Assessment of Metformin Induced Vitamin-B12 Deficiency amongst Type-II Diabetic Patients Vis-À-Vis Evaluating the Quality of Life

Sashidhar Reddy¹, Aditya Sai Rama Krishna K², Madaboina Suhas³, T. Sneha Rao⁴

ABSTRACT

Introduction: Metformin is the cornerstone medication in the management of Type 2 diabetes mellitus with estimates that it is prescribed to 120 million patients with diabetes around the world. Several studies reported that the long-term use of metformin lowers vitamin-B12 levels and its clinical manifestation can be misdiagnosed as diabetic peripheral neuropathy. Thereby, the present study was undertaken to assess the vitamin-B12 deficiency in type II Diabetic patients with long-term use of metformin, an association between vitamin B12 and peripheral neuropathy and evaluating the quality of life in (T2DM) patients.

Material and Methods: A prospective observational case-control study was conducted at the outpatient and inpatient departments of RVM Hospital. Total 34 (controls) healthy volunteers and 34 (cases) Type 2 diabetes patients using metformin for more than six months were recruited for the study. Data was collected using designed profile form, structured questionnaires and venous blood samples were collected in K2 EDTA vacutainers from the patients and centrifuged at 1500 rpm for 10 minutes, settled plasma was transferred into cryovials, estimating the parameters such as Vitamin-B12, HbA1c and CBP. The statistical analysis was done using SPSS (v25) software.

Results: The prevalence of serum vitamin B12 deficiency and borderline deficiency in T2DM patients is 20.5%, the mean HbA1C levels in our study T2DM subjects is < 9.07% which is showing either poor control of diabetes and lack of compliance with metformin medication. 83% of T2DM patients with 1-10 years of metformin use resulted in Vitamin-B12 deficiency.

Conclusion: Vitamin-B12 deficiency and Borderline deficiency prevalence in Type II Diabetes patients was found to be slightly high in our study population when compared to control subjects. The reliability test was done to evaluate their quality of life which concludes that the quality of life was poor amongst the study participants. Considering the regular screening of vitamin-B12 as a cost factor, the peripheral smear can be done in T2DM patients on long-term metformin to detect macrocytic anaemia followed by biochemical analysis periodically.

Keywords: Vitamin-B12, Metformin, T2DM, HbA1c and Peripheral Neuropathy

INTRODUCTION

Metformin, is the most commonly used oral hypoglycaemic agent, is the preferred drug among type 2 diabetes patients, mainly those with overweight and have normal kidney function. Numerous guidelines propose that it should be preferred drug with concurrent lifestyle modifications while initiating the therapy for type-2 DM patients. Metformin is the most widely used anti-hyperglycemic drug which is used as first-line treatment for patients with type 2 diabetes mellitus.

Vitamin B12 is a vital nutrient for health and plays a crucial role in the functioning of the brain, nervous system, and in the formation of red blood cells. In addition to anaemia, vitamin B12 deficiency may increase the severity of peripheral neuropathy in patients with T2DM. Furthermore, because vitamin B12 participates in the most critical pathway of homocysteine (Hcy) metabolism, a reduction in vitamin B12 would increase plasma concentrations of Hcy, which is strongly linked to cardiovascular disease in patients with T2DM and PCOS. (2) The various causes of vitamin B12 deficiency include nutritional deficiency, gastric mucosal damage, pernicious anaemia, drugs like metformin and Proton Pump Inhibitors (PPIs).

Hence various mechanisms have been proposed to explain vitamin B12 deficiency observed amongst Type-2 DM patients taking metformin therapy which are: changes in small intestinal motility which stimulates bacterial overgrowth and consumption of B12 by bacteria, changes in Intrinsic Factor (IF) levels which could adversely affect vitamin B12 absorption. Metformin may also inhibit the calcium-dependent absorption of vitamin B12 and intrinsic factor complex at the terminal ileum, this inhibitory effect can be reversed with calcium supplementation. Increase in metformin dose by 1g /day increases risk deficiency by greater than two-fold.

