VITAMIN B_{12} LEVELS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS ON METFORMIN

K.S. Akinlade[1], S.O. Agbebaku[1], S.K. Rahamon[1], and W.O. Balogun[2]

1. Department of Chemical Pathology, University of Ibadan/University College Hospital, Ibadan, Nigeria
2. Department of Medicine, University of Ibadan/University College Hospital, Ibadan, Nigeria

Correspondence:
Dr. K.S. Akinlade
Endocrinology/Metabolic Research Unit,
Department of Chemical Pathology,
University of Ibadan/
University College Hospital,
PMB 5116, Ibadan,
Nigeria.
Phone: +2348075010718
e-mail: ksakinlade@yahoo.co.uk

ABSTRACT

Background: **Due to the clinical benefits of metformin, its associated side effects such as vitamin B_{12} deficiency are usually overlooked and rarely investigated.**

Objective: **This study was carried out to determine the serum level of vitamin B_{12} in Nigerian patients with type 2 diabetes mellitus (T2DM) on metformin.**

Methods: Serum vitamin B_{12} level was determined using high performance liquid chromatography (HPLC) in 81 T2DM patients who have been on metformin for 5 years or more. Vitamin B_{12} deficiency was defined as serum concentration of <200 pg/dl, borderline deficiency as 200 – 300 pg/dl and >300 pg/dl as normal. Differences in vitamin B_{12} levels between different groups were assessed using Mann Whitney *U* test and $P<0.05$ was considered as statistically significant.

Results: **Vitamin B_{12} deficiency and borderline deficiency were recorded in 8.6% and 26.0% of the patients respectively. Vitamin B_{12} 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 B_{12} level when compared with patients on ≤1000 mg/day.**

Conclusion: **Low serum vitamin B_{12} level is associated with longer duration and higher dose of metformin use. Therefore, routine determination of vitamin B_{12} level in patients with T2DM on high dose of metformin and those with prolonged use of metformin might help in identifying patients that would benefit from vitamin B_{12} supplements.**

Keywords: Metformin, Type 2 diabetes mellitus, Vitamin B_{12} deficiency.

INTRODUCTION

Metformin is the most prescribed anti-diabetic drug in patients with type 2 diabetes mellitus (T2DM) and hence, considered a cornerstone in the treatment of T2DM.[1] It is an anti-hyperglycaemic agent that is usually well tolerated in most of the patients (except for mild gastrointestinal side effects) and it is characterized by excellent improvement in the cardiovascular morbidity and mortality associated with T2DM.[2]

Due to the numerous clinical benefits associated with metformin, some side effects with potential adverse health effects associated with its use are usually ignored and rarely investigated. One of such side effects is vitamin B_{12} deficiency.[3, 4]

Vitamin B_{12}, also called cobalamin, is a water-soluble vitamin involved in the optimal functioning of the hemopoetic, neuro-cognitive and vascular systems. It is involved in DNA synthesis, fatty acid metabolism and energy production.[5] Vitamin B_{12} exerts its physiological effects by facilitating the methylation of homocysteine to methionine which is later activated into S-adenosyl methionine that donates its methyl group to methyl acceptors.[6]. Similarly, vitamin B_{12} mediates the conversion of methyl malonyl coenzyme A (coA) to succinyl coA, a process when hindered, results in accumulation of serum methylmalonic acid (MMA) thereby causing defective fatty acid synthesis of the neuronal membranes.[7]

Reports have shown that there is an association between metformin use and vitamin B_{12} deficiency.[3, 4] However, the mechanism through which metformin induces vitamin B_{12} deficiency (VBD) in patients with T2DM is presently unclear. Some of the suggested mechanisms include alteration in small bowel motility, which stimulates bacterial overgrowth and consequential vitamin B_{12} deficiency. Others include

competitive inhibition or inactivation of vitamin B_{12} absorption, alteration in intrinsic factor levels and interaction with the cubulin endocytic receptor. Also, inhibition of the calcium dependent absorption of vitamin B_{12}–intrinsic factor (IF) complex at the terminal ileum has been suggested as one of the mechanisms.[8,9,10]

Although decrease in vitamin B_{12} levels following metformin use typically starts as early as the 4th month,[11] clinical features of vitamin B_{12} deficiency become apparent by 5 years owing to the large body stores in the liver that are not quickly depleted.[10] This is however influenced by increasing age and dose of metformin.[12, 13]

