Screening the Effect of Metformin on Serum Vitamin B$_{12}$ and Blood Homocysteine Levels in Patients with Type 2 Diabetes Mellitus

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Abstract

Vitamin B$_{12}$ deficiency is a side effect of metformin use in patientswith type 2 diabetes mellitus (T2DM) and associated with increase in plasma homocysteine levels.The aim of present work was to screen vitamin B$_{12}$ and homocysteine levels in patients with T2DM on metformin therapy. Patients with T2DM collected from Fayoum University hospital outpatient clinics were screened and divided into three groups. Group 1 was thirty patients on metformin therapy for more than one year without vitamin B$_{12}$ supplementation; group 2 was thirty patients on metformin therapy for more than one year on vitamin B$_{12}$ supplementation and group 3 was patients not receiving metformin therapy (control group). All patients were subjected to complete history taking, including peripheral neuropathy and laboratory investigations. The mean±SD serum vitamin B$_{12}$ level was significantly higher in the metformin and vitamin B$_{12}$ supplementation users compared to metforminonly and non-metformin users (p<0.001), 629.9±249, 216±109 and 354.6±177.6 ng/L, respectively. Homocysteine levels were similar in the three groups. There was a significant correlation between metformin dose and vitamin B$_{12}$ levels (P<0.05), also between vitamin B$_{12}$ deficiency and hyperhomocysteinemia (P<0.01, r=-0.45). There was significant relation between homocysteine and macrovascular complications (r= 0.33, P<0.01) and between vitamin B$_{12}$ and peripheral neuropathy (P<0.01, r=-0.303). Vitamin B$_{12}$ deficiency was found to be associated with metformin use in T2DM patients with significant correlation with diabetic peripheral neuropathy and hyper-homocysteinemia, whereas vitamin B$_{12}$ supplementation with metformin could prevent those effects.

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Introduction

Type-2 diabetes mellitus (T2DM) is a progressivedisease which is characterized by theelevation of glycated haemoglobinlevel (HbA1c) and decline in beta cells function. When lifestyle modification fails to achieve adequate glycaemic control, oral hypoglycemic agents are introduced as a treatment approach. [1,2]

If metformin is not contraindicated and can be tolerated, it should be considered as the first-line therapy in newly diagnosed T2DM patients with HbA1cless than 9%. [3] This is attributed to the advantages of weight loss, good initial efficacy in decreasing fasting plasma glucose in overweight and obese individuals with T2DM, and low cost [4].

Gastrointestinal disturbance is a well-known side effect of metformin.It also can cause vitamin B_{12} deficiency by reducing its absorption.

The increased frequency of vitamin B_{12} deficiency among T2DMpatients had been reported in several cross sectional studies [6-8] and case reports [9-11]. The prevalence of vitamin B_{12} deficiency ranged from 5.8% to 33% [6,12,13].

Metformin induced vitamin B_{12} deficiency is almost always overlooked and rarely taken into consideration [9,14]. Many patients on metformin develop vitamin B_{12} deficiency, consequently developing neuropathy and anaemia which is falsely attributed to underlying Diabetes mellitus by physicians and so never addressed [5].

Furthermore, metformin, by decreasing vitamin B_{12} levels, can increase plasma homocysteine level which is strongly linked to cardiovascular disease in patients with T2DM [15].

Hence, the study was designed to evaluate the prevalence of metformin induced vitamin B_{12} deficiency and the consequent elevation in homocysteine levels in type 2 diabetic Egyptian patients and whether the deficiency prevalence will be reduced by vitamin B_{12} supplementation.

Material and Methods

The study was performed in one center, Fayoum University hospital in Egypt. Local hospital research ethics committee approval was obtained. Patients were collected from the outpatient clinics. T2DM were screened and divided into three groups. Group 1 (GP1) included patients on metformin without vitamin B$_{12}$ supplementation for at least 12 months. Group 2 (GP2) patients on metformin and vitamin B$_{12}$ supplementation for at least 12 months. Control group (GP3) comprised patients of T2DM with no history of metformin use in last 2 years. All patients gave signed informed consent. Patients were evaluated by:

1. Examination for peripheral neuropathy by assessing the pain and numbness in extremities [stocking and glove distribution of diabetic peripheral neuropathy (DPN)].

3. Fasting Serum levels of vitamin B$_{12}$ and homocysteine (HC) plasma levels were measured in all patients using ELISA immunoassay.

The severity of vitamin B$_{12}$ deficiency was split into three groups: mild vitamin B12 deficiency, defined as a vitamin B$_{12}$ level of 160-180ng/L; moderate deficiency (120-160ng/L); and severe deficiency (<120ng/L). Macrocytic anaemia was defined as the presence of a low Hb in conjunction with an MCV of >96fl. [16] Plasma HC ≥10 μmol/L was regarded as hyperhomocysteinemia [17].

