Therefore we aim to undertake this study to evaluate the prevalence and severity of Vitamin B12 deficiency in patients with chronic metformin use and evaluate how it is related with diabetic neuropathy.

Aims and objectives :

1. To evaluate the prevalence and severity of Vitamin B12 deficiency in T2DM patients who were on metformin therapy.
2. To evaluate Vitamin B12 deficiency in T2DM patients on metformin therapy and its relation to peripheral neuropathy.

Material and methods :

This study was undertaken in the Department of Medicine in collaboration with the Medicine OPD, Medicine Wards, and Rajiv Gandhi Centre for Diabetes and Endocrinology, Jawaharlal Nehru Medical College and Hospital, AMU, Aligarh.

Type of study design : Retrospective, observational study.

Duration of study : From Dec 2020 to Nov 2022.

Study population : T2DM patients who were on metformin therapy for at least 6 months.

Ethics approval : The study was approved by the Institutional Ethics Committee of J.N.M.C. & H., A.M.U., Aligarh.

Inclusion Criteria :

1. Patients with age ≥ 18 yrs who were diagnosed with T2DM according to the WHO diagnostic criteria.
2. T2DM patients who were on metformin therapy.

Exclusion criteria :

1. Age < 18 years
2. Patients with H/O gastrectomy, colectomy, gastric iliac surgery, inflammatory bowel disease, or Pernicious anemia
3. Patients who are strictly on a vegetarian diet.
4. History of alcohol abuse and smoking.
5. Pregnant woman.

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6. Known patients of malabsorption syndrome
7. Patients on Vitamin B12 supplements
8. Patients on drugs that cause peripheral neuropathy.

Evaluation of Diabetic Neuropathy :

Relevant history is taken for the symptoms of diabetic neuropathy using the Michigan Neuropathy Screening Instrument questionnaire.

Physical examination is done for the signs of diabetic neuropathy using the Michigan Neuropathy Screening Instrument-Physical Examination tool.

A nerve conduction study was also done in the bilateral upper limbs and lower limbs for the assessment and quantitative confirmation of peripheral neuropathy using the Natus XCalibur NCV Machine.

Evaluation of Vitamin B12 deficiency :

Blood samples were taken from all selected T2DM patients who were on metformin therapy.Serum Vitamin B12 levels were measured by **chemiluminescence immune assay** (CLIA).

Diagnosis of Vitamin B12 deficiency: The diagnosis of vitamin B-12 deficiency is made based on serum or plasma vitamin B-12 concentration, with deficiency currently defined as a concentration lower than **148 pmol/L (200 pg/mL)** and marginal/borderline status defined as a concentration of **148– 221 pmol/L(200 pg/ml – 300 pg/ml)** (12) (13). A value of more than 221 pmol/L(300 pg/ml) is defined as vitamin B12 adequate.

Blood samples were also taken for the assessment of Hemogram, RFT, HbA1C, Fasting Blood glucose, and Serum Lipids levels.

Statistical analysis :

In the present study, all the qualitative data were analyzed using the Pearson Chi-square test. Kruskal Wallis's one-way ANOVA test was also used for the analysis of quantitative data. All the test was performed using the computer program SPSS version 25.0

Observation and results :

Table 1. Distribution of T2DM patients on metformin therapy evaluated for peripheral neuropathy with MNSI Questionaire :

S.No.	MNSI-Q Score	No. of Cases	(%)	Ch^2- Value	P- Value
1	Negative	79	67.5	14.368	0
2	Positive	38	32.5		
3	Total	117	100		

Using the MNSI questionnaire, we found that 38(32.5%) patients were having a positive score, with symptoms of diabetic neuropathy.

