Association of Vitamin B_{12} and Metformin in Type II Diabetes Patients in Bahrain

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Abstract

Objective: Metformin therapy is the gold standard treatment for type 2 diabetic patients. Long term use of metformin has been associated with vitamin B_{12} deficiency. We aimed to study the level of vitamin B_{12} in type 2 diabetic patients on metformin, and to determine other factors affecting the level of vitamin B_{12}.

Methodology: Out of 344 patients with type 2 diabetes that were interviewed from January to December 2015; 304 (91.3%) were investigated for vitamin B_{12} levels deficiency. The patient's lab results from 2015-2016 were reviewed from the electronic hospital information system, patient data file.

Result: Most of the patients, 56.4% had a normal vitamin B_{12} level. In 91% of the patients the treating physician had already investigated for serum vitamin B_{12} level. Dyslipidemia and hypertension were significantly more (p=0.009 and 0.022 respectively) in patients with normal vitamin B_{12} level. Percentage of normal B_{12} level patients was higher (p=0.001)even in more than 20 years of metformin use; 46.7% of more than 20 years Metformin users had vitamin B_{12} level within 301-450 pg/mL and 33.3% had more than 450 pg/mL.

Conclusion: We recommend routine screening for basal vitamin B_{12} level for all patients with type 2 diabetes mellitus (T2DM) at the initiation of metformin therapy with subsequent annual follow up of the level.

Keywords: Vitamin B_{12}; Metformin; Type II diabetes; Bahrain

Introduction

Metformin is considered as the first-line antidiabetic agent in the treatment of type 2 diabetes mellitus (T2DM) due to its effect on glucose and lipid metabolism [1] simultaneously with the major protective role it plays against life threatening complications. It acts as an insulin sensitizer on insulin-targeted tissues; liver, muscle, and adipose tissues and reduces insulin resistance. Additionally it offers protection from cardiovascular diseases and heart failure [2,3]. Given its safety and efficacy record, it is routinely prescribed to approximately 120 million type 2 diabetes patients world-wide [4].

However various reports have recognized long-term treatment with metformin as a pharmacological cause of vitamin B_{12} deficiency [5-7]. On the other hand, the exact prevalence of vitamin B_{12} deficiency among diabetic patients described in different literature is not constant.

Diabetic neuropathy, affecting more than 90% of the patients is the commonest complication of diabetes [6]. The fact that vitamin B_{12} deficient neuropathy and diabetic neuropathy cannot be distinguished clinically [7] renders it difficult to determine which of the two pathology is actually responsible for the neuropathy in a diabetic patient with a coexisting vitamin B_{12} deficiency. Long term treatment with metformin therefore increases the chance of vitamin B_{12}

neuropathy be misdiagnosed as diabetic neuropathy or it may contribute in worsening the diabetic neuropathy [7].

In addition to neuropathy long term metformin therapy has been linked also to the development of anaemia [8]. Once again the mechanism of this could be the metformin induced vitamin B_{12} deficiency as it is a well-recognized cause of megaloblastic anemia.

As vitamin B_{12} deficient neuropathy and diabetic neuropathy both present with similar indistinguishable clinical manifestations, the objectives of this study were: 1) to determine the level of Vitamin B_{12} in T2DM patients on metformin; 2) to identify additional factors that may affect the Vitamin B_{12} level in T2DM patients on metformin; 3) the effect of metformin use in the level of vitamin B_{12} in type 2 diabetic patients.

Material and Methods

Type 2 diabetic adult patients attending the diabetic clinic of primary care department of a Bahrain Defense Force Royal Medical Services Military Hospital were randomly recruited between January 2015 to December 2015. Each participant was interviewed with a structured questionnaire and was asked about: personal data, duration of T2DM, duration and dose of metformin, other risk factors (dyslipidemia, hypertenstion, cerebrovascular diseases, Coronary Vascular Disease, smoking, hypothyrodsim, use of insulin and nephropathy) and symptoms of peripheral neuropathy. The interview consisted of a verbal consent followed by direct questions by the treating physician who recorded the answers manually. To evaluate

cerebrovascular diseases, patients were questioned about past history of cerebrovascular accident, transient ischemic attack or stroke. Peripheral neuropathy was assessed by asking the patient about presence of tingling sensation, heat sensation or paresthesia of the lower limb.

