**Thesis Title**

**Vitamin B12 Status in Long-term Metformin Treated Type 2 Diabetes Mellitus (T2DM) Patients /**

**Vitamin B12 Status in Patients with Type 2 Diabetes Mellitus on Long-term Metformin Therapy**

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**INTRODUCTION**

Diabetes mellitus (DM) is a metabolic disorder affecting about 424.9 million people worldwide with the majority of the patients (about 90%) having type 2 DM and it is apprehended that by 2045, some 628.6 million people, or one adult in 10, will have diabetes. In Bangladesh a total 6.9 million diabetic people in 2017 with national prevalence is 6.9% and it is estimated that in 2045 around 13.7 million of her people will have diabetes, with a prevalence of 14.95% making 9th ranked county in the world at that time.1(*Idf Diabetes Atlas*, 2017)

DM is associated with the development of the specific micro- and macro-vascular complications. Peripheral neuropathy is one of the microvascular complications of diabetes which carries the risk of foot ulcers and amputation.2 Almost 30% of people with diabetes aged 40 years or older have impaired sensation in the feet.3 Up to 50% of DPN may be asymptomatic and patients are at risk for insensate injury to their feet.4 Mørkrid et al. found 19.7% patients to have DPN among the type 2 diabetic outpatients at the BIRDEM hospital, Bangladesh.5

**Metformin**

Metformin (1,1-dimethylbiguanide hydrochloride) belongs to the biguanide class of drugs which are guanidine derivatives. Metformin has been used in Europe and Canada for more than 50 years (since 1957); the US Food and Drug Administration (FDA) approved it later in December 1994 (Bouchoucha et al., 2011). Past and recent recommendations from the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD), propose that metformin therapy (in the absence of contraindications) has to be initiated, concurrent with lifestyle intervention, at the time of DM diagnosis (Nathan et al., 2009; ADA, **2018)**.

The mechanisms of metformin action are little understood. It is effective only in the presence of insulin and improves hyperglycaemia primarily by suppressing glucose production by the liver (hepatic gluconeogenesis) (Kirpichnikov et al., 2002). In addition to suppressing hepatic glucose production, metformin increases insulin sensitivity, enhances peripheral glucose uptake, increases fatty acid oxidation, and decreases absorption of glucose from the gastrointestinal tract (Collier et al., 2006).

Side effects of metformin therapy are gastrointestinal upset and rarely lactic acidosis. Also, metformin treatment may be an iatrogenic cause for exacerbation of peripheral neuropathy in diabetic type 2 patients with depressed Cbl levels and elevated fasting tHcy and methylmalonic acid (MMA) levels (Wile&Toth, 2010).

**Vitamin B12**

Vitamin B12 is a water soluble vitamin which is obtained from foods, mainly those of animal protein origin.6 and requires intrinsic factor (IF) for absorption. The body uses it very vitamin B12 stores very economically, reabsorbing vitamin B12 from ileum and returning it to the liver; very little is excreted (Tietz text book of clinical biochemistry, 2006) Vitamin B12 is essential in the synthesis of neuronal myelin sheath and also in the synthesis of monoamines or neurotransmitters like serotonin and dopamine.7 Axonal demyelination, degeneration and later death are the hallmark of vitamin B12 deficiency induced neuronal damage that manifests as severe peripheral or autonomic neuropathy, sub acute combined degeneration of the spinal cord, delirium and dementia.8,9 In addition, vitamin B12 deficiency produces hyperhomocysteinemiawhich is an independent risk factor for atherosclerotic disease.10,11

**Relation of and Vitamin B12 with diabetes and metformin**

Various studies have shown increased prevalence of vitamin B12 deficiency in patients with type 2 diabetes mellitus on Metformin (Khan et al., 2017; Iftikhar et al., 2013)

Vitamin B12 deficiency is traditionally diagnosed by laboratory findings of low serum vitamin B12 levels, typically in the setting of megaloblastic anemia. However, subclinical B12 deficiency often presents with normal serum B12 levels and hematologic parameters (Snow, 1999).

