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Original article

Vitamin B12 Deficiency in Type 2 Diabetes Patients Using Metformin

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ABSTRACT

Background and aims*. Metformin is the first line of medical therapy for type 2 diabetes, the mechanism of metformin remains as yet incompletely understood but recent studies have reported a decrease in vitamin B12 in patients treated with metformin. This study was aimed to evaluate vitamin B12 status in type 2 diabetic patients (T2DM) treated with metformin compared to a control group, and also to evaluate the correlation between the vitamin status and the dose, the frequency of taking metformin, as well as with the age.* ***Methods****. This was a cross-sectional study conducted in T2DM patients, 150 patients on metformin and 150 patients without metformin (control group). Vitamin B12 analysed using fully automated modular analyser Roche COBAS e411 Immunoassay system, and Snibe MAGLUMI 2000.* ***Results****. The average duration of taking metformin is 10.05 +/- 6.58 years and the average dose is 691.30 +/- 198.41 mg/day. The serum level of vitamin B12 (Cobalamin) is significantly lower in patients taking metformin (216.6 pg/ml versus 555.1 pg./ml, p=0.001). About 31 of 150 diabetic patients on metformin (20.67%) presented a vitamin B12 deficiency (level < 200 pg./ml) versus a 7 without metformin (4.67%). Cobalamin deficiency (rate between 200 and 300 pg/ml) was noted in 50 patients (33.33%) of patients on metformin versus 16 (10.67%) without metformin. The current study reported a relationship between metformin and vitamin B12 deficiency in type 2 diabetic patients.* ***Conclusion****. Metformin impacted vitamin B12 levels and our recommendations screening test and treatment modalities should be established due to the widespread use of metformin in T2D patients.*

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INTRODUCTION

Metformin is part of the biguanide family, a group of drugs that come from guanidine, a derivative of Galega offininalis, a plant that has been used since medieval times to treat diabetes [1]. In the forties of the last century, metformin unintentionally obtained recognition for its ability to lower blood glucose—an observation noted while used to treat influenza [2]. Metformin was introduced in the late 1950s and was prescribed in large quantities as the treatment of choice for diabetes mellitus [3].

After over 60 years of its introduction as diabetes treatment, metformin has become the most frequently prescribed treatment for type 2 diabetes mellitus (T2DM) management in Europe and is part of the primary treatment scheme [4]. The mechanism of action of metformin has not yet been fully elucidated. The hypoglycaemic effect is probably mainly caused by the inhibition of hepatic gluconeogenesis [5]. At the cellular level, a blockade of the mitochondrial complex 1 of the respiratory chain presumably leads to an increase in the adenosine monophosphate/adenosine triphosphate (AMP/ATP) ratio and thus to indirect activation of the AMP-activated protein kinase (AMPK) [6,7]. However, the

signaling pathway involved in this activation, particularly at the hepatic level, remains controversial. Indeed, even though metformin has been shown to inhibit complex 1 of the mitochondrial respiratory chain, initial work concluded that it activated AMPK without modifying the cellular energy state [8].
Moreover, in 1998 the UK Diabetes Prospective Study Group demonstrated the anti-atherogenic effects of this drug [9], later it was found significantly reduce many components of the syndrome of insulin such as polycystic ovary syndrome, and obesity [10], all these effects occur due to a complex mechanism of action that until today is not fully known.
On the other hand, there are side effects associated with metformin and most of them are mild, these include diarrhoea, nausea, and gastrointestinal symptoms such as abdominal distress [11,12]. The side effects appearance is associated with the frequency and dose of metformin. Literature has revealed a correlation between metformin and a decrease in vitamin B12 absorption in the gastrointestinal tract, and such findings have been continuously published.
A systematic review on individuals with T2DM done by Chapman et al, in 2016 revealed that 10 out of 17 observational studies found that individuals that use metformin had significantly lower levels of vitamin B12 than non-metformin users. Another study of meta-analysis in this same review has shown that after 3 to 6 months of use, metformin shows a decrease in vitamin B12 levels [13].
In the early 1970s, the association between metformin and vitamin B12 deficiency has been reported in various studies [14]. According to Bauman et. al, the uptake of B12 intrinsic factor complex by ileal cell surface receptors is known to be a process dependent on calcium availability. Metformin affects calcium-dependent membrane action [15]. Moreover, several studies have revealed that the long-term use of metformin is linked with the malabsorption of vitamin B12 (cobalamin [Cbl]) along with a decrease in the vitamin B12 serum concentration from 30% to 14% [16].
Vitamin B12 is a cofactor for the enzyme methionine synthase necessary for the conversion of homocysteine to methionine, which explains the increase in serum homocysteine concentrations in vitamin B12 deficiency [17,18]. Similarly, vitamin B12 is a cofactor of methylmalonyl-CoA mutase which converts methylmalonic acid-CoA into succinyl-CoA, inducing, in the event of vitamin B12 deficiency, an accumulation of methylmalonic acid which can be measured in serum or urine [17].
The two usually recognized diagnostic criteria for vitamin B12 deficiency are, a low serum concentration of vitamin B12 (cobalamin) associated either with clinical and/or haematological signs of vitamin B12 deficiency either to an elevated serum concentration of homocysteine or methylmalonic acid [19].
Metformin has a significant impact on vitamin B12 levels in patients with T2DM. Therefore, the objective of this study was to investigate the effects of long-term therapy with metformin on vitamin B12 for type 2 diabetes patients (T2DM) and evaluate the correlation between the vitamin status and the dose, the frequency of taking Metformin, as well as with the age.