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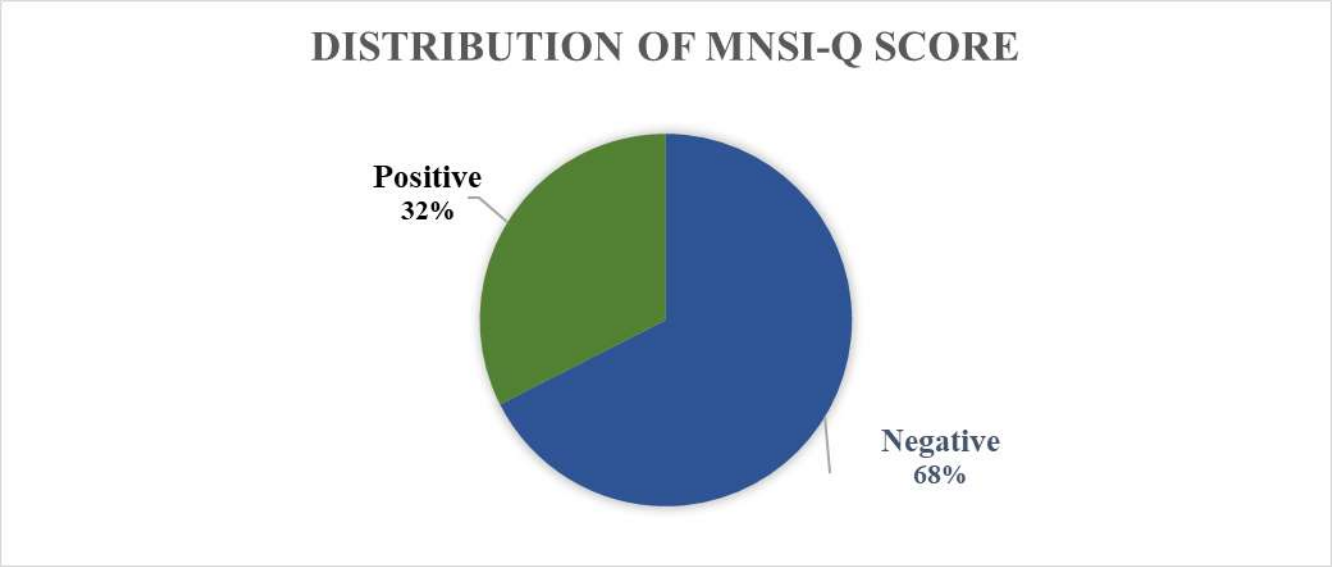

Table 2. Distribution of T2DM patients on metformin therapy evaluated for peripheral neuropathy with MNSI-Physical examination :

S.No.	MNSI-PE Score	No. of Cases	(%)	Ch^2- Value	P- Value
1	Negative	69	59.0	3.769	0.052
2	Positive	48	41.0		
3	Total	117	100		

Out of the 117 patients, the MNSI-PE score was positive in 48 (41%) patients, suggesting diabetic neuropathy in these patients.

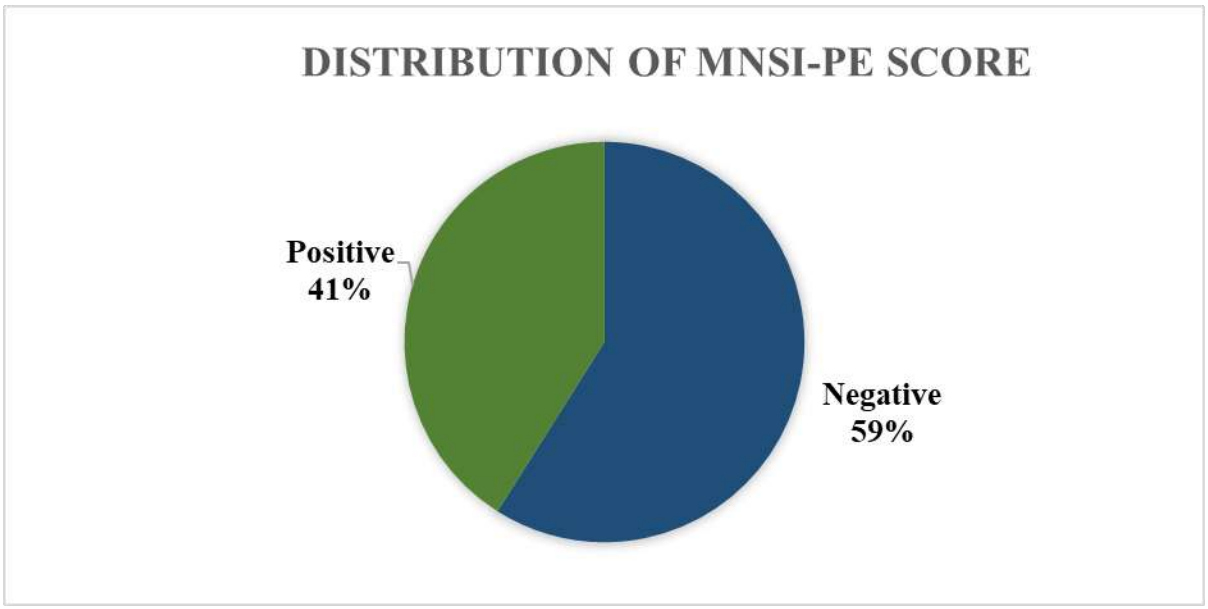

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Table 3 : Distribution of T2DM patients on metformin therapy evaluated for peripheral neuropathy with Nerve Conduction Study :

S.No.	Sensory Neuropathy	No. of Cases	(%)	Ch^2- Value	P- Value
1	Absent	62	53.0	0.419	0.518
2	Present	55	47.0		
3	Total	117	100		

When we evaluated the patients with nerve conduction studies, we found that 55 (47%) patients were having sensory neuropath.