A total of 344 patients who were above the age of 30 years, who are being treated by metformin were interviewed. Serum Vitamin B$_{12}$ Assay was ordered by the treating physician to 305 patients, so the rest of them were excluded. Type 1 diabetic patients were not included in this study. Nor were the T2DM below 30 years of age, not receiving metformin, or suffering from chronic renal insufficiency defined by serum creatinine level less than 3. Patients with a prior history of gastrostomy, bariatric surgery, ileum resection or Crohn's disease were also excluded.

After the completion of interview lab results from 2015-2016 were reviewed from the electronic hospital information system patient data file. Level of vitamin B$_{12}$, HbA1C, hemoglobin were recorded. Patients were considered low Vitamin B$_{12}$ level if it were less than 300 pg/ml.

Ethics Board approval was obtained from Bahrain Defence Force Royal Medical Services prior to commencement of this cross-sectional study.

Statistical Analysis

Data were collected and stored in a spreadsheet using Microsoft Excel 2010® software. Data management and coding were then done in Excel. Data were analyzed using SPSS® version 20.0 (IBM Inc., Chicago, Illinois, USA).

Descriptive analysis was done, where categorical variables were presented in the form of frequencies and percentages. Chi2 test was used to test for differences between the groups and test for associations. Results were compared between the Vitamin B$_{12}$ deficient group and Normal Vitamin B$_{12}$ group in two ways. 1) Number and percentage of each variable was calculated in each group and compared within the same group and 2) Percentage of each variable was compared between the 2 two groups. A p-value below 0.05 was interpreted as an indicator of statistical significance.

Result

The demographic data of the 305 participants' are presented in Table 1. The highest number of participants, (46.1%) was from 50 to 60 years old. Most of the patients, 172 (56.4%) had a normal vitamin B$_{12}$ level (p=0.023). The majority of patients, 193 (57.8%) were male. Bahraini nationals represented 68.9% of participants. Greater percentage (59.3%) of the patients were obese with a BMI higher than 30. About 32% of patients had T2DM duration of 1-5 years and 6-10yrs. Similarly longest period of time treated with metformin is 1-5 years 36.2% and 6-10yrs 33.2%. Majority of the patients, 220 (65.9%) were receiving total daily dose above 2000 mg. A good number of patients, 166 (49.7%) reported symptoms of peripheral neuropathy. In spite of laboratory result revealing Hb level below 12 mg/dl in 18% of the participants, only 12% of the patient presented with clinically apparent anaemia. The level of last HbA1c was between 6 to 7% in 105 (31.4%) participants.

Characteristic Total (n)	Vitamin B$_{12}$ deficiency	Normal Vitamin B$_{12}$	P value
Interviewed 334			
Vitamin B$_{12}$ Assay 305	133 (43.6)	172 (56.4)	0.028*
Sex (male, %)	81 (60.9)	93 (54.1)	0.232
Female	52 (39.1)	79 (45.9)	
Age (years%)			
30-60	96 (44)	122(56)	0.034*
Above 60	37 (42.5)	50 (57.5)	0.049*
BMI			
18.5-24.9	11 (37.9)	18 (62.1)	0.068
25-29.9	36 (39.6)	55 (60.4)	0.005*
>30	85 (46.7)	97 (53.3)	0.209
Duration of diabetes			
Below 1 year	3 (50.0)	3 (50.0)	0.998
1-5 years	43 (43.0)	57 (57.0)	0.048*
6-10 years	44 (47.8)	48 (52.2)	0.552
11-20 years	33 (41.3)	47 (58.8)	0.027*
Duration of Metformin use			

Below 1 year	5 (45.5)	6 (54.5)	0.68
1-5 years	45 (40.2)	67 (59.8)	0.003*
6-10 years	46 (47.4)	51 (52.6)	0.47
11-20 years	34 (48.6)	36 (51.4)	0.741
Above 20 years	3 (20.0)	12 (80.0)	0.001*
Daily dosage of Metformin			
≤ 1000 mg	35 (43.2)	46 (56.8)	0.084
1000-1500 mg	8 (38.1)	13 (61.9)	0.128
≥ 2000 mg	90 (44.3)	113 (55.7)	0.022*

Table 1: General characteristics of patients with and without vitamin B_{12} deficiency.