4. Information related to the duration of diabetes was obtained by questioning the patients.

Patients with adiagnosis of pernicious anaemia, malabsorption (coeliac disease, inflammatory bowel disease, gastrointestinal surgery), malnutrition (pure vegans, anorexia nervosa), iron deficiency anaemia, history of thyroid disease and thyroxine treatment and/or a history of other organ-specific autoimmune conditions (vitiligo, Addison's, primary ovarian failure, hypoparathyroidism) were excluded from the study.

Statistical analysis

All analysis of data was conducted through the use of IBM software SPSS 10.0 (SPSS Inc., Chicago, USA). The result of power analysis of the study for vitamin B$_{12}$ showed that sample size of 30 patients for each group is sufficient to get power of >80% and α=0.05. The two

ways ANOVA analysis followed by post hoc ANOVA using Tukey system were used to analyse the difference in vitamin B$_{12}$ and homocysteine levels between groups. Correlation was assessed using Pearson (for normal distribution, linear correlation) and Spearman (for non-normal, non-linear correlation) tests as specified. All tests were two-sided and p-values of <0.05 were considered to be statistically significant. Post hoc assessment of multiple interactions was conducted by Tukey as indicated.

Results

A total of 88 (65 female) T2DM, with mean±SD (range) age 49.8±11.3 (22-75) years old were screened in the study. 30, 30 and 28 patients were in GP1, GP2 and GP3, respectively. Patients' demographic and clinical characteristics are shown in Table 1. No significant difference between the three groups was found in age, gender distribution, duration of DM, peripheral neuropathy and macrovascular complications.

Table 1. Demographic and clinical characteristics of patients in each group

	Group 1	**Group 2**	**Group 3**	**p**
Age(years)	50.6 ± 11.25	49.5 ± 11.5	49.7 ± 11.7	0.89
GenderMales (%)	11(36.7%)	6(20%)	6(21.5%)	0.34
Females (%)	19(63.3%)	24(80%)	22(78.5%)	
Duration of DM (years)	5.18 ± 3.2	5.16 ± 4	6.65 ± 5.4	0.35
Peripheral neuropathy (Number of patients, %)	24(80%)	17(56.7%)	17(60.7%)	0.13
Macrovascularcomplications (Number of patients, %)	17(56.7%)	12(40%)	17(56.7%)	0.25

The mean±SD laboratory results of the three groups are shown in Table 2. Vitamin B$_{12}$ levels were significantly higher (<0.001) in users of daily vitamin B$_{12}$ supplementation (GP2) compared to the other two groups, with the lowest values in patients using metformin only (GP1).Peripheral neuropathy prevalence was found to be highest in metformin users without vitamin B$_{12}$ supplementation, 80% (24/30 patients), compared to those using metformin with vitamin B$_{12}$ supplementation, 56.7% (17/30 patients) and the control group, 60.7% (17/28 patients).

Table 2. The mean±SD laboratory results of the three groups

Parameter (mean±SD)	Group 1	Group 2	Group 3	p
Vit B12 (ng/L)	216±109	629.9±249	354.6±177	<0.001 *
Homocystiene(µmol/L)	8.6±4.01	6.9±2.46	7.3±2.97	0.094
Hb (g/dl)	11.67±1.73	12.40±1.48	12.85±1.25	0.014 *
HCT (%)	36.99±5.49	38.92±6.46	40.86±3.91	0.029 *
MCV (fL)	84.46±9.01	82.37±9.01	85.89±5.76	0.258

*: significant

Comparison between patients with peripheral neuropathy and patients without peripheral neuropathy is shown in Table 3. Vitamin B$_{12}$ levels were significantly lower (p=0.01) and metformindose were significantly higher (p=0.02) in patients with peripheral neuropathy.

7 (8%) patients had a mild vitamin B$_{12}$ deficiency, 5 in GP1, one in GP2 and one in control group. 18 (14%) had a moderate deficiency; 14 were in GP1 and 4 in GP3. None had a severe deficiency.In the vitamin B$_{12}$-deficient patients, 64% (16/25) were anaemic, while in patients with normal vitamin B$_{12}$ levels, 23.8 % (15/63) were anaemic. Table 4 shows the comparison between vitamin B$_{12}$ deficient patients and non-vitamin B$_{12}$ deficient patients in the two metformin groups (60 patients). Among the 60 metformin users, the daily dosage was 1000 mg or less in 34 subjects and more than 1000 mg in 26 subjects. Metformin dose and number of patients with neuropathy were significantly higher (p=0.006 and 0.027, respectively) in vitamin B$_{12}$ deficient patients.