The mechanism by which metformin therapy causes vitamin B12 deficiency is not clear, but it is thought to be due to either alterations in small bowel motility, which stimulate small bowel bacterial overgrowth and subsequent vitamin B12 deficiency, or by directly decreasing vitamin B12 absorption (Kumar et al., 2013; Albers et al., 2012).

The deficiency of vitamin B12 occurs on Metformin therapy as dose and duration dependent manner even within 6 weeks to 3 months of commencing Metformin (Chapman LE et al., 2016)

However, there are conflicting reports on the association between Metformin-induced B12

deficiency and neuropathy, with some reports showing an association (14, 15) (Sing AK et.al., 2013; Roy RP et al., 2016) while others have refuted this (11, 16–18) (Russo GT et al., 2016; Ahmed MA et al., 2016; Ma J et al., 2015; Elhadd *et al.*, 2018)

Various studies have shown increased prevalence of vitamin B12 deficiency in patients with type 2 diabetes mellitus.12,13,14,15,16,17,18,19,20 Vitamin B12 was found to be low in obese and even overweight persons with or without metabolic syndrome.21 Metformin use has been implicated as a cause of vitamin B12 deficiency in patients with diabetes.16,17,20 But vitamin B12 deficiency has been also found in diabetic patients not taking metformin.13,14,15 Barghouti et al. found that diabetic patients are less likely to have B12 deficiency in comparison to nondiabetic subjects.22

Peripheral neuropathy caused by diabetes mellitus and vitamin B12 deficiency may produce overlapping clinical pictures.23 Moreover; nondiabetic neuropathies may be present in patients with diabetes and may be treatable.4 The relatively high prevalence of B12 deficiency in diabetic patients makes it likely that at least a portion of peripheral neuropathy cases in diabetic patients may be attributable to B12 deficiency.12 It has been observed that, independent factors other than glycemic control are critical to the development of diabetic polyneuropathy and strict glucose control is not enough to ameliorate the onset and progression of T2DM diabetic polyneuropathy.24 Moreover, several published studies reported that therapeutic supplementation with B12 or vitamin B complex mixtures containing B12 significantly improved symptoms in diabetic neuropathy patients (Sun Y et al, 2005, Dominguez et al, 2012, Fonseca VA et al, 2013) .

Therefore, measurement of serum B12 level in patients with type 2 diabetes mellitus with or without Metformin therapy may help in exploring concurrent B12 deficiency, a condition which can be treated very easily. The present study aims to find out the serum vitamin B12 status in type 2 diabetic patients and also would attempt to correlate the deficiency status, if any with presence of peripheral neuropathy and metformin use, if so present.

**RATIONALE**

Type 2 DM is a serious health problem in our country and the prevalence of type 2 diabetes mellitus is increasing. Metformin is the preferred initial pharmacologic agent for the treatment of type 2 diabetes (if not contraindicated and if tolerated). It is used as first line therapy almost all patients all over the world. Several studies have shown Long-term use of metformin may be associated with biochemical vitamin B12 deficiency, and periodic measurement of vitamin B12 levels should be considered in metformin-treated patients, especially in those with anaemia or peripheral neuropathy. But there are conflicting reports on the association between Metformin-induced B12 deficiency and neuropathy have refuted this.

Studies on vitamin B12 in T2DM are very limited in Bangladesh . The frequency of vitamin B12 deficiency in patients with T2DM on Metformin therapy in Bangladesh is unknown and the measurement of serum vitamin B12 in T2DM patients on Metformin is not part of the standard annual review examination.