METHODS

Study setting

In this study, 150 patients with Type 2 diabetes mellitus (T2DM) on a metformin for at least 6 months were selected as a positive case and 150 non-diabetics on a metformin were selected as a control cases. This study was based in outpatient clinic at Diabetes and Endocrinology University Hospital Tripoli, Abu Miliana Clinic, and Mitiga Military Hospital (MMH), Tripoli, Libya in a period of time between June and August 2022.

Data collection

Patients' data were collected using recording patients file and pre-designed questionnaire. Data included, age, gender, body mass index BMI, smoking habits, diabetes diagnosis timeline, duration on metformin and dose of metformin daily. Haemoglobin (Hb) and glycosylated haemoglobin (HbA1c) testes result were included when available.
Venous blood samples were collected from the three centres using dedicated evacuated tubes, and serum vitamin B12 levels were assessed in a private laboratory at Al-Andalus Centre. Vitamin B12 analysed using fully automated modular analyser Roche COBAS e411 Immunoassay system, and fully-auto Chemiluminescence Immunoassay (CLIA) and Snibe MAGLUMI 2000. A three months' prior results (vitamin B12 level) were collected from recording patients file and used in this study.
COBAS e411 is a system analyser, random access, software-controlled system for immunoassay analysis. The e411 vitamin B12 assay employs a competitive test principle using intrinsic factor specific for vitamin B12. Vitamin B12 sample competing with added vitamin B12 that labeled with biotin for the binding sites on the ruthenium-labeled intrinsic factor complex [20].
Serum vitamin B12 deficiency was considered <200pg/ml, borderline deficiency was considered between 200 and 300 pg/ml, and normal value was considered above 300pg/ml. Blood hemoglobin level was used to determine the anemia <13g/dl in males, and <12g/dl in females [21].

Statistical analysis

The statistical analyses were performed using the Microsoft Excel. In this study two-tailed t-test were used to compare between two groups and the data expressed as mean and standard deviation and the P. values of 0.05 were considered statistically significant.

RESULTS

This study targeted 150 T2D patients (98 female /52 male) and 150 control (78 female/72 male) in their average age about 56.24 ± 12.72 years old, with extremes ranging from 20 to 84 years old, and 66% were females. The patients average weight was 77.79 ± 11.35 Kg (minimum 50 Kg and the maximum 103 Kg). The average duration of receiving metformin for 40 patients out of 150 patients was 10.05 +/- 6.58 years and the average dose was 691.30 +/- 198.41mg/day.

Serum level of vitamin B12 (Cobalamin) was significantly lower in patients receiving metformin (216.6 pg/ml versus 555.1 pg./ml, p=0.001), figure (3.1). About 31 of 150 diabetic patients on metformin (20.67%) presented a vitamin B12 deficiency (level < 200 pg./ml) versus a 7 not on metformin (4.67%). Cobalamin deficiency (rate between 200 and 300 pg/ml) was noted in 50 patients (33.33%) of patients on metformin versus 16 (10.67%) without metformin, table (3.1). The majority of vitamin B12 deficiency were found in metformin group aged 21-30 years' category represents 9 (25.71%) of participants, while the majority of low-normal was found in group aged 31-40 years' category represents 20 (44.44%) of participants. A significant negative association was noted between the age and serum vitamin B12., table (3.2a&b).

