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Prevalence of B12 deficiency among patients with type 2 diabetes mellitus using Metformin in Sulaimani governorate /Kurdistan region of Iraq

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Abstract

Background and objectives: Vitamin B12 deficiency is prevalent among patients with type 2 diabetes mellitus, especially those using Metformin for a long period of time. The aim of this study was to determine the association and prevalence of vitamin B12 deficiency among Kurdish patients with type 2 diabetes mellitus on metformin therapy and its associated clinical complications. **Methods:** This cross-sectional study was conducted in the Diabetes and Endocrine Center of Sulaimani city. Data, including past medical history, medication history and blood samples, were collected from 223 participants to measure fasting blood sugar, blood cells counts, HbA1c, renal function and serum B12 level. **Results:** This study found a high prevalence of vitamin B12 deficiency, 24.6% in patients with type 2 diabetes mellitus on Metformin therapy. There was a significant difference between mean B12 levels of those with B12 deficiency (145.24 ± 32.88 pg/ml) and those with normal serum B12 level (387.62 ±281.44 pg/ml). No relationship existed between vitamin B12 levels and the dose and duration of metformin use. However, there was a statically significant relationship between serum vitamin B12 and peripheral neuropathy. **Conclusions:** Prevalence of vitamin B12 deficiency among patients using Metformin is high, but there is no significant effect of the dose and duration of Metformin exposure on B12 concentration. Additionally, there's a significant relationship between the presence of neuropathy and B12 level.

Key words: Metformin,Peripheral neuropathy,Type 2 diabetes mellitus, Vitamin B12 deficiency .

Introduction

World Health Organization (WHO) estimated that, globally, there were 422 million adults aged over 18 years with Diabetes Mellitus (DM) in 2014. Diabetes caused 1.5 million deaths in 2012. Higher-than-optimal blood glucose level caused an additional 2.2 million deaths, by increasing the risks of cardiovascular and other diseases[1]. For Initial drug therapy, it is generally agreed that Metformin, if not contraindicated and if tolerated, is the most widely prescribed oral hypoglycemic agent in patients with Type 2 Diabetes Mellitus (T2DM) worldwide and it's one of the most cost-effective agents[2,3]. Due to the numerous clinical benefits associated with Metformin, some side effects with potential adverse health effects, associated with its use, are usually ignored. One of such adverse effects is vitamin B12 deficiency[4,5]. Vitamin B12 deficiency is prevalent among patients with T2DM, especially those using Metformin for a long period of time[6]. Ten to thirty percent of the patients have evidence of reduced vitamin B12 absorption[7].

Various mechanisms have been suggested, including alterations in intestinal mobility, bacterial overgrowth, and interactions with a complex of intrinsic-factor/vitamin B12 and cubilin, an endocytic receptor involved in the absorption of cobalamin. B12-intrinsic factor complex uptake by ileal cell surface, a calcium-dependent process, is also affected by Metformin because of impaired calcium availability4. In addition to anemia, vitamin B12 deficiency may increase the severity of peripheral neuropathy in patients with T2DM[8]. Neurologic features, when present, consist of the classic picture of subacute combined degeneration of the posterior and lateral spinal columns. The symmetrical neuropathy affects the legs more than the arms. It begins with paresthesia and ataxia associated with loss of vibration and position sense, and can progress to severe weakness, spasticity, clonus, paraplegia, and even fecal and uri-

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nary incontinence[9,10]. Association between Metformin and impaired vitamin B12 absorption has been described in previous literatures[11]. Currently, there is no published data regarding the prevalence of vitamin B12 deficiency among T2DM patients who are receiving Metformin in Iraq. The aim of this study was to determine the association and prevalence of vitamin B12 deficiency among Kurdish patients with type 2 diabetes mellitus on Metformin therapy and its associated clinical complications.

