PREVALENCE AND RISK FACTORS OF VITAMIN B\textsubscript{12} DEFICIENCY AMONG PATIENTS WITH TYPE II DIABETES ON METFORMIN: A STUDY FROM NORTHERN REGION OF UNITED ARAB EMIRATES

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INTRODUCTION

The UAE is ranked 16\textsuperscript{th} worldwide, with 19\% of the UAE population living with diabetes [1]. These statistics indicate that the region has high-risk factors for diabetes, mostly related to rising obesity rates and physical inactivity. A sedentary lifestyle and bad eating habits are cited as the main causes of the increasing prevalence of type 2 diabetes mellitus (T2DM) in the UAE. It is becoming increasingly clear that T2DM is associated with decreasing level of activity and an increasing prevalence of obesity.

According to the World Health Organization, the number of T2DM patients is expected to double within the next 25 years.

Metformin’s best known and most feared side effect, i.e., lactic acidosis almost never occurs if metformin is used appropriately [2]. The common side effects of metformin are gastrointestinal such as abdominal distress, soft stools, and diarrhea. In general, these side effects appear shortly after the initiation of metformin and promptly disappear after discontinuation. However, insidious or asymptomatic side effects resulting from long-term treatment, such as Vitamin B\textsubscript{12} deficiency, may not be easily detected without close attention. It is important to recognize the clinical consequences of Vitamin B\textsubscript{12} deficiency. Vitamin B\textsubscript{12}, which is a water-soluble vitamin plays a very fundamental role in DNA synthesis, optimal hematopoiesis, and neurological function. The clinical picture of Vitamin B\textsubscript{12} deficiency, hence, is predominantly of features of hematological and neurocognitive dysfunction [3].

Several studies have screened outpatients taking metformin for Vitamin B\textsubscript{12} deficiency. The association between metformin treatment and impaired Vitamin B\textsubscript{12} status dates back to the 1970s when the interaction was first proposed [4], and estimates of prevalence were stated to be close to 30\%. Since then, the prevalence of metformin-associated Vitamin B\textsubscript{12} deficiency has been reported in many different studies; however, the rates of incidence in such populations vary by a large amount. While many studies estimate the rate of prevalence to be around 10–20\% of treated patients [5,6], other studies have reported levels as low as 5\% of treated patients [7] and levels higher than 30\% [8].

The mechanism by which metformin therapy causes Vitamin B\textsubscript{12} deficiency is not clear, but it is thought to be due to either alteration in small bowel motility, which stimulate small bowel bacterial overgrowth and subsequent Vitamin B\textsubscript{12} deficiency, or by directly decreasing Vitamin B\textsubscript{12} absorption [9,10]. The absorption of the Vitamin B\textsubscript{12} intrinsic factor complex by cells of the terminal ileum is calcium dependent, and metformin alters intracellular handling of calcium, thereby reducing absorption [9,10]. The later theory is supported by the fact that the administration of calcium reverses metformin-induced Vitamin B\textsubscript{12} deficiency [10].

Although the clinical significance of Vitamin B\textsubscript{12} deficiency related to metformin treatment is debatable, monitoring for Vitamin B\textsubscript{12} deficiency may lead to increasing the considerable problem of peripheral neuropathy in non-insulin-dependent DM patients. Neuropathy, being an impending health abnormality occurring due to Vitamin B\textsubscript{12} deficiency, affects around 30\% diabetics who are over 40 years of age and states about having a diminished sensory perception in their feet [12]. A proposed
mechanism for these neurological effects is the disturbance of myelin synthesis due to impaired production of methionine [13]. Therefore, it is worthwhile to consider the prevalence of Vitamin B<sub>12</sub> deficiency among the growing T2DM population.

As metformin has been prescribed worldwide and treatment periods increase, the prevalence of metformin-induced Vitamin B<sub>12</sub> deficiency may have also significantly increased. However, the relationship between metformin use and Vitamin B<sub>12</sub> deficiency in the UAE population has not been widely investigated. This study focused on the prevalence of Vitamin B<sub>12</sub> deficiency in UAE patients with T2DM who were treated with or without metformin.

**METHODS**

**Study design, participants, and procedures**

This study is a descriptive, cross-sectional study. A total of 213 participants were randomly selected to be part of the study in Northern Regions of the UAE, from June 2014 to February 2015. 200 patients who fulfilled the inclusion criteria were included in the study.