There was a statistically significant negative correlation between vitamin B$_{12}$ and homocysteine (r= -.448; p<0.001) as shown in Figure 1.

Table 3: Patients with peripheral neuropathy vs. normal in total patients

Parameters	Patientswithperipheral neuropathy	Patients without peripheral neuropathy	p
Number of patients (%)	58/88 (66%)	30/88 (34%)	
Vitamin B12(ng/L)	351.4±239.8	497.5±259.2	0.01 *
HC (µmol/L)	7.85±3.1	7.14±3.6	0.34
Hb (g/dL)	12.23±1.6	12.43±1.5	0.57
MCV (fl)	83.1±8.1	86.1±8.1	0.11
Metformin Dose (mg/day)	995.7±792	606.7±604	0.02 *
duration with diabetes	6.02±4.9	4.9±3.24	0.27

(years)			

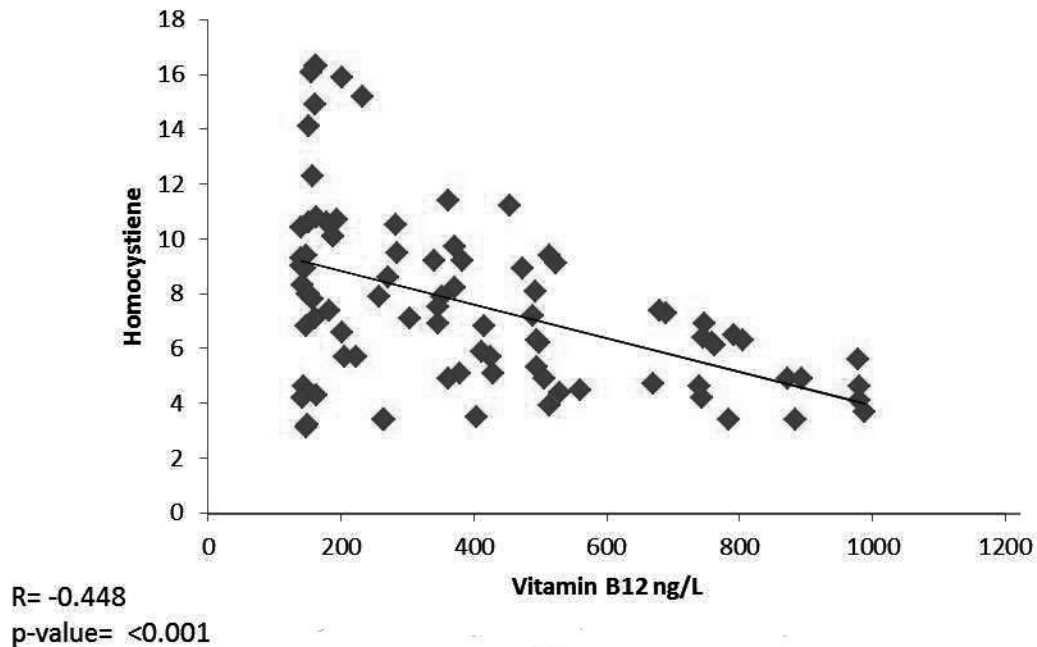MCV: mean corpuscular volume; HC: homocysteine; Hb: hemoglobin

Figure 1. The inverse relationship (R-value = -0.448) between vitamin B$_{12}$ levels (ng/L) and homocysteine levels (p=<0.001).

Homocysteine mean±SD was significantly higher (p=0.025) in patients with macrovascular complications than those without, 8.35±2.9 and 6.79±3.4, respectively.

Four patients had a macrocytic anaemia, three in GP1 and one in control group. There was significant correlation between vitamin B$_{12}$ and hemoglobin as well as hematocrit (r=0.347; p-value<0.001 and r=0.349; p-value <0.001, respectively).

Discussion

The prevalence of vitamin B$_{12}$ deficiency of all patients with T2DM in study was 28.4% (25/88); 20 (33.3%) patients of them were patients taking metformin. These results are quiet

close to the prevalence of vitamin B12 deficiency shown in previous studies, which ranged from 5.8 to 30% in patients taking metformin [6,12,16,18-20].

This wide variation in the reported prevalence could probably be explained by the variability of measurement methods of vitamin B$_{12}$ levels in thelaboratories (HPLC, radio assay etc.) and difference in definitions of vitamin B$_{12}$ deficiency.

There is no definite mechanism of metformin-induced vitamin B$_{12}$ deficiency. Several hypotheses are available; include bacterial overgrowth in the small intestine, changes in small bowel motility, changes in bacterial flora, competitive inhibition or inactivation of vitamin B$_{12}$ absorption or an effect of calcium on cell membranes [15,21,23].