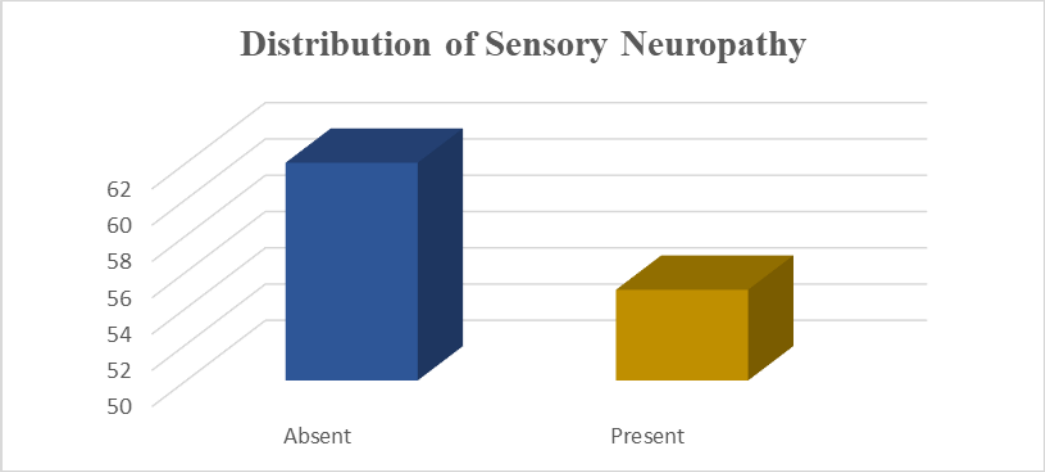

Table 4 : Distribution of T2DM patients on metformin therapy evaluated for peripheral neuropathy with Nerve Conduction Study.

S.No.	Sensorimotor Neuropathy	No. of Cases	(%)	Ch^2- Value	P- Value
1	Absent	100	85.5	58.888	0.000
2	Present	17	14.5		
3	Total	117	100		

Nerve conduction studies show, 17 (14.5%) patients were having mixed neuropathy(Sensorimotor neuropathy)

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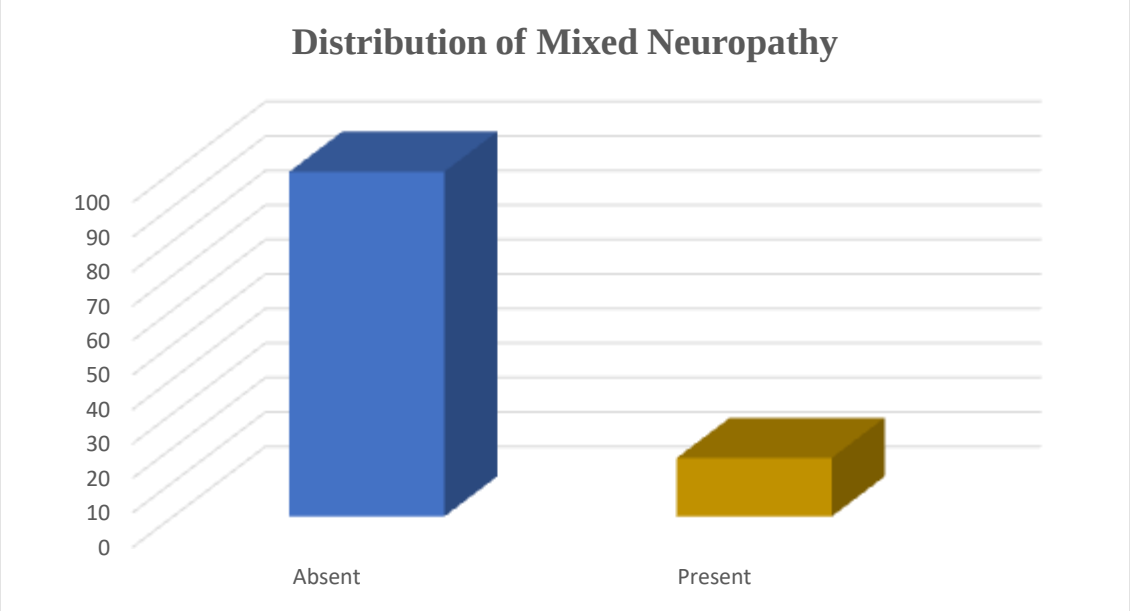

Table 5. Correlation of Duration of metformin therapy and Vitamin B12 levels.