All patients aged 45 years or older who were being treated for T2DM at the outpatient clinic were eligible for inclusion, regardless of metformin use. We excluded all participants with diabetes after necrotic pancreatitis, late-onset autoimmune disease of adults, and pure vegetarians who had a history of pernicious anemia, chronic renal insufficiency defined by a creatinine 3.0, prior bariatric surgery, gastrectomy, B<sub>12</sub> supplementation with B<sub>12</sub> shots or an oral Vitamin B<sub>12</sub> dose of 500 mcg/day, prior ileum resection, or Crohn’s disease.

Before and during their regular scheduled visit to their treating internist, patients were informed about this study. Subsequently, patients could consult one of the investigators to receive more information and sign informed consent when they had agreed to participate.

During this visit, a structured questionnaire was used to collect the data. It consisted of two parts; first part included demographic data and the second part included 17 study questions which consist of information about the level of Vitamin B<sub>12</sub>, duration of diabetes, hemoglobin A1c, metformin use, and metformin duration. Data on additional patient characteristics and diabetes complications were retrospectively searched for in the patient records. Vitamin B<sub>12</sub> level <150 pmol/L was considered deficient, whereas Vitamin B<sub>12</sub> level ≥150 pmol/L was considered normal.

**Statistical analysis**

All statistics were done with SPSS software version 20.0 (SPSS, Inc., Chicago, IL, USA). Associations between categorical values and B<sub>12</sub> deficiency were done with two analyses. Associations between continuous variables and Vitamin B<sub>12</sub> deficiency were done with Student’s t-test. Student’s t-test was also used to determine associations between metformin use and serum level of B<sub>12</sub>. A multivariate analysis using logistic regression was used to identify factors independently associated with Vitamin B<sub>12</sub> deficiency. Covariates chosen for the multivariate model were known or hypothesized biologic factors that would affect Vitamin B<sub>12</sub> deficiency. p<0.05 was considered statistically significant.

**RESULTS**

A total of 213 patients enrolled in the study. 13 patients were excluded: Nine were younger than 45 years of age and four were taking supplemental B<sub>12</sub> 500 mcg/day. The remaining 200 patients were included in the final analysis.

Table 1 summarizes the participants demographic data. The mean ± standard deviation (SD): age of the patients was 49.5 ±7.8 years; duration of diabetes 3.48 ±3.01 years; body mass index (BMI) was 28.51 ±5.25; hemoglobin A1c (HbA1c) was 6.7 ±0.62; metformin duration 3.35 ±1.88 years, and serum Vitamin B<sub>12</sub> level 158.5 ±43.36 pmol/L and ranged from 62 to 351 pmol/L among all patients. There were 96 (48%) patients with Vitamin B<sub>12</sub> deficiency (<150 pmol/L) and 104 (52%) patients with normal Vitamin B<sub>12</sub> level (≥150 pmol/L).

The majority of participants (84%) took metformin had neuropathy, hypertension, dyslipidemia, numbness or paresthesia, and depression, or mood changes 140 (70%), 183 (91.5%), 134 (67%), 136 (68%), 161 (80.5%), and 120 (60%), respectively. Regarding gender more than half of the patients were male 102 (51%) and 98 (49%) were female.

Table 2 summarizes key variable comparisons between patients with and without B<sub>12</sub> deficiency.

Regarding continuous variables, age, diabetes duration, metformin duration, BMI, and Hba1c were not associated with B<sub>12</sub> deficiency (p>0.05).

When a second X<sup>2</sup> was performed defining at risk for Vitamin B<sub>12</sub> deficiency as any serum level <150 pmol/L, the results indicated that patients using metformin were statistically at higher risk for Vitamin B<sub>12</sub> deficiency (odds ratio, 2.64; 95% confidence interval, 1.40–4.99).

A series of further analysis were subsequently performed to determine whether metformin duration was associated with B<sub>12</sub> deficiency. In this study population, there was no statistically significant association between the average prescribed metformin duration for patients with B<sub>12</sub> deficiency (mean±SD) 3.29±1.93 and patients without B<sub>12</sub> deficiency 3.43±1.84 (p=0.66).

A t-test was conducted to determine whether any difference existed in the B<sub>12</sub> level of individuals using metformin versus those who were not. Patients taking metformin had statistically significant lower level of B<sub>12</sub> (123.71 pmol/L vs. 190.69 pmol/L; p<0.001) (Table 3).