Risk Factors	Deficient n(%)	Normal n(%)	P value
Dyslipidimia	120 (90.2)	150 (87.2)	0.412
Hypertension	90 (67.7)	113 (65.7)	0.717
Insulin treatment	24 (18.0)	27 (15.7)	0.586
Smoking	19 (14.3)	24 (14.0)	0.934
CVD	15 (11.3)	17 (9.9)	0.694
Hypothyroidism	7 (5.3)	12 (7.0)	0.539
Cerebrovascular d	8 (6.0)	6 (3.5)	0.296
Periph. Neurop. Neuropathy	9 (6.8)	5 (2.9)	0.11
Nephropathy	120 (90.2)	150 (87.2)	0.412

Table 2: Number and Percentage of patients in Normal and Deficient group with different risk factors.

Risk Factors	Deficient n(%)	Normal n(%)	P value
Dyslipidimia	120 (44.4)	150 (55.6)	0.009*
Hypertension	90 (44.3)	113 (55.7)	0.022*
Insulin treatment	24 (47.1)	27 (52.9)	0.56
Smoking	19 (44.2)	24 (55.8)	0.285
CVD	15 (46.9)	17 (53.1)	0.623
Hypothyroidism	7 (36.8)	12 (63.2)	0.108
Cerebrovascular d	8 (57.1)	6 (42.9)	0.461
Periph. Neuropathy	65 (43.9)	83 (56.1)	0.036*
Nephropathy	9 (64.3)	5 (35.7)	0.137

Table 3: Number and Percentage of Risk factors in patients with and without vitamin B_{12} deficiency.

Low vitamin B_{12} was observed in 133 participants which represents 43.6% of the total number. In comparing the patients with low vitamin B_{12} and the normal, normal vitamin B_{12} was significantly more frequent in age 41 and above (p=0.044), females 79 (60.3%) (p<0.001), non-Bahraini population 58 (49.8%) (p=0.007). Fifty five (60.4%) patients with a BMI between 25-29.9 had normal vitamin B_{12} level. None of the risk factors we investigated-dyslipidemia, hypertension, insulin treatment, smoking, CVD, hypothyroidism, nephropathy showed any statistically significant association with vitamin B_{12} deficiency (Table 2). On the other hand dyslipidemia, hypertension was found significantly more with a p-value of 0.009 and 0.022 respectively, in patients with normal vitamin B_{12} level. Only 17 patients representing 5.1% of the sample complained of cerebrovascular diseases, and 8(57.1%) of them had low vitamin B_{12} compared to 6 (42.9%) normal, but the difference was not statistically significant (Table 3).`