S.No.	Duration of Metformin Therapy	Serum Vitamin B12 (pg/ml)		F- Value	P- Value
		Mean	Std. Deviation		
1	≤ 5 Year	840.62	317.17	5.344	0.002
2	6-10 Year	555.18	400.19		
3	11-15 Year	523.44	630.92		
4	16-20 Year	339.00	178.19		
5	Total	653.95	413.51		

The mean±SD value of vitamin B12 is lowest (339.00±178.19) in the patients who were on metformin therapy for 16-20 years and highest (840.62±317.17) in the patients who were on metformin therapy for ≤5 years. Duration of metformin therapy is significantly inversely associated with Vitamin B12 levels($F=5.344$, $P<0.05$).

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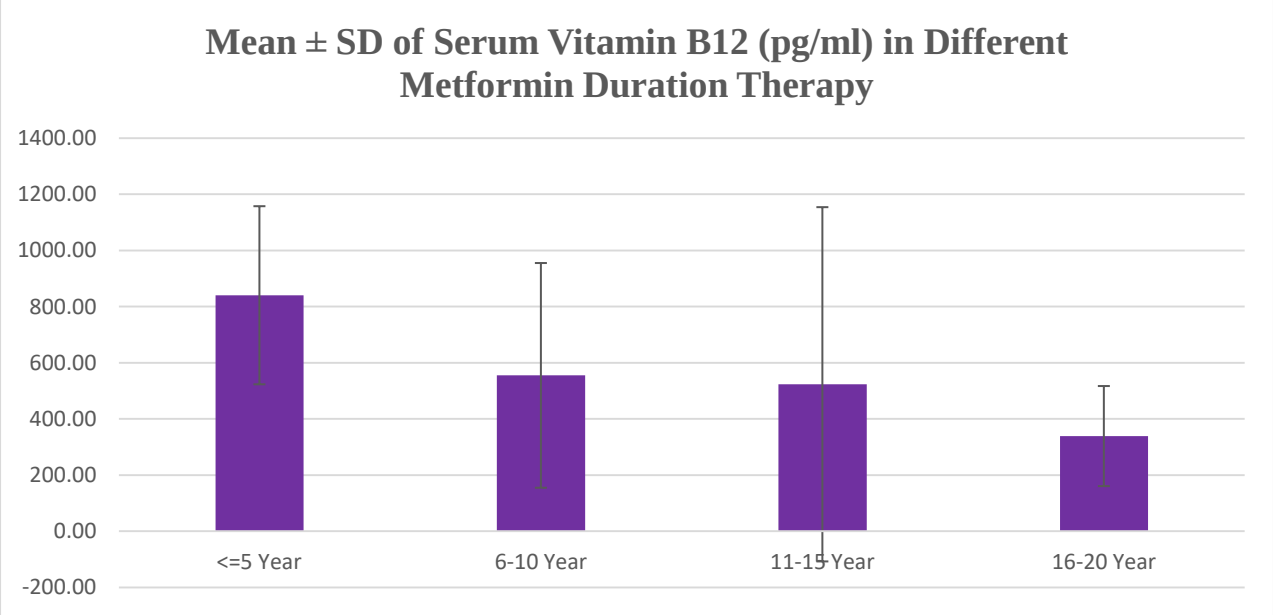

Table 6. Correlation of Dose of Metformin therapy with Vitamin B12 levels.

S.No.	Dose of Metformin	Serum Vitamin B12 (pg/ml)		F- Value	P- Value
		Mean	Std. Deviation		
1	500mg	799.83	285.11	4.271	0.007
2	1000mg	673.37	415.49		
3	1500mg	445.50	480.83		
4	2000mg	514.50	70.00		
5	Total	653.95	413.51		

1. The mean dose of metformin in our study is (∑=987.17).
2. The mean vitamin B12 levels is found to be (∑=653.95).
3. Mean±SD of vitamin B12 is highest (799.83±285.11) in patients taking 500mg of metformin and lowest (445.50±480.83) in patients taking 1500mg of metformin. Dose of metformin therapy is significantly inversely associated with Vitamin B12 levels(F=4.271, P<0.05).