Patients and methods

This cross sectional study was conducted in the Diabetes and Endocrine Center of Sulaimani City in a period of 6 months, from 1st November, 2017 to 31st April, 2018 (around 30 000 diabetic patients have been registered in this center and the majority of them are T2DM); 223 patients were enrolled (the sample size was calculated using Raosoft sample size calculator, taking 95% confidence level, 5% margin of error, and response distribution of 80%). All the included patients were Kurds in ethnicity, and previously diagnosed with T2DM. They have been on Metformin for more than 12 months, and patients of both genders were included. All patients with T2DM who has history of anemia and B12 deficiency, prior transfusion, alcohol intake, renal insufficiency, prior gastric surgery, patients on current parenteral or enteral nutritional support (including B12), proton pump inhibitors, oral contraceptive pills, those with malabsorption syndrome, vegetarians, lost data and lost follow up were excluded from the study. Informed written consent was taken from all participants, and the study was approved by both scientific and ethical committee of Kurdistan Board for Medical Specialties. Data collected for each patient included age, gender, residence, past medical and surgical history, transfusion history, drug history, duration of diabetes mellitus, dose of Metformin, duration of Metformin use, adherence to Metformin and evidence of peripheral neuropathy, using Subjective Peripheral Neuropathy Screen Questionnaire (SPNSQ).

Venous blood samples were collected using full aseptic technique; blood samples were taken on fasting and kept at - 30 degree Celsius in closed bottles, which were held in vertical position. Samples were analyzed on the same day for B12 levels using Cobas e 411- automated analyzer. Glycosylated hemoglobin (HbA1c) measurement was done using Cobas c 311- automated analyzer. Complete blood counts (CBC) were done using mythic 18 automated analyzer, and for renal function test we used-Cobas 6000. All the data was entered in computer software Statistical Package for Social Sciences (version 24.0). Descriptive statistics were applied to summarize the data. Mean and standard deviation (±SD) was calculated for all the quantitative variables, i.e. age, duration of metformin use, dose of metformin, serum vitamin B12 levels and glycosylated hemoglobin. Frequency and percentages were calculated for qualitative variables, i.e. gender and vitamin B12 deficiency. Data was analyzed using Chi square-test, Anova test and t test. A p-value of ≤ 0.05 was considered statistically significant.

Results

Out of 223 patients, there were 157 females (70.4 %), with a female to male ratio of 2.37:1. The age ranged between 33-85 years, with the mean age of 57.4±10.13 years. The mean ±SD duration of diabetes was 8.41±5.66 years. The mean ±SD duration of Metformin exposure and Metformin dose was 7.25±5.22 years and 1620.69±676.63 mg, respectively. The range of glycosylated hemoglobin (HbA1c%) levels was 5.59%- 15.7%, and the mean was 9.0±2.1. Out of 223 patients, 53 of them (23.8%) had poor concordance for their regular daily Metformin intake, and of that number, 12 patients (22.6%) were among deficient vitamin B12 group. Further, 182 patients (81.6%) were complaining of neuropathic pain, and 44 of them (19.7%) were among deficient group, Table 1.

Table (1): Baseline characteristics of participants.

Variables	No. = 223 (100%)
Sex	
Male	66 (29.6%)
Female	157 (70.4%)
Age (Mean ±SD)	57.4 ±10.13
Duration of T2DM in years (Mean ±SD)	8.41±5.66
Duration of Metformin use in years (Mean ±SD)	7.25±5.22
Dose of Metformin in mg (Mean ±SD)	1620.69 ±676.63
Serum B12 level (pg/ml)	
<191	55 (24.6%)
191 - 200	5 (2.2%)
>200	163 (73%)
Neuropathic pain	
Yes	181 (81.2)
No	42 (18.8)
HbA1c %	9.0 ±2.10

The range of MCV was 57-100.3 fL, and the mean MCV of patients on Metformin was 80.34±5.96, which was lowest among those with normal serum B12 level although statically not significant (p-value= 0.053), Table 2 and Table 4. The mean WBC count was 8.68±7.77 x109/L and the mean platelet count among them were 256.18±118.43 x109/L.

Serum B12 levels ranged between 50- 893 pg/ml. There was no significant association between gender and serum B12 levels among the B12 deficient group and non-deficient group (p-value = 0.545). The correlation between HbA1c and B12 deficiency was also not significant statistically (p-value= 0.152), Table 2.

Table (2): Hematological findings among patients on Metformin

Hematological test	Range	Mean±SD
HbA1C %	5.59-15.7	9.0±2.1
Hb (gm/dl)	82-161	137.5±70
MCV (fL)	57-100.3	80.34±5.96
PLT (x10^9/L)	109-1780	256.18±118.43
WBC (x10^9/L)	3.9-17.9	8.68±7.77

The prevalence of vitamin B12 deficiency was 24.6%, (55 out of 223 patients) (Figure 1).