The correlation between serum B_{12} levels and the duration of metformin was determined in two different ways. Firstly we compared the patients who were using metformin for the same duration. There was significant difference in the 1to 5 years users and above 20 years users. At the same time in both this group the percentage of participant with normal B_{12} level were significantly higher. Most of the patients, 59.8% (p=0.003) using metformin for the last 1-5 years had normal B_{12} level. Similarly, 15 patients were receiving metformin for more than 20 years and 12 among them(80%) showed normal vitamin B_{12} level (p=0.001) (Figure 1). However when the duration was evaluated against the different levels of metformin, all of the patients who had a level below 100 were using metformin from 11 to 20 years, which was only 2.3% of that subgroup. On the other hand the longest time of metformin users which in this study is more than 20 years; none of them had serum B_12 level below 100. Moreover 46.7% of this group had vitamin B_{12} level within 301-450 pg/mL and 33.3% had more than 450 pg/mL (Figure 2). Also the patients receiving metformin less than one year; showed 45.5% participant B_{12} level within 301-450 pg/mL (Figure 2).
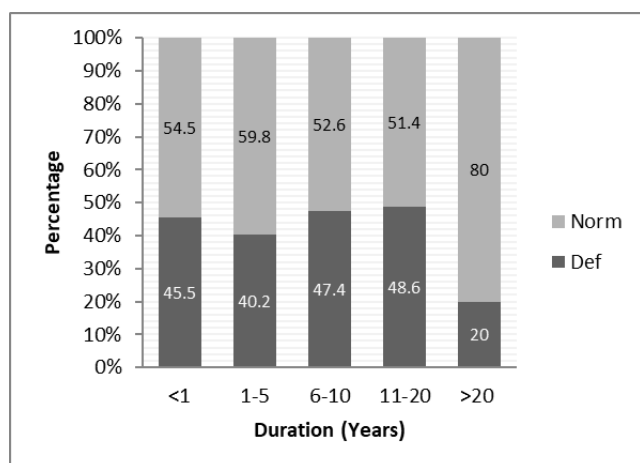
Figure 1: Percentage of Vitamin B₁₂ Deficient and Normal patients according to duration of metformin use. Significant difference in 1-5 years (p=0.003) and above 20 years (p=0.001).
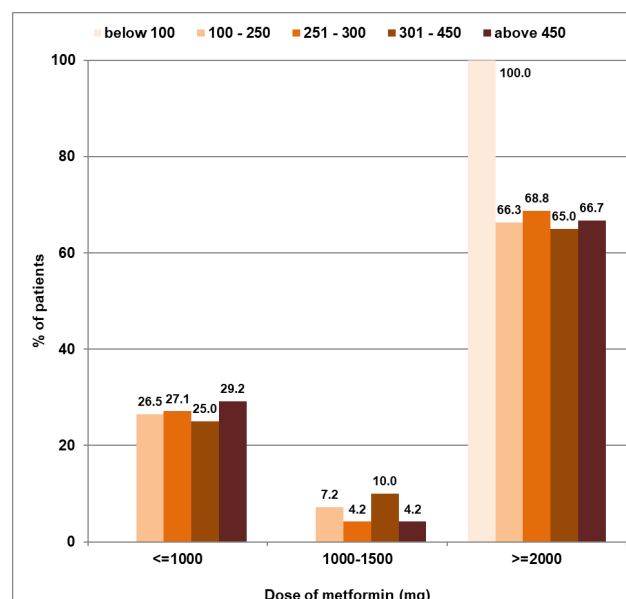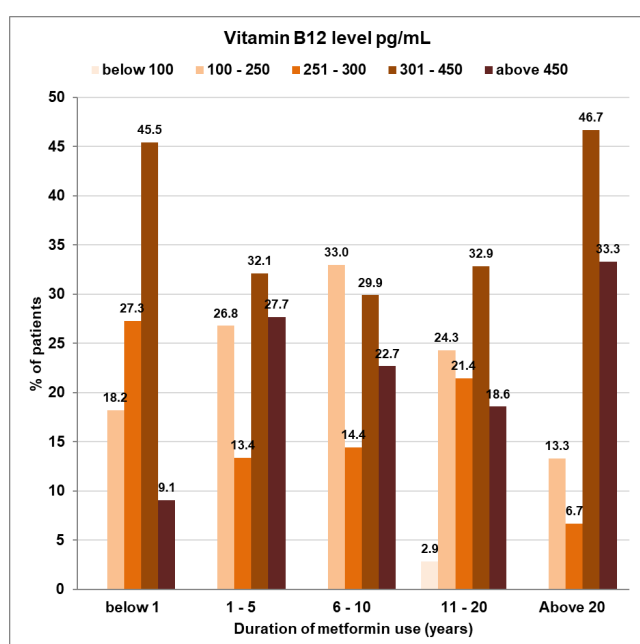

Figure 2: Percentage of Patients on different Vitamin B₁₂ level (pg/mL) according to the duration of Metformin Use.