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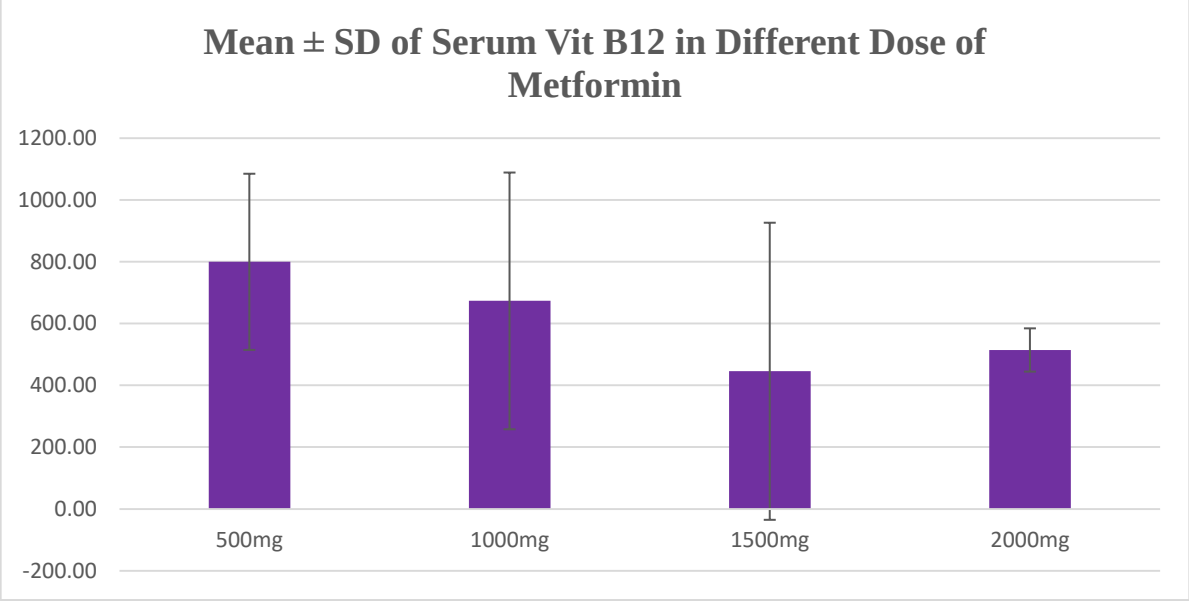

Table 7. Correlation of Peripheral Neuropathy with Vitamin B12 levels.

S.No.	Peripheral Neuropathy	Vitamin B12								Ch^2-Value	P-Value
		Low		Borderline		Normal		Total			
		Case	(%)	Case	(%)	Case	(%)	Case	(%)		
1	Present	12	54.5	7	87.5	35	40.23	54	46.2	7.355	0.025
2	Absent	10	45.5	1	12.5	52	59.77	63	53.8		
3	Total	22	100.0	8	100	87	100	117	100.0		

Of the total patients(n=117) included in this study, it was possible to establish the presence of peripheral neuropathy either by MNSI or Nerve Conduction studies in 54 patients(46.2%).

In our study, of the patients who were having diabetic neuropathy, a normal level of vitamin B12 was found in 40.23% (35 out of 87 patients); and in those who did not have diabetic neuropathy normal levels of vitamin B12 were seen 59.77%(52 out of 87 patients)

In our study, the patients who were having diabetic neuropathy, low level or vitamin B12 deficiency is observed in 54.5% (12 out of 22 patients); and those who did not have diabetic neuropathy low level of vitamin B12 was seen 45.5%(10 out of 22 patients).\

In our study, of the patients who were having diabetic neuropathy, borderline levels of vitamin B12 was found in 87.5% (7 out 8 patients); and in those who did not have diabetic neuropathy borderline level of vitamin B12 was seen 12.5%(1 out of 8 patients).