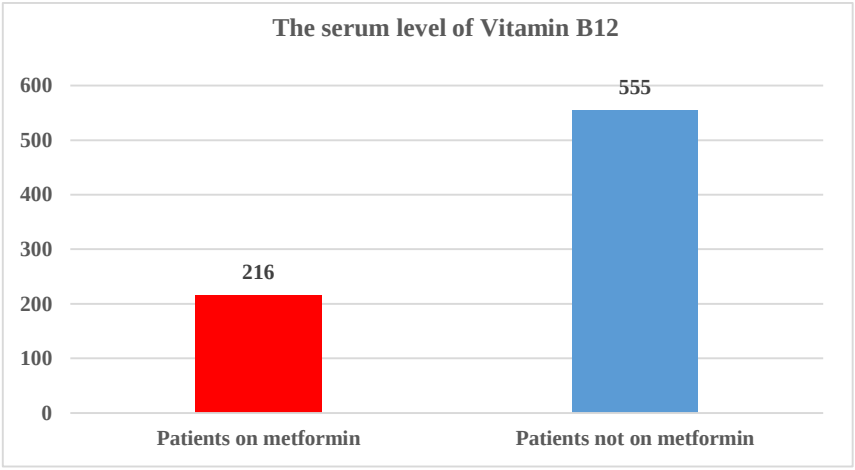

Figure 1. The average level of vitamin B12 deficient on metformin and not on metformin cases. The red bar represents patients on metformin, whereas blue bar represents patients not on metformin (control group).

Table 1. Association of vitamin B12 deficiency and metformin

Vitamin B12 Levels	Deficient (<200 pg/ml)	low-normal (200-300pg/ml)	Normal (>300 pg./ml)	Total
Patients on metformin	31 (20.67%)	50 (33.33%)	69 (46%)	150(50%)
Patients not on metformin	7 (4.67%)	16 (10.67%)	127 (84.66%)	150 (50%)
Patients (total)	38 (12.66%)	66 (22%)	196 (65.34%)	300 (100%)

Table 2. Age and percentage of vitamin B12 level of patient on metformin.

Patients on metformin/ ages	Deficient Level	low-normal Level	Normal Level	Total
10-20	0 (0%)	0 (0%)	1 (100%)	1
21-30	9 (25.71%)	16 (45.71%)	10 (28.58%)	35
31-40	8 (17.78%)	20 (44.44%)	17 (37.78%)	45
41-50	4 (18.18%)	5 (22.73%)	13 (59.09%)	22
51-60	3 (15%)	2 (10%)	15 (75%)	20
61-70	5 (22.73%)	7 (31.82%)	10 (45.45%)	22
71-80	0 (0%)	0 (0%)	3 (100%)	3
81-100	2 (100%)	0 (0%)	0 (0%)	2
Total	31 (20.67%)	50 (33.33%)	69 (46%)	150

Table 3. Age and percentage of vitamin B12 level of patient not on metformin (control group).

Patients not on metformin/Ages	Deficient Level	low-normal Level	Normal Level	Total
10-20	0 (0%)	2 (10%)	18 (90%)	20
21-30	2 (4.76%)	3 (7.14%)	37(88.1%)	42
31-40	3 (7.5%)	2 (5%)	35 (87.5%)	40
41-50	0 (0%)	2 (11.76%)	15 (88.24%)	17
51-60	0 (0%)	3 (23.08%)	10 (76.92%)	13
61-70	1 (10%)	2 (20%)	7 (70%)	10
71-80	0 (0%)	2 (40%)	3 (60%)	5
81-UP	1 (33.33%)	0 (0%)	2 (66.67%)	3
Total	7 (4.67%)	16 (10.67%)	127 (84.66%)	150

DISCUSSION

Several studies have shown that using metformin has a significant impact on vitamin B12 levels in patients with T2DM, recently in 2019, the American Diabetes Association reported and recommended, that regular screening of VitB12 levels in patients with T2DM on metformin-treated, especially who had anemia or peripheral neuropathies [22]. This study points out that no published recommendations have required regular screening tests for vitamin B12 deficiency for those patients.