Vitamin B12 deficiency also leads to development of peripheral neuropathy which may be indistinguishable clinically from diabetic peripheral neuropathy. Furthermore, recent studies have shown that vitamin B12 deficiency is common in patients with diabetes mellitus. Treatment of diabetic peripheral neuropathy with vitamin B12 supplementation resulted in clinical improvement of symptoms. Vitamin B12 deficiency leads to hyperhomocysteinemia which is an established cardiovascular risk factor. So, now the question arising whether the diabetic patients should undergo routine screening for vitamin B12 deficiency or routine supplementation of vitamin B12 should be given to diabetic patients. Present study aimed to find out the serum vitamin B12 status in patients with type 2 diabetes mellitus.

**RESEARCH QUESTION**

What is the status of vitamin B12 in T2DM patients who were treated with long-term metformin in comparison to those patients who were not treated with metformin?

**HYPOTHESIS**

There is significant difference between the vitamin B12 levels of the 2 groups.

Vitamin B12 is significantly decreased in Metformin treated T2DM group

**OBJECTIVES**

**Primary objective:**

* To determine serum vitamin B12 level in patients with type 2 diabetes mellitus on lon-term Metformin therapy

**Secondary objectives:**

* To determine serum vitamin B12 level in patients with type 2 diabetes mellitus not on metformin therapy
* To determine presence of peripheral neuropathy in patients with type 2 diabetes mellitus.
* To determine serum vitamin B12 level in patients with type 2 diabetes mellitus.
* To observe association between vitamin B12 level with presence of peripheral neuropathy, metformin use and glycemic status (HbA1c).

**METHODOLOGY**

**STUDY DESIGN**

Cross-sectional comparative type of study. /Observational cross-sectional study

**STUDY PERIOD**

This study will be conducted within the period starting from October 2018 to April 2020.

**STUDY POPULATION & PLACE**

Study population will be Non-pregnant adults with type 2 DM currently on Metformin therapy attending either OPD (Outpatient care) or IPD (Inpatient care) of Sylhet MAG Osmani Medical College Hospital (SOMCH) and equal number of age and sex matched T2DM patients who are not on metformin therapy.

**SAMPLE SIZE AND STATISTICAL BASIS OF IT:**

**Z 2 pq**

**d2**

**Sample size: n =**

Z = is standard normal variate (at 5% type 1 error (P<0.05) it is 1.96 and 1% type 1 error (P<0.01) it is 2.58). As in majority of studies P values are considered significant below 0.05 hence 1.96 is used in the formula.

p = Expected proportion in population based on previous studies (40%).

q= 1-p

d = Absolute error or precision. Usually it is taken as 20% (0.2).

Sample size (n) = [1.962 × 40 × 60] / [(20 × 40) /100]2

= 144.06

So intended sample size (n) = 150

**SAMPLING METHOD:**

Purposive non-probability sampling. **/** Convenience sampling method

**Data collection technique:**

Semi-structured data collection sheet

**SELECTION CRITERIA:**

**Inclusion criteria:**

* Non-pregnant adult patients having type 2 diabetes mellitus for at least one year.

( and control group - who have no history of metformin use for atleast 1 year

* Dosage of metformin of at least 1000 mg/day
* ( and control group – on others drugs

**Exclusion criteria:**

* Patients with diagnosis of pernicious anaemia, vitamin B12 supplementation, malabsorption (celiac disease, inflammatory bowel disease, and gastrointestinal surgery), malnutrition (pure vegans, anorexia nervosa), iron deficiency anaemia, history of thyroid disease and thyroxin treatment and/or a history of other organ-specific autoimmune conditions (vitiligo, Addison’s disease, primary ovarian failure, hypoparathyroidism)
* Known cases of Chronic Kidney Disease(CKD)/ End stage renal disease(ESRD)
* Chronic (3 months or more) use of acid suppressants i.e. Proton pump inhibitors or h2 receptor blockers
* Concomitant drugs use that affect vitamin b12 level (e.g. Corticosteroids, phenytoin, dihydrofolate reductase inhibitors)

**STUDY PLAN:**

Subjects will be recruited on the basis of inclusion and exclusion criteria

**↓**

Before sampling, written informed consent will be taken from each subject after explaining the steps and purpose of the study