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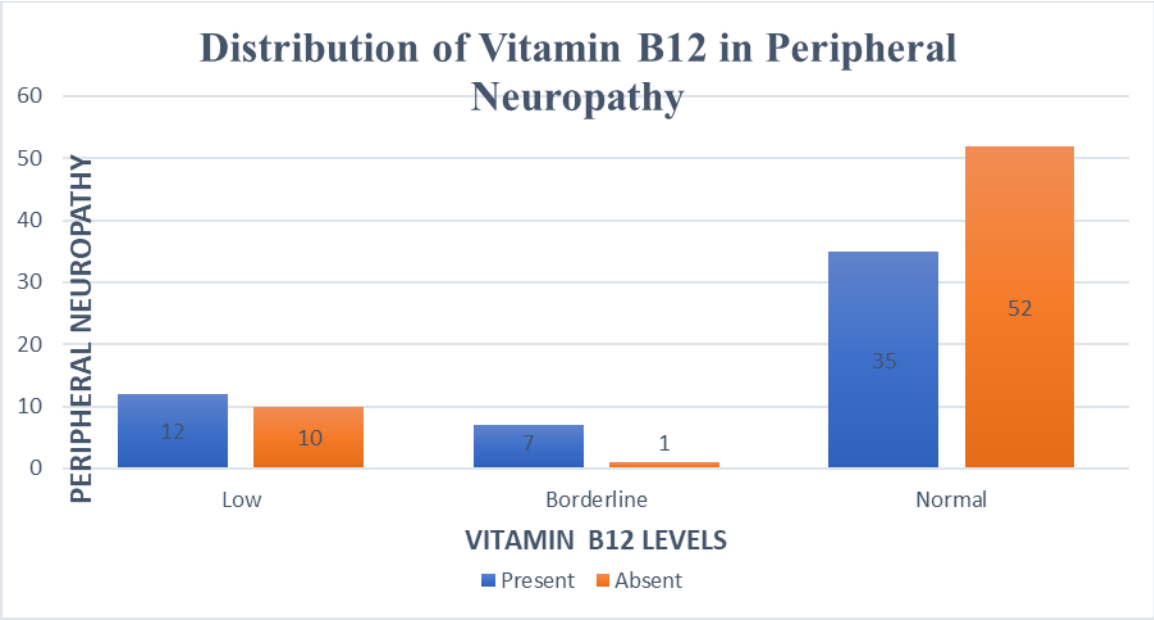
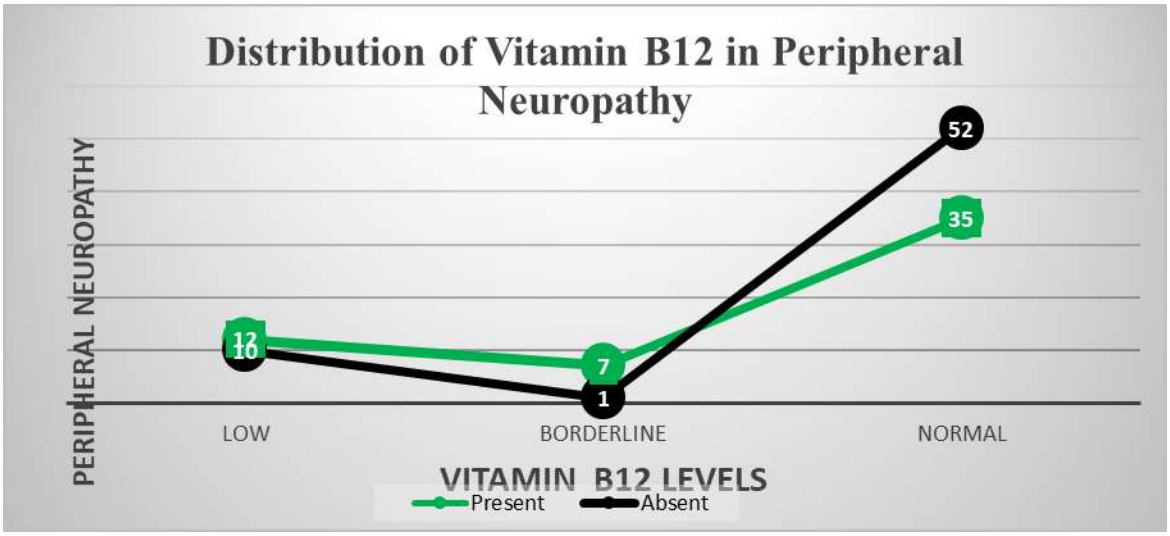
In our study, the prevalence of altered vitamin B12 levels(low or borderline) in patients with diabetic neuropathy who were on metformin therapy was 25.64%.

In our study, the overall prevalence of vitamin B12 deficiency is 18.

Diagnosis of diabetic neuropathy in T2DM patients on metformin therapy was associated with lower levels of vitamin B12 and it is statistically significant(X=7.355,p<0.05).





Discussion : In this study of a selected population of 117 patients, diagnosed with T2DM, we found that deficiency of vitamin B12 is highly prevalent in our population (18.8% of patients in our study); however, it is not as common as some earlier research reporting prevalence from 28 to 41% indicated (14)(15)(16) Using metformin increases the chance of developing vitamin B12 deficiency in diabetic patients, according to a recently published meta-analysis (17). Past studies have shown that in metformin-treated individuals, the prevalence of vitamin B12 deficiency ranges from 5 to 40% when arbitrary cutoff points for vitamin B12 deficiency are used. The findings of our current study are comparable to those studies using the similar cut off points(18)(19).

Populations at risk for vitamin B12 deficiency :

Elderly people, pregnant women, and some ethnic and racial groups are at particular risk for developing vitamin B12 deficiency(20).