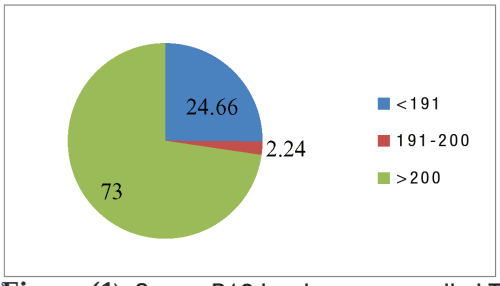

Figure (1): Serum B12 level among enrolled T2DM patients.

Both the dose and duration of Metformin had no effects on B12 level among the studied patients. Mean dose of Metformin among B12 deficient patient was 1614.96 (±683.05), while in patients with normal B12 levels, the mean dose was 1648.92 (± 686.3), p-value= 0.868.

In the current study, there was a significant relationship between the presence of neuropathic pain and B12 concentration (p-value<0.001). Most prominent symptom was numbness and paresthesia in the legs with burning sensation. Out of 223 patients, 182 (81.61%) were complaining of neuropathic pain; 44 (19.7%) of them were among B12 deficient group, 133 (59.6%) of them were among non- deficient group, and 5 (2.2%) cases were of borderline group, Table 3.

Table (3): Demographic characteristics of studied patients in correlation with serum B12.

Demographics	Serum B12 level No. (%)			p-value
	Deficient < 191 pg/ml	Borderline 191-200 pg/ml	Normal > 200 pg/ml	
Number of patients No. (%)	55 (24.66%)	5 (2.24%)	163 (73%)	N/A
Male No. (%)	19 (8.52%)	2 (0.89 %)	45 (20.20%)	
Female No. (%)	36 (16.14%)	3 (1.34%)	118 (52.91%)	0.545
Age in years (Mean , SD)	56.83 (±10.04)	57 (± 8.27)	57.06 (±9.75)	0.993
Duration of T2DM in years (Mean, SD)	8.09 (± 5.22)	11 (± 5.56)	8.43 (± 5.82)	0.535
Duration of Metformin use in years (Mean, SD)	7.14 (± 5.1)	8.1 (± 4.53)	7.26 (± 5.31)	0.916
Dose of Metformin in mg (Mean , SD)	1648.92 (± 686.30)	1490 (± 368.10)	1614.96 (±683.05)	0.868
Drug (metformin) compliance No. (%)				
Yes	43 (19.29%)	4 (1.79 %)	123 (55.15%)	0.725
No	11 (4.93%)	1 (0.44%)	41 (18.40%)	
Neuropathic pain No. (%)				
Yes	44 (19.74%)	5 (2.24%)	133 (59.64%)	0.000
No	11 (4.93%)	0 (0.0%)	30 (13.45%)	

N/A: Not Applicable

There was a significant difference between mean B12 levels of those with B12 deficiency (145.24 ± 32.88 pg/ml) and those with normal serum B12 level (387.62 ±281.44 pg/ml), p-value < 0.001. The range of hemoglobin (Hb) level of patients on Metformin was 82-161 gm/dl, and the mean was 137.5±70 gm/dl. The prevalence of anemia among all patients was 41 cases (18.38%), in which only 14 cases were among deficient vitamin B12 group while most of them were among normal vitamin B12 group (26 cases), and only 1 case among borderline group. This was statically not significant (p-value = 0.288), Table 4.

Table (4): Correlation between laboratory findings and serum B12 level among studied patients

Variables	Serum B_{12} level			p-value
	Deficient < 191 pg/ml	Borderline 191-200 pg/ml	Normal > 200 pg/ml	
Number of patients No. (%)	55 (24.66%)	5 (2.24%)	163 (73%)	N/A
Hb (gm/dl)	128.32 (± 13.44)	132.6 (± 16.1)	129.8 (± 10.60)	0.589
WBC (x10^9/L)	9.26 (±8.6)	6.5 (±2.32)	8.55 (±7.58)	0.069
PLT (x10^9/L)	266.80 (±67.34)	246 (± 34.49)	243.04 (±56.23)	0.036
HbA1c % (Mean, SD)	8.66 (± 1.97)	8.69 (±2.62)	9.13 (± 2.13)	0.348
MCV (fL)	81.77 (± 7.86)	83.10 (±5.29)	79.75 (± 5.08)	0.053
B_{12} level (Mean , SD)	145.24(± 32.88)	195.70 (± 2.78)	387.62(±281.44)	0.000
Anemia No. (%)				
Yes	14 (25.45%)	1(20%)	26 (15.9%)	0.288
No	41(74.55%)	4(80%)	137 (84.1)	

N/A: Not Applicable

Mean serum level of B12 for those who are on Metformin for more than five years and for those less than 5 years was 332.85±230.42 pg/ml and 311.57±293.66 pg/ml, respectively (p-value= 0.547), Table 5.