The effect of metformin in decreasing plasma vitamin B$_{12}$ levels appears to be dependent on treatment duration and dose [22-24] of metformin and considered as a high risk factor for vitamin B$_{12}$ deficiency.

Several previous studies reported that metformin has no effect on homocysteine or small effect, if any [4,25-27]. However, patients using metformin without vitamin B$_{12}$ supplementation in the present study showed high prevalence of vitamin B$_{12}$ deficiency. They had a relatively higher homocysteine level than that of patients using vitamin B$_{12}$ supplementation. However the difference within the three groups was not statistically significant that might be due to the number of patients studied. So, the decrease in vitamin B$_{12}$ concentrations was associated with an increase in homocysteine levels [22]. It was clearly shown that homocysteine concentrations increased with decreasing levels of vitamin B$_{12}$ and the correlation between them was negative and statistically significant (r =-0.448, p<0.01).

The relationships between diabetes mellitus, metformin, vitamin B$_{12}$ and HC can be explained by the fact that lowering serum vitamin B$_{12}$ may occur in patients with diabetes mellitus regardless of metformin use. [19] It is further lowered by metformin directly by reducing vitamin B$_{12}$ absorption [17]. Vitamin B$_{12}$ deficiency and the accompanying elevation in homocysteine levels have been reported to cause a distinct sensory neuropathy that closely mimics diabetic neuropathy. [5] Diabetic neuropathy is also known to get worse with co-existing vitamin B$_{12}$ deficiency [5]. We revealed a significant correlation between B$_{12}$-deficiency and incidence of neuropathy (r=-.303; p=0.004). Generally, electromyography or

nerve conduction tests are used to confirm diagnosis of peripheral neuropathy [28]. But, they are not routinely performed at the outpatient level.

Anaemia was found to be prevalent in all groups of patients. However the prevalence was higher in vitamin B_{12} deficient metformin group 55% than in patients with normal serum vitamin B_{12} levels 37.5%, compared to 32% in the control group. The high incidence of anaemia in patients with T2DM is common and this may be due to the association of T2DM with shortened red blood cell lifespan or possibly mild renal impairment. This was confirmed in our study as vitamin B_{12} correlation with Hb and HCT was statistically significant [16]. There were no significant differences in the mean MCV between with and without vitamin B_{12} deficiency groups.

With regard to HC, as an important risk factor in vascular diseases in patients with diabetes, its levels were significantly higher in diabetic patients with cardiovascular diseases (CVD) than in normal subjects [29,30]. We found significant correlation between homocysteine levels and macrovascular complications (e.g. hypertension, angina, and stroke) in diabetic patients (r=0.33, p<0.01). These results cannot confirm the relation between homocysteine and CVD complications because the difference in levels between groups was too small and non-significant. Also several factors are implicated in cardiovascular complications in diabetic patients.

Some population-based studies show little or no association between high homocysteine levels and CVD risk [31]. Hence, a causal role for homocysteine in the development of CVD is still debated.

The limitations of our study were; vitamin B_{12} baselines were not examined prior starting metformin therapy and the dose of vitamin B_{12} supplementation was not recorded; this study did not confirm whether the high prevalence of neuropathy was related to vitamin B_{12} deficiency, further evaluation of HbA1c would exclude other contributing factors.

Possibly one year duration, limited number of subjects in the study and the correlation analysis performed in the subgroups with even smaller number of patients; may be insufficient for vitamin B_{12} deficiency and elevation in homocysteine to be remarkable and give more representative results. Homocysteine levels are also affected by folate levels [32],

making it difficult to contribute any elevation in homocysteine level to vitamin B_{12} deficiency only. Due to observational nature of the analysis, a complete matching of the metformin and the non-metformin groups was not obtained.

Conclusions

In conclusion, type 2 diabetes mellitus patients using metformin therapy for long time had lower vitamin B_{12} levels than those not using metformin. Although, the management of vitamin B_{12} deficiency in metformin treated patients has not been established, we noticed that concomitant use of vitamin B_{12} supplementation could correct metformin-induced vitamin B_{12} deficiency. Hence, regular screening of serum vitamin B_{12} level in patients under long-term metformin therapy is recommended and dose should be reduced when patients develop deficiency. Since metformin has been reported to cause vitamin B_{12} deficiency by reducing its absorption, further evaluation is needed to clarify the pathological mechanisms and studies comparing various doses of supplemental vitamin B_{12} through different routes are warranted. Vitamin B_{12} deficiency also resulted in elevation in homocysteine levels; however the effect is seems to be small and insignificant.

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