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Older people are more likely to have vitamin B12 deficiencies, especially those over 65, who have a cobalamin deficiency prevalence of 10%–15%.(20) (21) (22).In our study, we found that mean±SD vitamin B12 is lowest (504.07 ± 599.20 pg/ml) in the 66-75 year age group and highest (825.53 ± 30570 pg/ml) in the 35-45 year age group, and it is inversely associated with age. This result is statistically significant($F=2.538$, $P<0.05$). Numerous age-related comorbidities and underlying diseases, as well as malabsorption and/or poor dietary intake, are potential causes of vitamin B12 deficiency in elderly people.

Pregnancy can alter the mother's vitamin B12 status by enabling the transfer of cobalamin to the fetus and child(20). Prevalence estimates of vitamin B12 deficiency during pregnancy have been found to range from less than 10% in Canada and Brazil to more than 70% in some regions of Turkey and India(20). Total plasma vitamin B12 levels gradually decrease during pregnancy, and this reduction is typically accompanied by a mild increase in MMA levels, suggesting a functional depletion in the status of intracellular cobalamin.(23)(24). The extensive anatomical and physiological changes that prevent the use of the known reference ranges used for the assessment of cobalamin levels in non-pregnant women make it difficult to assess vitamin B12 status and to assess the prevalence of vitamin B12 deficiency during pregnancy. In our study, we did not include pregnant diabetic women, therefore prevalence estimates of vitamin B12 levels in this group of patients are not available.

Additionally, it has been noticed that different ethnic and racial groupings have variable prevalence rates of vitamin B12 deficiency. This is probably because of genetic, cultural, and religious practises that predispose different populations to varying amounts of dietary animal product intke, especially red meat. In a recent study of a random sample of South Asian patients showed a very high prevalence of B12 deficiency (22% to 46%) compared with estimated rates in the general population (3% to 5%)(25).In our study which is done in the south Asian region, in diabetic patients on metformin therapy, the prevalence of Vitamin B12 deficiency is 18.8%.

Metformin-induced Vitamin B12 deficiency :

According to various studies, people with diabetes who take metformin have a reported prevalence of vitamin B12 deficiency that ranges from 6% to 50%(26).In our study, the prevalence of vitamin B12 deficiency with metformin use is 18.8%. Additionally, various studies have found an association between the dose and duration of metformin therapy and vitamin B12 levels. A cross-sectional research on 550 T2D patients taking metformin found that higher daily and cumulative dosages of the drug were highly linked with lower HoloTC and cobalamin concentrations (1 mg/d increase in daily dose and 10 g rise in cumulative dose) (mean treatment duration of 64 mo and mean daily dose of 1306 mg)(26). However, researchers did not find a relation between cobalamin/HoloTC concentrations and the length of metformin use(26). In a recent prospective observational study, Shivaprasad et al.(27) evaluated the effect of metformin dose and duration on vitamin B12 levels in 2887 T2D patients. They observed that 24.5% and 34.5% of metformin users had vitamin B12 levels below 200 pg/mL and between 200 and 300 pg/mL, respectively. In our study, we found that the mean±SD of vitamin B12 is highest (799.83±285.11) in patients taking 500mg of metformin and lowest (445.50±480.83) in patients taking 1500mg of metformin. Dose of metformin therapy is significantly inversely associated with Vitamin B12 levels($F=4.271$, $P<0.05$).In our study, 18.8% and 6.8% of metformin users had vitamin B12 levels below 200 pg/mL and between 200 and 300 pg/mL, respectively. Additionally, we also found mean±SD value of vitamin B12 is lowest (339.00±178.19) in the patients who were on metformin therapy for 16-20 years and highest (840.62±317.17) in the patients who were on metformin therapy for ≤5 years. Duration of metformin therapy is significantly inversely associated with Vitamin B12 levels($F=5.344$, $P<0.05$).

In our study, we found that a higher prevalence of low or borderline vitamin B12 levels is seen in female patients(mean±SD of vitamin B12 in females is 589.57±373.46 pg/ml), patients taking a higher dose of metformin(1500mg to 2000mg), and patients with diabetic neuropathy. A higher dose of metformin is associated with lower levels of vitamin B12, which has been demonstrated in previous studies.(28)(29).

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Metformin-induced vitamin B12 deficiency and its implications for diabetic neuropathy :

In diabetic individuals with preexisting neuropathy, metformin-induced vitamin B12 deficiency can worsen nerve damage. In a recent prospective case-control study of 150 adults with diabetes by Hashem et al (30) found that patients who used metformin for the previous 6 months had a significantly lower cobalamine level as compared to the control group who did not receive metformin. Moreover,the patients who were on metformin had a significantly higher frequency of diabetic neuropathy. Larger doses and longer duration of metformin therapy were also independent predictors of diabetic neuropathy.