The current study indicates that the prevalence rate of vitamin B12 deficiency was 12.66% (38 out of 300). Vitamin B12 deficiency levels differed in the two groups, in on metformin group was 20.67% (31 out of 150) and in the control group was 4.67% (7 out of 150). Our assessment and Saudi Arabia study [23] revealed that the deficiency of vitamin B12 levels is occurred less in older patients than younger diabetic patients (figures 3.3, 3.4), and this may due to the supplements used by the older patients. Unexpected, the patients age showed a significant negative correlation with serum vitamin B12 levels as well as significant differences between the vitamin B12-deficient and normal groups.

This study also demonstrates that the prevalence of vitamin B12 deficiency is significantly greater in the metformin group than in the control group. This result is identical to previous studies with a variable rate. In 2015, Beulens et al 2015 reported a prevalence of 28.1% among metformin-treated patients in a cross-sectional study of 550 participants [24], whereas a study in Korea reported a prevalence of 22.2% among metformin-treated patients of 247 participants [25]. These studies showed that treatment with metformin was associated with a decrease of vitamin B12 levels, conversely, Aroda et al 2016, and the National Health and Nutrition Examination Survey reported a much lower prevalence of 4.3% and 5.8%, respectively [26,27]. The difference prevalence of vitamin B12 levels may due to the different serum vitamin B12 test methods and different of the metformin doses. Moreover, B12 insufficiency was significantly higher in the metformin group 33.33% (50 out of 150) compared with the control group 10.67% (16 out of 150). This indicates that B12 insufficiency was generally not found in our population, and after initiation of metformin in people with T2DM, the B12 insufficiency may develop into B12 deficiency.

The two usually recognized diagnostic criteria for vitamin B12 deficiency are, a low serum concentration of vitamin B12 (cobalamin) < 200 pg/ml associated either with clinical and/or hematological signs of vitamin B12 deficiency either

to an elevated serum concentration of homocysteine or methylmalonic acid [19]. A real difficulty is that quite different results can be obtained depending on the vitamin B12 assay kit, whereas the ranges of normal values are very close for all kits [18]. The risks of falsely normal concentrations are high with ELISA techniques: 22-35% depending on the kits [18]. Mass spectrometry, which is more reliable, is not yet very developed in most of the EU. Serum folic acid testing is warranted in the initial investigation of vitamin B12 deficiency, especially if homocysteine levels are elevated (renal failure) than serum methylmalonic acid concentrations for the diagnosis of vitamin B12 deficiency [28].
Conversely, a few factors can falsely influence vitamin B12 concentrations upwards, such as biotin supplementation (for hair loss) due to interference with certain vitamin B12 dosages, and in certain diseases or cancers, (hematologic malignancies) [17]. Megaloblastic macrocytic anemia is the main manifestation (sometimes normocytic in case of associated iron deficiency, especially in case of Biermer's disease or malabsorption). The other main signs are neurological, ranging from paresthesias and sensory disturbances to exceptional combined sclerosis of the marrow, glossitis, infertility, thrombosis, malabsorption, skin hyperpigmentation, and hair loss [29]. Several definitions of vitamin B12 deficiency are proposed in the literature, and the most relevant standard definition was published by George G Klee as follows, serum level < 200 pg.mL−1 and serum level of homo-total cysteine < 13 pmol.L−1 or methyl-malonic acid level > 0.4 μmol.L−1 in the absence of renal failure, folate, or vitamin B6 deficiency [30]. The most commonly surrogate markers used for the detection of vitamin B12 deficiency are homocysteine and methylmalonic acid (MMA) [31]. However, the tests were not available at our facility.
Finally, Possible reasons for the variations in vitamin B12 levels, are the cut-off levels used in the studies, diverse cultural beliefs, patient characteristics, and dietary habits, it is possible that diet has an important role in preventing vitamin B12 deficiency significantly, as the diabetic patient should have at least 3 to 4 small meals daily.

CONCLUSION

This study aimed to associate between vitamin B12 deficiency in T2DM patients on metformin compared to control group. Metformin has several advantages over other glucose-lowering drugs and therefore, remains a cornerstone in the treatment of type 2 diabetes. American Diabetes Association (ADA) and EASD mention B12 deficiency as a potential side effect of metformin therapy. Still, they do not yet provide clear recommendations for its screening and correction due to the lack of a reliable evidence base.
One of the proposed solutions to the problem is annual screening for B12 deficiency and include B vitamins in the treatment regimen for all patients with type 2 diabetes receiving metformin. MCV gradually increases while Hb remains within the normal range, making it difficult to detect. Therefore, regular blood film checks and vitamin B12 measurements are recommended for patients on metformin.

Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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