Figure 3 shows correlation between the daily doses of metformin with vitamin B₁₂ deficiency. All the patients who had serum B₁₂ level less than 100 was receiving metformin on a dose of 2000 mg/day.

The patients who complained of peripheral neuropathy, 56% of them had normal vitamin B₁₂ level, which was significantly (p=0.036) more than the lower vitamin B₁₂ group.

Figure 3: Percentage of Patients on different Vitamin B₁₂ level according to the Dose of Metformin.

No statistically significant difference existed between the HbA1c level of the low vitamin B₁₂ and normal group, larger portion of both groups had HbA1c level between 6-7%; 37.6% for low and 29.1% for the normal vitamin B₁₂. Then again, when compared separately for each HbA1c level, significantly larger portion (64.6%) of the normal vitamin B₁₂ level patients had HbA1c level higher than 8% (p=0.004) in Table 4.

Characteristics	Vitamin B₁₂ deficiency, n (%) ≤ 300 pg/mL	Normal Vitamin B₁₂, n (%) >300 pg/Ml	P value
Level of last HbA1c	Comparison of deficient and normal		
Below 5.9%	6 (4.5)	9 (5.2)	
6-7%	50 (37.6)	50 (29.1)	
7.1-8%	36 (27.1)	44 (25.6)	0.445
8.1-9%	17 (12.8)	31 (18.0)	
Above 9%	24 (18.0)	38 (22.1)	
Level of last HbA1c	Comparison between HbA1c Levels		
Below 5.9%	6 (40.0)	9 (60.0)	0.282
6-7%	50 (50.0)	50 (50.0)	0.998
7.1-8%	36 (45.0)	44 (55.0)	0.207
8.1-9%	17 (35.4)	31 (64.6)	0.004*
Above 9%	24 (38.7)	38 (61.3)	0.012*

Table 4: Correlation between level of HbA1c and level of Vitamin B₁₂.

Anemia and hemoglobin level did not have any statistically significant difference between low and normal vitamin B$_{12}$.

Discussion

In our study we found that low vitamin B$_{12}$ level was prevalent in 43.6% of T2DM patients on metformin. Higher current daily dose and duration of metformin use have positive association with low vitamin B$_{12}$ level. The present study could not establish any association between the low vitamin B$_{12}$ level and BMI, Symptoms of peripheral neuropathy, anemia and hemoglobin level. The normal vitamin B$_{12}$ level patients had higher HbA1c level. Also this study found that more than 90% of patients were already checked for vitamin B$_{12}$ deficiency.

Prevalence of vitamin B$_{12}$ deficiency among diabetic patients shows a wide range of variation from 5.8% to 52% [5,8-15]. This discrepancy between different studies may be due to the fact that, each of the studies followed their own reference ranges for normal value of vitamin B$_{12}$, along with difference in age, study settings, and dose and duration of use of metformin [7].

De Jager et al. [5,8,9] studied patients from Netherlands, United States and Sweden. Their prevalence was much lower than the present study; 5.8 to 9.9%. Another study recent study conducted in South Africa found a moderately higher prevalence of 28.1% [7]. The difference might not reflect the difference in geographical distribution. Rather this might be explained by their cut off point ranging between 145-150 pmol/L compare to our 300 pmol/L. We chose this cutoff point following a recent study that conducted in a large sample of 700 patients in Korea, chose serum level ≤ 300 pg/mL their cut off point for vitamin B$_{12}$ deficiency [19]. Even with this high cutoff point their prevalence was 9.5%. The high prevalence in the current study might be explained by both the early detection of vitamin B$_{12}$ deficiency and high number of deficient patients. More than ninety one percent of the participants were already investigated for vitamin B$_{12}$ deficiency by their treating physicians.