Various studies have reported that an average of 10-30% deficiency of patients taking metformin for longer duration and at higher dosage have shown vitamin B12 deficiency. Vitamin B12 level should be done in patients with type 2 diabetes, especially those taking metformin therapy for a longer duration. Clinical symptoms in vitamin B12 deficiency include alteration in mental status, megaloblastic

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anaemia and neurological damage. In the similar context, diabetic neuropathy can also present with symptoms such as paresthesias, numbness and tingling in hands and feet; these symptoms could give rise to confusion between diagnosis of vitamin B12 deficiency and diabetic peripheral neuropathy. Hence in early stages progression of neurological damage could be managed by early detection of vitamin B12 and this vitamin B12 deficiency may lead to permanent neurological damage if it is misdiagnosed as diabetic neuropathy.2

In an Indian study conducted among 441 healthy middle-aged men, the vitamin B12 deficiency was observed among 67% of the study participants.3 Vegetarian concentrations <150 pmol/L where diet was a significant factor associated with low vitamin B12 levels in this study on multivariate analysis. In another cross-sectional study among 175 healthy elderly Indian subjects aged >60 years, vitamin B12 deficiency was observed among 16% of the study participants. Evidence from early clinical observation, however, indicated a prevalence of 30% for vitamin B12 malabsorption among patients undergoing long-term metformin treatment.4 Also, low vitamin B12 levels have been reported to be associated with poor nerve conduction velocities and poorer responses to light touch by monofilament detection. The reality is that Type II diabetes influences patients’ lives, where the mere presence of diabetes deteriorates a person’s quality of life (QoL).5,6 World Health Organization (WHO) introduced the first definition of health as “A state of complete physical, mental, and social well-being not merely the absence of disease”. WHO, furthermore, introduced QoL as an estimation of well-being as well as a measurement of health and the effects of health care.7 Thereby quality of life is a significant health outcome in its own right, representing the ultimate goal of all health interventions. Persons with diabetes have a lower quality of life than persons without chronic illness, but their quality of life is better than in patients with other serious chronic diseases. Duration and type of diabetes are not consistently associated with quality of life; on the other side better, glycemic control is correlated with better quality of life. As they deal with their condition on a daily basis, they have to make countless decisions in an often to approach the nondiabetic metabolic state. Insulin therapy in diabetes can substantially affect the quality of life positively by reducing symptoms of high blood sugar, or negatively by increasing symptoms of low blood glucose.8

The aim of the study was to investigate the risk of long-term use of Metformin induces Vitamin-B12 deficiency amongst Type II diabetic patients and evaluate their quality of life. The Objectives stand to determine the levels of Vitamin B12 in diabetic patients concerning control population, to Study the association between vitamin B12 and peripheral neuropathy and lastly assess their quality of life by using WHO-BREF scale.

MATERIAL AND METHODS

An aggregate of 34 patients with type 2 diabetes mellitus (T2DM) and 34 healthy volunteers between the age gathering of 30 to 70 years were enrolled in the planned case-control study. All the T2DM patients were on metformin and have been on the medication for at least a half year.

Inclusion criteria

Cases: Type-II Diabetic Patients > 35 years age and use of Metformin > 6 months. Controls: Non-diabetics (age and sex matched).

Exclusion criteria

Patients with Pernicious Anemia, newly Diagnosed T2DM, Pregnant Women, Type-I Diabetes, Vegetarians, IBD, Gastrectomy, Colectomy and Hypothyroidism, were excluded for this study. Also, patients with recent intake of oral or intramuscular vitamin- B12 medication were excluded.

Informed Consent: All the participants were enrolled in this study after obtaining informed consent from each of the participants.

Sample collection and storage: After overnight fasting of about 6 – 8 hours, 2 ml of venous blood was collected from each subject enrolled into the study, and the separated serum was stored in cryo-vial tubes. The samples were centrifuged at 1500 rpm for 10 minutes to obtain serum samples which were kept at -80 C until analyzed for the vitamin-B12 level. Determination of serum vitamin B12 AND HbA1c levels: Serum vitamin B12 levels of the patients were determined using CENTOR-XP instrument by chemiluminescence method, and HbA1c levels were estimated using standard HPLC.

STATISTICAL ANALYSIS

The serum B12 levels were compared with metformin years of use by Chi-square cross tabulation. Also, the NTSS-6 scores were compared with vitamin B12 levels among all metformin-treated T2DM patients using a Scatter plot. The Reliability test using Cronbach's alpha equation was done to evaluate the quality of life. Data with Gaussian distribution were presented as mean ± standard deviation. All the statistical analysis was performed using SPSS (version 25.0) and Microsoft Excel.

