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Prevalence of Low Serum Vitamin B12 and Associated Factors in Type 2 Diabetic Patients on Metformin Therapy at Muhimbili Diabetic Clinic in Dar Es Salaam, Tanzania

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Abstract

Background:

Vitamin B12 deficiency is a health problem worldwide and increases when coupled with metformin use, especially in view of the rising incidence of Type 2 diabetes- mellitus and metformin being used as a 1st line oral hypoglycemic drug. Hematological and neurological manifestations of vitamin B12 deficiency are often overlooked and misdiagnosed as aging or diabetic neuropathy delaying appropriate management. This shows the need of proper guidelines for the screening and early detection of low vitamin B 12 levels among Type 2 diabetic patients to prevent complications and permanent nerve damage.

Objective:

To determine the prevalence of low vitamin B12 levels among type 2 diabetes patients on metformin therapy attending the MNH diabetic clinic.

Rationale:

Prevalence of low levels of vitamin B12 among Type 2 diabetes-mellitus patients on metformin therapy in Tanzania is not known, screening for low levels of vitamin B12 is not part of standard care among Type 2 diabetes- mellitus patients on metformin. Knowing the prevalence will provide evidence based screening for vitamin B12 levels as part of standard care.

Methodology:

A hospital based cross sectional study on adults Type 2 diabetes mellitus patients on metformin therapy attending the MNH diabetic clinic. Three hundred and twenty eight Type 2 diabetes mellitus patients were enrolled, for a period of 6 months and data was collected through a structured questionnaire on demographic characteristics, clinical assessment for presence of peripheral neuropathy using DNS and DNE scores and laboratory work done for measuring serum vitamin B12 levels and mean corpuscular volume.

Results:

Serum vitamin B12 levels were low (< 220 pmol/L) in 40 patients (12.5%) which was significantly associated with high dose of metformin, long duration of metformin use, presence of anemia, macrocytosis and peripheral neuropathy ($p = 0.001$).

Conclusion:

Low vitamin B12 level in metformin-treated diabetic patients was found be more in patients aged more than 40 years, patients who used higher metformin dose for a long period of more than five years. Low vitamin B12 levels were also associated with higher percentage of peripheral neuropathy compared to those with normal levels of serum vitamin B12.

"As the cost of vitamin B12 medicine is reasonable it is proposed that all patient with type II diabetes on treatment to be supplemented'.

To prevent development of peripheral neuropathy.

Background

Metformin is one of the first line oral hypoglycemic agent belonging to the biguanide class used for the treatment of T2DM(3) and it is initiated concurrently with lifestyle modification at the time of diagnosis (4).

Metformin acts by increasing insulin-mediated glucose utilization in peripheral tissues, decreasing hepatic glucose output and increases intestinal glucose utilization via non-oxidative metabolism (5). Metformin is effective as a monotherapy in lowering fasting blood glucose concentrations by approximately 20 percent and A1C by 1.5 % effective also in weight reduction or weight stabilization (5,7).and it has anti-lipolytic effect

Mechanism of metformin induced vitamin B12 deficiency is not clearly known, but some theories to explain it have been speculated. One of the mechanism being alteration of small bowel motility and stimulation of bacterial overgrowth which occur with metformin use causing vitamin B12 deficiency by directly decreasing vitamin B12 absorption in the terminal ileum(9).

Another proposed mechanism is through interruption of terminal ileum calcium absorption. The absorption of Intrinsic factor- vitamin B12 complex by the terminal ileum epithelial cells is calcium dependent. Metformin have been reported to alter the intracellular handling of calcium and therefore reducing absorption of vitamin B12. This theory is supported by the fact that the administration of calcium reverses metformin induced vitaminB12 deficiency(13).

The clinical features of vitamin B12 deficiency vary among individuals and there is no single symptom or a cluster of symptoms which are specifically associated with low level of vitamin B12. The most frequent reported symptoms of low vitamin B12 are hematological and neurological symptoms It may classically be documented by low serum vitamin B12 and other features however functional vitamin B12 deficiency may occur at any serum level(14).

Neurological and or neuropsychiatric symptoms may precede hematological symptoms/ signs and are the frequently presenting manifestations. These includes peripheral neuropathy ranging from paraesthesia, reduced peripheral sensation, decrease or absent deep tendon reflexes, diminished vibration or light touch sensation, ataxia of dorsal type. alteration in cognitive function and sub-acute combined degeneration of the spinal cord and dementia. Vitamin B12 deficiency which present as a peripheral neuropathy but without anemia is often misdiagnosed as diabetic neuropathy and if untreated could lead to progression of central or peripheral neuronal damage(15).