**↓**

Study subjects will be asked of and examined for relevant clinical information

**↓**

Data will be collected using prescribed data collection sheet

**↓**

5 ml of venous blood will be taken from each subject maintaining all aseptic precautions

**↓**

Serum will be separated and stored in room temperature until assay

**↓**

Assay of vitamin B12 and HbA1c of collected samples will be done on the same day of blood sample collection (preferably within 08 hours) without causing any harm to quality

**↓**

Patients having borderline B12 deficiency will be tested for serum Methylmelonic acid

**↓**

Data will analyzed using computer based SPSS program (version 23.0)

**Main outcome variables:**

* Glycaemic status
* Duration of diabetes
* Dose of Metformin
* Serum vitamin B12 level

**Checklist of variables:**

1. **Anthropometric variables:**

* Body Mass Index (BMI)
* Waist Circumference (cm)
* Blood Pressure (mmHg)

1. **Clinical variables:**

* Symptoms of peripheral neuropathy: pain, numbness, tingling, weakness, ataxia, upper limb symptoms
* Knee and Ankle jerks
* Pinprick, Temperature, Light touch, Vibration and Position sense

1. **Biochemical variables:**

* Random/Fasting plasma glucose
* Serum creatinine
* Haemoglobin concentration, MCV (mean corpuscular volume)
* HbA1c
* Serum vitamin B12

**QUALITY ASSURANCE**

Regular instruction from the supervisor will be taken. Collected data will be checked periodically.

**Operational Definitions:**

1. **Peripheral neuropathy:** Peripheral Neuropathy will be screened by using Toronto Clinical Neuropathy Scoring System and presence of peripheral neuropathy will be interpreted as following:

No neuropathy (1): 0-5 points; Mild neuropathy (2): 6-8;

Moderate neuropathy (3): 9-11; Severe neuropathy (4): 12+

1. **Serum vitamin B12 level status:**

* Deficiency: ≤200 pg/ml
* Borderline deficiency: >200 to ≤300 pg/ml
* Normal: >300 pg/ml.28

1. **Controlled Daibetes:** here, controlled diabetes is defined asdiabetic patients having HbA1c ≤7.0%.**22**
2. **Uncontrolled Diabetes:** Here, Uncontrolled diabetes is defined as HbA1c >7.0% .

**WORK PLAN**

September, 2018 – August, 2020 (2 Years)

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Activities** | **Month Year** | **Month Year** | **Month Year** | **Month Year** | **Month Year** | **Month Year** | **Month Year** | **Month Year** | **Month Year** | **Month Year** |
| **Designing the Study** | Sep, 2018 |  |  |  |  |  |  |  |  |  |
| **Review of Literature** | Sep, 2018 | Oct, 2018 | Nov, 2018 |  |  |  |  |  |  |  |
| **Development and Approval of Proposal** |  |  | Nov, 2018 |  |  |  |  |  |  |  |
| **Development of Data Collection Tools** |  |  |  | Dec, 2018 |  |  |  |  |  |  |
| **Pretesting Questionnaire** |  |  |  |  | Jan, 2019 |  |  |  |  |  |
| **Data Collection, Entry and Analysis** |  |  |  |  |  | Jan, 2019 to Dec, 2019 |  |  |  |  |
| **Report Writing** |  |  |  |  |  |  | Jan, 2020 |  |  |  |
| **Submission and Approval** |  |  |  |  |  |  |  | Feb, 2020 | March, 2020 |  |
| **Printing, Binding and Final Submission** |  |  |  |  |  |  |  |  |  | Apr, 2020 |

**Procedures of preparing and organizing materials:**

Those patients, who fulfill the inclusion criteria and exclusion criteria, will be included in the study. Then sample will be collected from either IPD or OPD Sylhet MAG Osmani Medical College Hospital (SOMCH). Informed written consent will be taken from each study subject after complete explanation of the steps and purpose of the study. Data will be collected by using semi-structured questionnaire and will be recorded in the data collection sheet. Then 5 ml venous blood was taken from each subject in two separate test tubes maintaining all aseptic precaution. Then serum was assayed for HbA1c and vitamin B12 on the same day of blood sample collection (preferably within 08 hours). Separated serum was discarded if vitamin B12 assay could not be done within the same day.