Assessment of vitamin B_{12} deficiency in patients with T2DM is of clinical importance. It can present as peripheral neuropathy and may be mistaken for diabetic neuropathy in patients on metformin treatment.[14] Also, low vitamin B_{12} levels have been reported to be associated with poor nerve conduction velocities and poorer responses to light touch by monofilament detection.[15] This may lead to unnecessary use of anticonvulsants, tricyclic antidepressants and other medications for diabetic neuropathy.[14, 16, 17]

Usually, the established clinical benefits of metformin use in patients with type 2 diabetes mellitus (T2DM) make its side effects to be overlooked and rarely investigated especially, in Nigeria. This study was therefore, carried out to evaluate the serum levels of vitamin B_{12} in patients with T2DM on metformin.

MATERIALS AND METHODS

Subjects

A total of 81 patients with type 2 diabetes mellitus (T2DM) within the age range of 45 to 80 years were recruited into this cross-sectional study using a convenient sampling method. The patients were those attending the Endocrinology Clinic of the Department of Medicine and the Metabolic Research Unit of the Department of Chemical Pathology, University College Hospital, Ibadan, Nigeria. All the patients were on metformin and have been on the drug for a minimum of 5 years.

Exclusion criteria

Patients with gastrectomy, small bowel resection, liver disease, chronic kidney disease and thyroid disease were excluded from this study. Also, patients with recent intake of oral or intramuscular vitamin B_{12} supplement, patients on histamine 2 receptor blocker and vegetarians were also excluded.

Informed Consent and Ethical Approval

Participants were enrolled into this study after obtaining a written informed consent from each of them. Also, an ethical approval (UI/EC/14/0119) was obtained from the University of Ibadan/University College Hospital Joint Ethical Committee.

Blood pressure measurement and body mass index calculation

After at least 10 minutes of rest, BP was obtained with the patient in supine position using a Mercury Sphygmomanometer. Height (m) was taken using a Stadiometer. Body mass index (BMI) was calculated as the ratio of body weight (kg) to the square of height (m^2).

Sample collection and storage

After an overnight fast of about 8-10 hours, 5 ml of venous blood was collected from each participant and dispensed into plain bottles. The samples were allowed to retract and then spun at 4000 rpm for 10 minutes to obtain serum samples which were kept at -200C until analysed for vitamin B_{12} level.

Determination of serum vitamin B_{12} level

Serum vitamin B_{12} levels of the patients were determined using high performance liquid chromatography (HPLC). Vitamin B_{12} deficiency was defined as serum concentration of <200 pg/dl and borderline deficiency as 200–300 pg/dl.[18] Concentrations >300 pg/dl were considered as normal.

Statistical analysis

The distribution of vitamin B_{12} levels and the characteristics of the patients were assessed using histogram with normal curve. Thereafter, Student's t-tests and Mann-Whitney *U* were used to determine differences in means or medians of the variables as appropriate. Data with Gaussian distribution are presented as mean ± standard deviation while data with non-Gaussian distribution are presented as median (interquartile range). *P*-values less than 0.05 were considered to be statistically significant. All analyses were performed using SPSS (version 20.0).

RESULTS

Vitamin B_{12} deficiency and borderline deficiency were respectively observed in 8.6% and 26.0% of the patients. About 65.4% of the patients were not deficient of vitamin B_{12}.

Based on duration of metformin use, all the patients were divided into 2 groups: <10 years and ≥10 years. As shown in Table 1, the median vitamin B_{12} level was significantly lower in participants who have used

Table 1: Characteristics of the patients and serum levels of vitamin B_{12} based on duration of metformin use

	<10years (n = 50)	≥10years (n = 31)	*P* - value
Age (years)	60.76 ± 8.26	63.90 ± 7.94	0.095
Height (m)	1.62 ± 0.09	1.61 ± 0.08	0.851
Body weight (kg)	67.74 ± 11.47	69.10 ± 10.82	0.599
Systolic BP (mmHg)	126.00 ± 16.78	127.10 ± 16.77	0.776
Diastolic BP (mmHg)	76.24 ± 10.02	74.52 ± 11.21	0.474
BMI (kg/m^2)	25.89 ± 4.00	26.45 ± 3.10	0.512
Vitamin B_{12} (pg/dl)	429.48 (304.17 – 510.42)	299.63 (261.12 – 373.05)	0.004*