The association between metformin treatment and impaired vitamin B12 levels dates back to the 1970s when the interaction was first proposed(16,17) and estimates of prevalence were stated to be close to 30%. However different studies report different prevalence ranging from as high as 30% to as low as 5% (3,17,18).

Duration of metformin use influences development of low vitamin B12 levels(24). The time point at which metformin become a factor in the risk of vitamin B12 deficiency and some form of clinical presentation is between 5–10 years of metformin treatment A study done in Brazil among 128 type 2 diabetic patients on metformin to assess the duration of development of symptoms after exposure to metformin therapy showed that patients who were exposed to metformin for more than 3 year developed symptoms after 5 years (21). But according to the British Columbia guideline for individuals on metformin, the risk of developing low levels of B12 after being on metformin therapy was as early as after 4 months (25)..

Dosage of metformin is another factor which influences development of low serum vitamin B12. An increase in the dosage of metformin correlates with low serum vitamin B12. It has been suggested that a dosage of 1000mg per day is expected to results in a measurable reduction of vitamin B12 of 40pmol/L (3). An association between metformin dose and low serum B12 was observed in a study done in Korea among Type 2 diabetic patients which showed that vitamin B12 levels were lower in patients receiving

equal or more than 2,000 mg/day than those receiving 1,000-2,000 mg/day and those receiving equal or less than 1,000 mg/day (24).

The aim of this study was to determine the prevalence of low serum vitamin B12 levels and the associated factors among metformin treated Type 2 diabetes mellitus i.e. the association between duration and dosage of metformin therapy and low serum vitamin B12 and to determine whether metformin induced low serum vitamin B12 levels is associated with megaloblastic anemia and peripheral neuropathy.

Methods

Patients attending the Muhimbili outpatient diabetic clinic for their regular visits and those who concerted to participate in the study were included. The study was done from July 2015 to March 2016. Adult Type 2 Diabetes Mellitus patients on metformin therapy were included in the study the patients who met inclusion criteria were consecutively included in the study.

Informed consent verbally and written was obtained from all participants and were interviewed using a structured questionnaire. The demographic characteristics and information on duration of diabetes, dose and duration of metformin use, use of other diabetic medications were obtained. Recruitment of the participants was done twice per week and approximately 40 participants were recruited per week over a period of 3 months.

Clinical Measurements

Weight was measured using a Secca weighing machine after removal of shoes and heavy clothes and recorded to the nearest 0.5kg. Height was measured using a calibrated stadiometer in centimeters and recorded to the nearest 0.05cm. The body mass index was then calculated by an equation of weight (kg) divided by height in m2. According to WHO classification, obesity was defined as BMI > 30 kg/m2, overweight, BMI = 25–29.9 kg/m2, underweight, BMI <18kg/m2, Normal BMI = 18 – 24.9kg/m2 Peripheral neuropathy: Information on the presence or absence of peripheral neuropathy was obtained after asking for clinical symptoms of peripheral neuropathy using the Diabetic Neuropathy Score (DNS) and filled in the questionnaire Then all patients were examined for neuropathy using the Diabetic Neuropathy examination (DNE) (26,27). The assessment of fine touch, vibration sense and ankle reflex were used in the DNE. Fine touch was tested using a 10g Semmes Weinstein monofilament, which was applied to a non-callused site on the dorsum of the first toe just proximal to the nail bed in one leg. Patients were asked to respond whether they felt the pressure or not (yes/no).Inability to perceive the sensation was considered as abnormal sensation (27). Assessment of vibration sensation was done using a 128 Hz tuning fork which was applied at the distal plantar aspect of the big toe on both feet and the response was considered abnormal when the patient lost vibratory sensation while examiner still perceived it (27). Ankle reflex was assessed with a patellar hammer while the patient was sitting and the foot dorsiflexed and gently struck the Achiles tendon. If reflex response did not occur the test was repeated with reinforcement and response was recorded as present or absent.

The findings were then computed to obtain the Diabetic Neuropathy Examination (DNE) score which was used to quantify the neuropathy after symptoms assessment and a score > 3 was considered as presence of neuropathy(27) Overall presence of neuropathy was assessed by either the DNE and/ or DNS scores with patient's neuropathy described by DNE and/ or DNS score separately (27).