**Statistical analysis:**

Data will be analyzed by using SPSS program (version 23.0). Results will be expressed in frequencies or percentages (mean ± SD and median). Comparison of vitamin B12 level in subgroups will be done by Student’s unpaired t-test. Frequency of reduced vitamin B12 using recommended cut-off values will be compared in between subgroups by Chi-square test. Pearson’s correlation test will be used to observe correlation among different variables (HbA1c, vitamin B12, fasting glucose, age, metformin use etc.). P value ≤0.05 will be considered significant.

**Ethical Implications:**

* Ethical clearance will be taken from the ethical committee of Sylhet MAG Osmani Medical College prior to commencement of study.
* Informed written consent will be taken from the patients and study related information will be explained in local language to patients.
* Before data collection, the respondents will be told that they are at liberty to participate and to decline to answer any question during the study. The respondents will be given assurance that the findings of the interview/ investigation/ examination will not be used/ disclosed to any unauthorized person or authority other than the research purpose

**BUDGET CONSIDERATIONS**

|  |  |
| --- | --- |
|  |  |
| **Medicines & Reagents** | BDT 1,70,000 |
| **Purchasing Literature from the Internet** | BDT 5,000 |
| **Stationary** | BDT 5,000 |
| **Questionnaire Adaptation** | BDT 5,000 |
| **Printing and Photocopying** | BDT 15,000 |
| **Data Analysis** | BDT 10,000 |
| **Report Preparation and Presentation** | BDT 10,000 |
|  | **Grand Total = BDT 2,20,000** |

**Source of funding:** Myself

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**Appendix I**

**Data Collection Sheet**

|  |  |
| --- | --- |
| ◙ ID: | ◙ Mobile No.: |
| ◙ Patient’s Name: | ◙ Age (years): |
| ◙ Sex: Male (1), Female (2) | ◙ Duration of Diabetes (years): |
| ◙ Smoking Status: Smoker (1), Non-smoker (2) | ◙ Body Mass Index (Kg/M2): |
| ◙ Waist Circumference (cm): ◙ Blood Pressure (mmHg): | |
| ◙ Anti-Diabetic Drugs:  ◙ Metformin (1): Duration of Use (years): Daily Dose (mg):  ◙ Other OADs (2): Name of the Drug: ◙ Insulin (3) | |

**◙ Peripheral Neuropathy Scoring (Toronto Clinical Neuropathy Scoring System):**

No neuropathy **(1)**: 0-5 points; Mild neuropathy **(2)**: 6-8;

Moderate neuropathy **(3)**: 9-11; Severe neuropathy **(4)**: 12+

|  |  |  |
| --- | --- | --- |
|  | Right | Left |
| **Symptom scores** | **Present=1**  **Absent=0** | **Present=1**  **Absent=0** |
| Pain |  |  |
| Numbness |  |  |
| Tingling |  |  |
| Weakness |  |  |
| Ataxia |  |  |
| Upper-limb symptoms |  |  |
| **Reflex scores** | **Absent=2**  **Reduced=1**  **Present=0** | **Absent=2**  **Reduced=1**  **Present=0** |
| Knee Reflexes |  |  |
| Ankle Reflexes |  |  |
| **Sensory Test Scores** | **Abnormal=1**  **Normal=0** | **Abnormal=1**  **Normal=0** |
| Pinprick |  |  |
| Temperature |  |  |
| Light touch |  |  |
| Vibration |  |  |
| Position |  |  |
| Totals |  |  |