Table 2: Characteristics of the patients and serum levels of vitamin B_{12} based on metformin dose

	≤1000mg/day (n = 56)	>1000mg/day (n = 25)	*P* - value
Age (years)	61.96 ± 8.06	61.96 ± 8.78	0.998
Height (m)	1.61 ± 0.09	1.62 ± 0.07	0.607
Body weight (kg)	65.84 ± 9.48	73.68 ± 12.89	0.003*
Systolic BP (mmHg)	127.86 ± 16.15	123.20 ± 17.73	0.248
Diastolic BP (mmHg)	75.04 ± 9.67	76.80 ± 12.15	0.486
BMI (kg/m^2)	25.30 ± 3.01	27.91 ± 4.39	0.002*
Vitamin B_{12} (pg/dl)	417.29 (295.94 – 505.49)	306.98 (244.22 – 389.36)	0.004*

metformin for ≥10 years compared with patients who have used metformin for <10 years.

Similarly, patients were divided into 2 groups based on metformin dose. It was observed that the median level of vitamin B_{12} was significantly lower in patients who were on metformin at a dose of >1000 mg/day compared with patients who were on metformin at a dose of ≤1000 mg/day. In contrast, the mean body weight and BMI were significantly higher in patients who were on metformin at a dose of >1000 mg/day compared with patients who were on metformin at a dose of ≤1000 mg/day (Table 2).

DISCUSSION

Reports have shown that metformin use have a significant impact on the concentration of vitamin B_{12} in patients with T2DM.[19,20,21] The percentages of patients with vitamin B_{12} deficiency and with borderline deficiency observed in this study are in line with the report of Nervo *et al.*[22] This observation could be an indication of nutritional deficiency or a consequence of metformin use.

The observed lower level of vitamin B_{12} in patients with T2DM who have been on metformin for 10 years or more compared with patients with <10 years history of metformin use corroborates earlier studies.[12,21,23,24] De jager *et al.*[13] showed that the negative impact of metformin use on vitamin B_{12} level becomes profound with increasing years of metformin use. This observation has been attributed to either or a combination of alteration in small bowel motility (which stimulates bacterial overgrowth with consequential vitamin B_{12} deficiency), alteration in intrinsic factor levels, interaction with the cubulin endocytic receptor and inhibition of the calcium dependent absorption of vitamin B_{12}-intrinsic factor complex at the terminal ileum.[8,10,11] Also, our observation could indicate depletion in the liver vitamin B_{12} store, secondary to increased duration of metformin use.

It has been shown that each 1g daily intake of metformin caused a ratio of 2.88 increase in the risk of developing vitamin B_{12} deficiency.[12] This possibly explains our observed lower level of vitamin B_{12} level in patients taking more than 1000mg of metformin per day compared with patients on 1000mg or less dose of metformin per day. Several other studies have also reported similar findings.[13,25,26] Although the mechanism through which high dose of metformin causes vitamin B_{12} deficiency is presently not well understood, our observation probably indicates that there is heightened inhibition of vitamin B_{12} absorption which could cause rapid depletion of the liver store of vitamin B_{12} in patients taking high dose of metformin.

An association between obesity and poor glycaemic control has been reported.[27] Nagrebetsky *et al.*[28] showed that there is a significant association between lower BMI and lower glycated haemoglobin (HbA_{1c}) concentration, an index of glycaemic control. These reports could explain the observed higher BMI in patients on high dose (>1000 mg/day) of metformin compared with patients on low dose. This observation

is not surprising as the relationship between obesity and insulin resistance is well established.

Small sample size and non-inclusion of age-matched control group are some of the limitations of this study.

CONCLUSION

It could be concluded from this study that low serum vitamin B_{12} level is associated with longer duration and higher dose of metformin use. Therefore, routine determination of vitamin B_{12} level in patients with type 2 diabetes mellitus on high dose of metformin and those with prolonged use of metformin might help in identifying patients that would benefit from vitamin B_{12} supplements.

Author Contributions

KSA and SKR conceptualized and designed the study, SOA and WOB recruited the patients and collected the blood samples, SOA did the laboratory assay, SOA and SKR did the statistical analysis, KSA, SKR, SOA and WOB prepared the manuscript, KSA and SKR edited and reviewed the manuscript, KSA supervised the entire process.

Conflict of Interest

The authors declare no conflict of interest.

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