Serum Vitamin B12 And Mean Corpuscular Volume (MCV) Measurements:

8 mls of blood were taken through the venipuncture for serum B12, put in a yellow capped vacutainer with clot activator but without anticoagulant. The samples were immediately sent to the laboratory for measurements. Serum vitamin B12 levels were measured using a Cobas e411 Immunoassay system analyzer (21) using ECL technology which has a coefficient variation of approximately 10%. Low vitamin B12 level was defined as serum level of < 220pmol/L.

For complete blood count (CBC), blood was taken in purple caped bottle with EDTA (EDTA K3 bottle). Hemoglobin level was measured to assess presence of anemia which was defined as hemoglobin <11.5g/dl in females and hemoglobin< 13g/dl in males. The mean corpuscular volume (MCV) to assess presence of macrocytosis, which was defined as MCV of more than 96 fL was also done.

Statistical Analysis

Data was entered into SPSS for analysis. Clinical characteristics were expressed as a mean + standard deviation (SD) or percentage, Chi squared was used to test the difference in the proportion of categorical variables. Pearson correlation analysis was performed to assess the linear relationship between serum vitamin B12 and metformin use. Multiple logistic regression analysis was performed to assess the independent predictive effect of the variables as the risks for low vitamin B12. P–value of < 0.05 was considered as statistically significant.

Ethical Clearance

Ethical Clearance was obtained from MUHAS Research and Publication Committee. Informed consents were obtained from participants after the aim of the study and benefits to the participants explained thereafter patients were enrolled in the study. Confidentiality was maintained throughout the study, data collected and entered into the computer and unauthorized person did not have access to the information.

Results

Sociodemographic Clinical and Laboratory Findings of the Study Participants

A total number of 328 participants were enrolled in the study with 8 participants excluded due to presence of microcytosis and iron deficiency anemia. Out of 320 remaining participants, 215 (67.2%) were females and 163 (50.9%) were aged between 40–60 years and 88 (27.5%) were obese. Majority of the participants (92.2%) were residing in Dar es Salaam. Almost all participants (91.2%) had at least attained primary school education and more than half (60.6%) were employed and 112 (35%) participants had been diabetic for more than 10 years One hundred and forty (43.8%) of the participants used higher dose of metformin of more than 1000mg/day of metformin and nearly half of the participants used metformin for a longer duration of > 10 years. Fifty percent of the recruited diabetic patients were found to have peripheral neuropathy as assessed by DNS and or DNE scores. Approximately half of the patients (50.3%) had anemia, which of macrocytic type in fifty-nine (18.4%) and normocytic in two sixty-one (81.6%).

Association Between Vitamin B12 Deficiency and Socio Demographic and Clinical Characteristics

Forty (12.5%) participants had low serum level of vitamin B12. Low serum vitamin B12 level was significantly associated with age of 40 years or above than age below 40years who all had normal vitamin B12 levels, (p=0.026). Patients with longer duration of diabetes of more than 10 years had higher proportion of patients (25.9%) who had low vitamin B12 levels compared to 5.3% of those who were diabetic for 10 years or less. A high daily metformin dosage of more than 1g per day was significantly associated with low serum vitamin B12 levels compared to those who were using a daily metformin dose of 1g/day or less (20.7% versus 6.1% respectively) p<0.001 In addition, low level of vitamin B12 was significantly associated with longer duration of metformin use with patients who used metformin for more than 5 years having low vitamin B12 levels compared with those who used metformin for 5 years or less. Presence of anemia, macrocytosis and peripheral neuropathy were significantly associated with presence of low levels of serum B12 levels. There was no statistically significant association between low vitamin B12 levels and sex, employment status, place of residence, education level, and BMI of the patients **(Table 1)**.

Table 1: Association Between Vitamin B12 Deficiency and Socio Demographic and Clinical Characteristics

Variables	Low serum B12 levels N=40 (12.5%)	p-value value
Age		
< 40 years	0 (0)	0.026
40 – 60 years	24 (14.7)	
> 60 years	16 (13.2)	
Sex: Female	26 (12.1)	0.753
Employment Status		
Employed	23 (11.9)	0.750
Unemployed	14 (13.9)	
Retired	3 (16.7)	

Place of Residence		
Dar es Salaam	37 (12.5)	1.000
Upcountry	3 (12)	
Education Level		
Non-formal	7 (23.8)	0.345
Primary	21 (15.8)	
Secondary	6 (7.9)	
College/University	6 (8.6)	
No formal	1 (10)	
Body Mass Index (BMI)		
Underweight	0 (0)	0.052
Normal	6 (6.1)	
Overweight	17 (12.9)	
Obese	17 (19.3)	
Duration of Diabetes		
≤ 10 years	11 (5.3)	< 0.001
> 10 years	29 (25.9)	
Daily Metformin Dosage		
≤ 1000g	11 (6.1)	< 0.001
> 1000g	29 (20.7)	
Duration of Metformin Use		
≤ 5 years	7 (4.5)	< 0.001
> 5 years	33 (20)	
Anemia	33 (20.5)	< 0.001
Mean Corpuscular Volume		
Normocytosis	6 (2.3)	< 0.001
Macrocytosis	34 (57.6)	
Peripheral neuropathy	37 (23.1)	< 0.001