**Investigations:**

|  |  |
| --- | --- |
| ◙ HbA1c (%): | Normal (1), High (2) |
| ◙ Serum Vitamin B12 (pgm/mL): | Normal (1), Borderline Deficient (2), Deficient (3) |
|  |  |
| ◙ Serum Creatinine: | ◙ eGFR (CKD-EPI Formula; ml/min/1.73m2): |
| ◙ Hb (gm/L): | ◙ MCV (fL): |

Data Collector’s Sign & Date:

Name:

**Appendix-II**

**Informed Written Consent:**

**Title of the Study**: Vitamin B12 Status in long-term Metformin treated Type 2 Diabetes Mellitus Patients

1. Investigator’s Name: Dr. Abu Kamran Rahul
2. Institution: Sylhet M.A.G. Osmani Medical College Hospital, Sylhet.
3. Do you know the type, purpose and procedure of this study? Yes / No.
4. Are you sure that you will not face any physical, psychological and social risk for this study? Yes / No.
5. Are you sure this study will not cause any physical or psychological harm? Yes / No.
6. Do you have freedom to refuse, participate or withdraw? Yes / No.
7. Do you loss any fundamental human rights due to participation in this study? Yes / No.
8. Do you feel that the confidentiality of your information will be maintained? Yes / No.
9. Do you know that you will get no remuneration or travel expenses due to participation in this study? Yes / No.

**Consent Form (English)**

Getting full information about the purpose, procedure and utility of this study, I give consent to participate in this study. I have not been influenced by anybody or groups or my fundamental human rights have not been violated due to participation in this study.

I am assured that confidentiality of all gathered information will be maintained and will be used for only study purpose and my personal information will not be disclosed to others.

My participation in this study is entirely voluntary. My decision whether or not participate will not prejudice my medical care. I have right to withdraw my consent and discontinue participation at any time without prejudice to me or affect on my medical care.

I will not get any renumeration due to participation in this study.

I am willingly giving signature to this consent form.

Signature of the participant

সম্মতি পত্র

এই গবেষণা কর্মের উদ্দেশ্য, পদ্ধতি ও উপযোগিতা সম্পর্কে পূর্ণ ধারণা পাইয়া এবং নীতিগত বৈশিষ্ট্য সমূহের প্রতি আমার সম্মতি প্রকাশ করিতেছি। গবেষণা কর্মে অংশগ্রহণের জন্য আমি কোন ব্যাক্তি বা গোষ্ঠীর দ্বারা প্রভাভিত হই নাই অথবা আমার মৌলিক মানবাধিকার ক্ষুণ্ণ হয় নাই। আমি নিশ্চিত হইয়াছি যে, এই গবেষণা থেকে সংগৃহীত তথ্যাবলি সম্পূর্ণ গোপন রাখা হইবে। এই তথ্যাবলি কেবলমাত্র গবেষণার কাজেই ব্যাবহার করা হুইবে। আমার ব্যাক্তিগত তথ্যাদি গবেষণাকারী ছাড়া অন্য কাঁরও নিকট প্রকাশ করা হইবেনা।

এই গবেষণায় আমার অংশগ্রহণ সম্পূর্ণ আমার ইচ্ছাধীন। আমি ইচ্ছা করিলে গবেষণায় অংশগ্রহণ নাও করিতে পারি, তাহাতে আমার চিকিৎসার তারতম্য হইবেনা। যে কোন মুহূর্তে আমি আমার সম্মতি প্রত্যাহার করিবার অধিকার রাখি। আমার এই প্রত্যাহার আমার চিকিৎসার উপর কোনরূপ প্রভাব ফেলিবেনা। অতএব, যথাযথ পর্যালোচনা সাপেক্ষে আমি স্বপ্রণোদিত হইয়া এই সম্মতিপত্রে স্বাক্ষর করিতেছি।

অংশগ্রহণকারীর স্বাক্ষর / বাম বৃদ্ধাঙ্গুলির ছাপ

সাক্ষীর স্বাক্ষর / বাম বৃদ্ধাঙ্গুলির ছাপ গবেষকের স্বাক্ষর ও তারিখ