In this study we did not find statistically significant difference in presence of cerebrovascular diseases between the participants with normal and low vitamin B$_{12}$ levels. When compared with total study population, in both deficient and normal vitamin D group, 48% was positive for peripheral neuropathy (p=0.987). In view of the fact that peripheral neuropathy is by itself is a complication of the T2DM, we further analyzed the data of the patients showing symptoms of peripheral neuropathy independently. That analysis revealed that significantly larger portion (56.1%) of the patients with peripheral neuropathy infact had normal vitamin B$_{12}$ level (p=0.036). Similar to our study the result of another study published in 2016 could not establish any association with the presence of diabetic peripheral neuropathy in T2DM patients and use of metformin [16]. Similar observation was reported from a study comparing metformin user and nonuser diabetic patients [17]. There was no difference in neurological function between the both groups.

The present study found that larger daily dose of metformin has a positive correlation with vitamin B$_{12}$ deficiency (Figure 3) [18]. Several previous studies have already reported this inverse effect of dose of metformin on serum vitamin B$_{12}$ level [7,12,15,19-21].

In our study we found weak association of duration of metformin use with low vitamin B$_{12}$ level. All the patients who had below 100 vitamin B$_{12}$ level were receiving metformin for 11-20 years. On the other hand almost half of the patients who were receiving metformin

for more than 20 years had a serum vitamin B$_{12}$ level between 300 to 450 pg/mL (Figure 2). Several other studies have recognized longer duration of metformin use as an independent risk factor for vitamin B$_{12}$ deficiency in T2DM patients [5,7,19-22]. de Jager et al. [5] revealed that a -19% (95% confidence interval -24% to -14%; P<0.001) mean decrease of vitamin B$_{12}$ level was observed in long term metformin users in comparison to placebo group. Ahmed et al. [7] demonstrated that metformin use duration was significantly longer (11 *vs.* 8 years, P=0.015) in B$_{12}$-deficient participants. Ko et al. [19] observed that metformin use of more than 4 years is associated with Vitamin B$_{12}$ deficiency (P<0.001). Similar to their observation, Ting et al. [21] demonstrated that Vitamin B$_{12}$ deficiency has significant association with metformin use of 3 years or more the adjusted odds ratio was (2.39, 95% confidence interval, 1.46-3.91) (P=0.001). Contrary to all these observations, Chen et al. [17] did not find any significant association between metformin duration and vitamin B$_{12}$ deficiency. In our study when we compared vitamin B$_{12}$ deficient and normal patients receiving metformin for less than 1 year, 1-5 years, 6-10 years, 11-20 years and longer than 20 years. Our result revealed no significant association between these two groups in each year category (Figure 1).

Level of HbA1c in relation to serum vitamin B$_{12}$ levels was also calculated. We demonstrated that high HbA1c level is found more frequently in T2DM patients with a normal vitamin B$_{12}$ level. Previously two other studies [7,14] supported similar association. Ahmed et al. [7] tried to explain this association from the perspective of patient compliance. The gastrointestinal adverse effects of metformin may promote poor compliance in patients, especially with high dose of the drug. The poor or noncompliance may consequently lead to poor glycaemic control and higher HbA1c.

One major limitation of this study is that we did not look for the number of patients already on vitamin B$_{12}$ supplementation (some of the patient may take vitamin B$_{12}$ supplementation from other hospitals) there is a possibility that a good number of patients are receiving it. And that might be the reason for our study result not showing much significant difference between the variables of both the groups.

Conclusion

Our result indicates that vitamin B$_{12}$ deficiency is prevalent among type 2 diabetic patients treated with metformin, especially those on high dose of medication and longer duration. There is no association of vitamin B$_{12}$ deficiency with diabetic neuropathy. Also this study revealed that the majority of the patients are being investigated for Vitamin B$_{12}$ deficiency, which is a very positive sign for the health care system. So we recommend routine screening for basal vitamin B$_{12}$ level for all patients with T2DM at the initiation of metformin therapy with subsequent annual follow up of the level. Although we could not establish any association between diabetic neuropathy and Vitamin B$_{12}$ deficiency, further studies are needed to identify symptoms that will help to distinguish these two separate entities.

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