Association Between Peripheral Neuropathy and Various Factors

Presence of peripheral neuropathy was significantly associated with age, as peripheral neuropathy increased with age, patients with diabetes duration of more than 10 years and a daily metformin dosage of more than 1gram. Peripheral neuropathy was also significantly more common in those who used metformin for more than 5 years, patients having anemia and higher mean corpuscular volume. However, there was no significant association between neuropathy and BMI **(Table 2)**.

Table 2: Association Between Peripheral Neuropathy and Various Factors

Variables	Peripheral neuropathy N= 150(49.7%)	p-value
Age group (years)		
<40	4 (11.1)	< 0.001
40 – 60	80 (49.1)	
>60	75 (62)	
Body Mass Index (BMI)		
Underweight	1 (50)	0.124
Normal	52 (53.1)	
Overweight	56 (42.4)	
Obese	50 (56.8)	

Duration of diabetes		
≤ 10years	55 (26.4)	<0.001
> 10 years	104 (92.9)	
Daily metformin dosage		
≤ 1000 g	43 (23.9)	< 0.001
> 1000 g	116 (82.9)	
Duration of metformin use		
≤ 5 years	20 (12.9)	< 0.001
> 5 years	139 (84.2)	
Anemia	92 (57.1)	0.007
MCV		
Macrocytosis	108 (41.4)	< 0.001
Normocytosis	51 (86.4)	

Logistic Regression Analysis

A multivariate analysis was performed involving factors/variables for which $p < 0.001$ in the univariate analysis. These factors included age of patients, duration of diabetes, duration of metformin use, dose of metformin, macrocytosis and presence of peripheral neuropathy. Among the factors which were put in the multivariate model only macrocytosis was shown to be an independent predictor of low levels of vitamin B12 [OR 4.5; 95 CI (1.1- 1.3), $p<0.001$]. The higher the MCV becomes for a patient, the more likely it was for a patient to have low levels of vitamin B12.

Presence of peripheral neuropathy was a weak predictor of low levels of vitamin B12. Patient with peripheral neuropathy were at least four times [OR 4.5,95%CI (0.8, 25.3) $p = 0.09$] more likely to be at high risk for low levels of vitamin B12 compared to those without peripheral neuropathy (Table 6).

Table 3: Regression analysis of the factors associated with low serum vitamin 12 levels

Variables	Low vitamin B12 (N=40)	OR	95% CI	p-value
Age		0.99	0.94 – 1.03	0.573
DM Duration (>10 years)	29 (72.5%)	1.002	0.99 – 1.01	0.702
Metformin dose (>1g/day)	29 (72.5%)	1.00	0.999 – 1.001	0.715
Metformin duration(>5 years years)	33 (82.5%)	1.01	0.99 – 1.02	0.387
MCV (macrocytic)	34	1.18	1.12 – 1.25	0.000
Neuropathy	37 (92.5%)	4.47	0.79 – 25.34	0.09

Discussion

In this study, prevalence of low serum vitamin B12 in Type 2 diabetic patients on metformin at MNH was 12.5%. This prevalence lies within prevalence found elsewhere from other studies since there is a wide range of prevalence ranges from as low as 5% to a higher prevalence of 36.8(28).The wide range of prevalence could be attributed to the variation in the different countries having varying economic status, use of different cut off values for interpretations of low vitamin B12, and different techniques for vitamin B12 analysis or assays (8). In this study those patients who used metformin for duration of more than five years had significantly high proportion of patients with low vitamin B12 levels than those who had used metformin for less than five years duration.

Usually the decrease in vitamin B12 levels following metformin use starts as early as the fourth month and clinically overt features of vitamin B12 deficiency manifest by five to ten years owing to the large body stores in the liver which are not easily depleted(15).