In this study we have used values of

- <200 pg/ml for definite vitamin B12 deficiency (147.6pmol/L)
- 200 to 300 pg/ml for borderline vitamin B12 deficiency. (147.6 - 221.4pmol/L)
- >300 pg/ml for normal vitamin B12 levels (221.4pmol/L)

RESULTS

The Clinical features of the study population are shown in Table 1. The prevalence of serum vitamin B12 deficiency in T2DM patients is 20.5% when compared to age-matched controls with 8.82%. As shown in table 1 the mean HbA1C levels in our study T2DM subjects is < 9.07% which is reflecting either poor control of diabetes despite high doses of metformin or lack of compliance. As depicted in table 2, 83% of T2DM patients with 1-10 years of metformin use resulted with Vitamin-B12 deficiency. As shown in Fig.1 (7/34) fall under Vitamin- B12...
Figure-1: Scatter plot for NTSS-6 scores vs. vitamin B12 levels among all metformin-treated T2DM patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Of Participants</td>
<td>68</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Age</td>
<td>46.10 ±14.6</td>
<td>53.38 ± 12.11</td>
<td>38.61±13.22</td>
</tr>
<tr>
<td>Sex (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>32 (47.1)</td>
<td>16 (47.1)</td>
<td>16 (47.1)</td>
</tr>
<tr>
<td>Male</td>
<td>36 (52.9)</td>
<td>18 (52.9)</td>
<td>18 (52.9)</td>
</tr>
<tr>
<td>HbA1c%</td>
<td>9.07 ±2.67</td>
<td>9.07 ±2.67</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin-B12 level, pg/ml</td>
<td>349 ± 156.3</td>
<td>350.1±175.2</td>
<td>349.6±137.49</td>
</tr>
<tr>
<td>Vitamin-B12 deficiency (&lt;200 pg/ml), in (%)</td>
<td>13(19.1)</td>
<td>7 (20.5%)</td>
<td>3 (8.82%)</td>
</tr>
</tbody>
</table>

Table-1: Clinical Characteristics of the study population

<table>
<thead>
<tr>
<th>Metformin years of use</th>
<th>&lt; 200 pg/ml</th>
<th>200 – 300 pg/ml</th>
<th>300 – 400 pg/ml</th>
<th>400 – 500 pg/ml</th>
<th>&gt;500 pg/ml</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;6 months</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>1 – 10 years</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>3</td>
<td>4</td>
<td>24</td>
</tr>
<tr>
<td>11 – 20 years</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>21 – 30 years</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table-2: Metformin years of use and Vitamin-b12 levels Cross tabulation in cases

Table-3: Reliability Test Statistics of WHOQOL-BREF scale in the study population

<table>
<thead>
<tr>
<th>Domain</th>
<th>Cronbach's Alpha Equation</th>
<th>No. of Items</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical health</td>
<td>.750</td>
<td>7</td>
</tr>
<tr>
<td>Psychological</td>
<td>.633</td>
<td>6</td>
</tr>
<tr>
<td>Social relationships</td>
<td>.437</td>
<td>3</td>
</tr>
<tr>
<td>Environment</td>
<td>.797</td>
<td>8</td>
</tr>
</tbody>
</table>