Table (5): Relationship between B12 level and duration of Metformin exposure.

Duration of metformin use in years	Number of patients N %	B12 level pg/ml	p-value
<5.0	80 (35.87%)	311.57±293.66	0.547
≥5.0	143 (64.13%)	332.85±230.42	

Discussion

Finding the relationship between serum B12 level and Metformin is not novel and it has been proven, but as per literature review, no data is available on this topic in Iraq, particularly among Kurdish ethnic group. The background of variable clinical response of Metformin among different individuals and the measurement of serum vitamin B12 in T2DM patients on Metformin is not part of the standard follow up in Sulaimani Diabetic and Endocrine Center. We tried to estimate the prevalence of B12 deficiency among registered T2DM patients on Metformin.

In our study, we found that 24.6% of the patients are vitamin B12 deficient. Our results are in-line with those of recently published studies.

However, the prevalence of vitamin B12 deficiency varies according to the cut off value for serum vitamin B12 level used[19]. Serum methyl malonic acid should be measured in patients with possibly low serum vitamin B12 levels[20]. Moreover, measurement of additional biomarkers, such as holotrans cobalamin, methylmalonic acid, red blood cell-B12, and plasma concentrations of methylation indices would provide a more comprehensive evaluation of true deficiency[21,22].

Although most studies associate vitamin B12 deficiency to prolonged use of Metformin, our study didn't show any association between the prolonged use of Metformin and vitamin B12 deficiency[23].

In addition, our study showed no relation between the dose of Metformin and serum vitamin B12 level, while some studies provided the opposite result, stating that the reduction of vitamin B12 may be induced by Metformin in a dose dependent manner[24]. This is because besides the non-biological and biological factors, which play a role in the mechanism of action of Metformin and its therapeutic effect, there's significant role of Metformin pharmacogenetics at the individual level of clinical response of Metformin[25]. Additionally, different countries have different ethnic groups and eating habits which will play a role in the way a body respond to Metformin.

On further analysis, we also found that there's a significant relationship between the presence of neuropathy and vitamin B12 deficiency. Our result is consistent with prior studies which have reported the risk of vitamin B12 deficiency as well as associated complications (anemia, neuropathy) with Metformin use[8,24].

In contrast to our study, Ahmed et al. analyzed no difference in the presence of neuropathy between those with normal and deficient vitamin levels among black South African descent[16]. That study was the first study to report differences in vitamin B12 levels among different ethnic groups with Metformin-exposed T2DM. Additionally, different studies had used different methods for assessing and scoring neuropathy among their patients which would give different results and under the lights of recent animal studies which they have shown positive impact of Metformin on neuropathy status through neuroprotective mechanisms[26]. Metformin induces neuropathy by enhancing vitamin B12 deficiency and this may also justify the contradictory nature of results obtained by different studies.

Although HbA1c was comparable among vitamin B12 deficient group and the normal vitamin B12 group, Akabwai et al. have demonstrated association between vitamin B12 deficiency and suboptimal glycaemic control[14].

Our study has several limitations; we didn't have facility to measure other additional biomarkers, such as holotranscobalamin, methylmalonic acid, red blood cell-B12, and plasma concentrations of methylation indices. In addition, we didn't assess folate level which may be present concomitantly with B12 deficiency. More comprehensive and thorough examination for neuropathy would be of great clinical significance as we have used Subjective Peripheral Neuropathy Screen Questionnaire, which is symptom dependent and subjective.

Conclusions

Prevalence of vitamin B12 deficiency among patients using Metformin is high with no significant effect of dose and duration of Metformin exposure on B12 concentration. Additionally, there's a significant relationship between the presence of neuropathy and B12 level.

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