Therefore the time point at which metformin use is expected to cause low levels of vitamin B12 and clinically evident features is between 5 to 10 years of treatment (22). However there have been occurrence of early manifestation of metformin associated vitamin B12 deficiency as early as one year from commencement of treatment and sometimes as early as four months (18,21). Another association which differs with the current study was drawn from the study done in Korea, which found that the relation of low levels of vitamin B12 with duration of metformin treatment may simply be the observation of the age related malabsorption that has been observed in other studies but when tested with age adjustment of the regression model for the relationship between duration of metformin treatment and serum vitamin B12 level it resulted in a reduction of the significance of duration of metformin to an insignificant level(18). In this study, a significant positive association was observed between high dose metformin and low vitamin B12.Patients who received metformin dose of more than 1000mg/day were more likely (20.7%) to have lower serum vitamin B12 levels compared to those who received metformin dose of less or equal than 100omg/day. In a study done in China, a statistically significant correlation between the dose of metformin and low vitamin B12 levels was found i.e the higher the dose, the lower the average vitamin B12 level. This is similar to the findings observed in the study done in Ireland in which there was a two-fold increase in risk of developing low vitamin B12 levels with each 1g/day dose increment(12).

Classically, low vitamin B 12 levels are related to megaloblastic anemia or macrocytosis (MCV of more than100 fL). As expected in the current study anemia and macrocytosis measured by MCV were significantly higher in patients with low serum B12 levels compared to patients who had normal levels of serum vitamin B12. Similar results were also found in Brazil study whereby anemia was found to be prevalent in patients with low vitamin B12, i.e 31% in the low vitamin B12 metformin group compared to metformin group with normal serum vitamin B12 levels(20).

However, one study observed that mean MCV level in the subjects with vitamin B 12deficiency was not greater than 100 fL, and the prevalence of megaloblastic anemia was approximately 0.5% and there was no difference which was found in the mean MCV between the groups with and without low vitamin B 12 levels concluding that. the anemia was likely caused by other factors like chronic illness(18). In addition, previous reports have indicated that up to 30% of patients with low serum vitamin B12 levels had normal MCVs. Furthermore, there is a masking of the macrocytic expression of megaloblastic anemia by coexisting factors like thalasaemia and chronic illness. Another explanation of high incidence of anemia in patients with T2DM could be a reflection of the association of T2DM with shortened red cell lifespan or possibly mild renal impairment. However, since there was no statistically significant correlation between the average hemoglobin or MCV and vitamin B12 levels in the groups, it could not be concluded that low vitamin B12 levels was an associated factor. Possible explanations for the lack of association with anemia or macrocytosis could also be due to the fact that these side effects require longer periods of deficiency in order to manifest (20).

It is well known that the risks of both type 2 diabetes and low vitamin B12 level increase with age. It is estimated that 21.2% prevalence of diagnosed diabetes among adults are ≥65 years of age and it has been observed that 6 to 20% prevalence of low vitamin B12 levels occurred among adults ≥60 years. In a case-control study that included155 diabetic patients with vitamin B12 deficiency, both age and duration of diabetes emerged as independent risk factors for occurrence of low vitamin B12 levels after adjusting for potential confounding factors(23).

The current study findings report,37 patients (23.1%) among those who had low serum vitamin B12 had typical manifestation of diabetic peripheral neuropathic symptoms (not statistically significance). Similarly, Singh, A et al exhibited differences in presence of neuropathy which were attributed to low serum vitamin B12level in a metformin treated population compared to a non-metformin treated population although they use Toronto clinical neuropathy screening system for assessment of neuropathy in their study. (9). Patients with metformin induced vitamin B12 deficiency developed some neurological symptoms such as impaired vibration sensation and proprioception which were potentially a result of neurological damage(30) which are difficult to distinguish them clinically from diabetic neuropathy or other causes of peripheral neuropathy. A previous study reported an association between low b12 and poor nerve conduction velocities with poorer response to light touch via monofilament detection which can also be found in other causes of peripheral neuropathy (21).

Conclusion

The current study finding reports a high prevalence of vitamin B12 deficiency in metformin-treated Type 2 diabetic mellitus patients at MNH. The metformin associated low serum vitamin B12 levels was observed to be significantly more in patients aged more than 65 years, those using metformin for more than five years, and using a higher dose of metformin of more than 1000mg/day. Peripheral neuropathy was also significantly more prevalent in patients with low serum vitamin B12 levels.

Recommendations

Due to the large proportion of metformin-treated T2DM patients having lower vitamin B12 levels it is therefore recommended that older patients on high metformin dosage, having used for more than five years should be regularly screened for of vitamin B12 levels or routinely supplemented with vitamin B12. It is important to screen patients and create awareness to this common problem. Therefore, we suggest that the measurement of vitamin B12 should become an essential part of the annual review in all patients with type 2 diabetes mellitus on metformin therapy.

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