DISSCUSSION

In the present study, the prevalence of vitamin-B12 deficiency in T2DM patients receiving metformin is 20.5% when compared to the age-matched non-diabetic study controls is 8.82%. De Jager et al.\textsuperscript{9} study included 196 T2DM patients, resulted with 9.9% prevalence of vitamin-B12 deficiency with 4.3 mean metformin years of use. According to Reinstatler et al.\textsuperscript{10}, the study included 575 metformin-treated patients which led to a 5.8% prevalence with 5 mean metformin years of use. Hermann et al.\textsuperscript{11} study resulted with 8% prevalence with an average of 5.2 years of metformin use. Beulens et al.\textsuperscript{12} study recruited 550 study subjects which resulted in 28.1% prevalence by consuming metformin for 5.3 years of an average, while a holotranscobalamin deficiency occurred in 3.9% of the patients. A 1 mg/day increase in daily metformin dose was associated with 0.042 decrease in cobalamin concentrations. Sun-Hye ko Et. al\textsuperscript{13} study has shown that the prevalence of vitamin B12 deficiency in metformin-treated type 2 diabetes patients was 9.5% (n = 76), and the mean vitamin B12 level was 662.5 ±
246.7 pg/mL. Vitamin B12 deficient patients had a longer duration of metformin use and higher daily metformin dose than non-deficient patients. KW Liu et al.\textsuperscript{14} study has shown the metformin group had a significantly higher prevalence of certain deficiency (29% vs 5%, \textit{p}=0.001) and possible deficiency (52% vs.27%, \textit{p}<0.03). Odds ratios of definite and possible deficiency in the metformin group were 7.40 and 2.92, respectively. A cross-sectional study by Pflipsen et al.\textsuperscript{15} involving 203 outpatient T2DM patients at a large military primary care clinic in the USA documented a prevalence of definite vitamin B12 deficiency of 22%. Similar studies done in Europe have reported a prevalence of about 27%.\textsuperscript{16,17} Akabwai et al.\textsuperscript{18}, Study has shown that the prevalence of vitamin B12 deficiency was 10.7%. Hemoglobin level < 12 g/dl and glycaemic hemoglobin ≥ 7% were associated with vitamin B12 deficiency. Adaikalakoteswari Et. al\textsuperscript{19} Study has shown that the prevalence rates of vitamin B12 deficiency (<191 ng/L) were 27% and 12% in Europeans and Indians, respectively and higher in metformin-treated type 2 diabetes patients. K. S Akindale, Et. al\textsuperscript{20} study has shown Vitamin B12 level was significantly lower in patients who have been on metformin for >10 years compared with patients with <10 years history of metformin use. Similarly, patients who were on metformin at a dose of >1000 mg/day had significantly lower vitamin B12 level when compared with patients on <1000 mg/day.

De Groot-Kampfuis et al.\textsuperscript{21} has conducted a study at Secondary care out patient diabetes clinic, The Netherlands on 164 metformin-treated patients with the mean age of 62.6 years whose duration of metformin use was 4.9 years with no exclusion of renal impaired patients was found the prevalence rate of 14.1%.

Marwan A. Ahmed et al.\textsuperscript{22} has conducted a study at Outpatient diabetes clinics of 2 tertiary hospitals, South Africa on 121 metformin-treated patients with the mean age of 58.5 years whose duration of metformin use was 9.6 years with an exclusion of renal impaired patients was found the prevalence rate of 28.1%. Hence effective screening of vitamin-B12 status is required in long term metformin therapy.\textsuperscript{23,24}

Our study showed a clear relationship between the dosage and length of metformin use and vitamin B12 deficiency vs NTSS scores and QOL in T2DM patients. The present study has been conducted at Out-patient department and In-patient wards of RVM Hospital, India on 34 metformin-treated patients with the mean age of 53.3 years whose duration of metformin use was 2.83 years with an exclusion of renal impaired patients was found the prevalence rate of 20.5%.

**Limitations**

The study has only measured serum vitamin B12 levels to assess the vitamin status and did not measure homocysteine levels, and the absence of data regarding compliance to metformin is also a limitation in our study. Compliance can have an impact on both the response to metformin and the levels of vitamin B12. A much larger study sample is needed to conclusively answer the secondary aim of investigating the relationship between metformin years of use and vitamin B12 deficiency and to show a clinically significant difference as small as 10% to be statistically significant.

**CONCLUSION**

Vitamin-B12 deficiency and Borderline deficiency prevalence in T2DM patients was found to be high in our study population when compared to control subjects. The reliability test was done to evaluate their quality of life which concludes that the quality of life was poor amongst the study participants. Considering the regular screening of vitamin-B12 as a cost factor, the peripheral smear can be done in T2DM patients on long-term metformin to detect macrocytic anaemia followed by biochemical analysis periodically.

**REFERENCES**

2. Jeetendra S, Tushar B. Metformin Use and Vitamin B\textsubscript{12} Deficiency in Patients with Type-2 Diabetes Mellitus. 2016;3:67–70.


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