Our study population had a 46.2% prevalence of diabetic neuropathy, which is consistent with prevalence rates from other studies, which showed that diabetic neuropathy prevalence rates range from 39 to 40%(31)(32). Moreover, we found a significant correlation between having lower levels of vitamin B12 and having a positive diagnosis of diabetic neuropathy. A high prevalence of low vitamin B12 levels (54.5% of patients) and an even higher prevalence of borderline vitamin B12 levels (87.5% of patients) were also observed in patients with diabetic neuropathy who were receiving metformin treatment.

Numerous observational studies have been conducted to determine whether a lack of vitamin B12 and diabetic neuropathy are related (33)(34)(35) .In a study by Gupta *et al.* (33), a positive correlation between metformin use duration and diabetic neuropathy and a negative correlation between metformin use duration and vitamin B12 levels is seen. In our study, we found that diabetic neuropathy(DSPN) is positively correlated with the duration of metformin therapy and it is statistically significant (X^2=19.870,P<0.05). Additionally,we also found diagnosis of diabetic neuropathy in T2DM patients on metformin therepy was associated with lower levels of vitamin B12 and it is statistically significant(X=7.355,p<0.05).

However, there are also studies such as in a recent meta-analysis, where sufficient evidence to link vitamin B12 deficiency to diabetic neuropathy is not found (20).

Another study that used vitamin B12 supplementation in diabetic patients with neuropathy found no conclusive results regarding neuropathy symptom improvement(36).

Given the large prevalence of diabetes and the fact that metformin is typically used as the first line of pharmacological management, it is crucial to look into a common side effect of this medication, vitamin B12 deficiency, which exacerbates multiple complications of diabetes. The population at risk of vitamin B12 deficiency caused by metformin is quite high and according to the findings of this study, screening for vitamin B12 deficiency is justified, particularly in at-risk populations like those with diabetic neuropathy who take high doses of metformin.

Summary : Our result is consistent with recent observations and meta-analyses that show a strong correlation between long-term metformin therapy and increased prevalence of vitamin B12 deficiency. The precise mechanisms underlying the vitamin B12 deficiency caused by metformin are yet unclear, despite the fact that it is highly likely that these mechanisms are connected to the decreased absorption of vitamin B12 in the small intestine.

Given the high global prevalence of diabetes, the widespread use of metformin as an insulin-sensitizing agent for treating insulin resistance, prediabetes, T2DM, and PCOS, as well as the chronic nature of treatment for such conditions, it is imperative to recognize vitamin B12 deficiency as a potential side effect of long-term and high-dose metformin therapy. Patients on metformin frequently go undetected for vitamin B12 deficiency since there are no established standards for screening this problem in this population. We believe initial screening and subsequent intermittent periodic testing of vitamin B12 status in selected high-risk populations of metformin-treated patients, such as old age patients and patients who are on high doses of metformin therapy, may be cost-effective in order to promptly detect and treat vitamin B12 deficiency.

Additionally, more research is required to determine how frequently people using metformin should have their vitamin B12 status checked. Therefore, more study is necessary to develop guidelines for the detection, prevention, and treatment of metformin-induced vitamin B12 deficiency. We also

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acknowledge the necessity for these guidelines to be supported by large prospective studies.

Vitamin B12 supplementation is an easy, safe, and efficient way for patients with metformin-induced vitamin B12 deficiency to halt the onset or exacerbation of peripheral nerve injury, anemia, and/or other clinical manifestations of vitamin B12 deficiency. There are currently no established protocols for the management of vitamin B12 deficiency brought on by metformin therapy. Therefore, healthcare professionals should think of this condition, especially while treating high-risk populations to prevent the worsening of nerve damage as well as a substantial deterioration of neuropathy resulting from the concomitant development of metformin-induced cobalamine deficiency and provide vitamin B12 supplementation when required.

Conclusion :

This study was conducted in the department of medicine, JNMCH, AMU with the title "Correlation of vitamin B12 levels and peripheral neuropathy with metformin therapy in type 2 diabetes mellitus" from Dec 2020 to Nov 2022. The results of the study are summarized below :

1. The prevalence of vitamin B12 deficiency in type 2 diabetes mellitus patients who are on long-term metformin therapy is 18.8%.
2. The prevalence of altered vitamin B12 levels(low or borderline) in type 2 diabetes mellitus on metformin therapy is 25.64%.
3. The dose of metformin therapy is significantly inversely associated with Vitamin B12 levels.
4. The duration of metformin therapy is significantly inversely associated with Vitamin B12 levels.
5. Diagnosis of diabetic neuropathy in T2DM patients on metformin therapy is associated with lower levels of vitamin B12 and it is statistically significant.

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