**The prediction equation**

Vitamin B<sub>12</sub> Deficiency = 1.336 + 0.003 (BMI) − 0.001 (Age) + 0.003 (DM duration) + 0.006 (Metformin duration)

BML age, duration of diabetes, and metformin duration values (independent variables) were included in multiple regression test as predictors for Vitamin B<sub>12</sub> deficiency level (outcome dependent variable). Only metformin duration was the significant predictor (p<0.001). We randomly selected 10 patients from our list to calculate the predicated Vitamin B<sub>12</sub> deficiency. The results were very predictive as follows:

**Table 1: Characteristics of outpatient patients with type 2 diabetes**

| Characteristics (N=200) | Value |
|------------------------|-------|
| Age (years) (mean±SD)  | 49.5±7.8 |
| Serum Vitamin B<sub>12</sub>, pmol/L (mean±SD) | 158.5±43.36 |
| Duration of diabetes (years) (mean±SD) | 3.48±3.01 |
| Body mass index (kg/m2) (mean±SD) | 28.51±5.25 |
| Hemoglobin A1c (%) (mean±SD) | 6.73±0.62 |
| Metformin duration (years) (mean±SD) | 3.35±1.88 |
| Metformin use n (%) | 140 (70) |
| Gender Male n (%) | 102 (51) |
| Female n (%) | 98 (49) |
| Insulin use n (%) | 43 (21.5) |
| Sulfonylurea use n (%) | 76 (37) |
| Retinopathy n (%) | 40 (20) |
| Neuropathy n (%) | 183 (91.5) |
| Nephropathy n (%) | 31 (15.5) |
| Hypertension n (%) | 134 (67) |
| Ischemic heart disease n (%) | 14 (7) |
| Dyslipidemia n (%) | 136 (68) |
| Numbness or paresthesia n (%) | 161 (80.5) |
| Depression or mood changes n (%) | 120 (60) |
| Memory changes n (%) | 43 (21.5) |

SD: Standard deviation

**Table 2: Summary of key variable comparisons**

| Predictor | Without deficiency | With deficiency | p-value |
|-----------|--------------------|-----------------|---------|
| Metformin use | 102 (51%) | 98 (49%) | 0.318 |
| Metformin duration | 3.35±1.84 | 3.43±1.84 | 0.66 |
| BMI | 28.51±5.25 | 26.7±3.0 | 0.003 |
| Age | 49.5±7.8 | 50.5±8.2 | 0.67 |
| DM duration | 3.43±1.84 | 3.48±3.01 | 0.66 |

**Table 3: Summary of key variable comparisons**

| Predictor | Without deficiency | With deficiency | p-value |
|-----------|--------------------|-----------------|---------|
| Metformin use | 102 (51%) | 98 (49%) | 0.318 |
| Metformin duration | 3.35±1.84 | 3.43±1.84 | 0.66 |
| BMI | 28.51±5.25 | 26.7±3.0 | 0.003 |
| Age | 49.5±7.8 | 50.5±8.2 | 0.67 |
| DM duration | 3.43±1.84 | 3.48±3.01 | 0.66 |
The mean percentage of the prediction was 81% that is high enough for prediction equation to be a powerful tool for reproducing testable results.

Multivariate logistic regression analysis showed that in this study sample, metformin duration significantly contributed (p<0.0001) to the prediction of Vitamin B\textsubscript{12} deficiency among patients with T2DM. Age and metformin use were insignificant predictors of Vitamin B\textsubscript{12} deficiency (Table 4).

**DISCUSSION**

In this cross-sectional study, we are aware of that was specifically designed to define the prevalence of B\textsubscript{12} deficiency in patients with T2DM. In our cohort, we identified 48% of T2DM patients with B\textsubscript{12} deficiency [14]. In multivariate models, metformin use was a positive and age use was a negative predictor of this deficiency. Of all patients (regardless of metformin use), 15.5% were nephropathy and 91.5% had neuropathy. It is possible that the chronic disease T2DM in itself is sufficient to explain the nephropathy in this population. Furthermore, 3.29 years of metformin use was related to a higher prevalence of neuropathy than when no metformin was used.

Our study showed that the prevalence of B\textsubscript{12} deficiency among secondary care treated T2DM patients was 80% in metformin users (median metformin use 3.29 years) and 20% in non-metformin users. Previous studies assessing T2DM patients on metformin have reported the prevalence of Vitamin B\textsubscript{12} deficiency to range from 5.6% to 33%. This wide variation in the reported prevalence could probably be explained by the varied study definitions of Vitamin B\textsubscript{12} deficiency. Tomkin, in 1972, was the first described cohort in T2DM patients using metformin for <5 years, a prevalence of 5.6% among metformin users was found [15]. In the cross-sectional study by Pflipsen et al found a prevalence of 22.6% among patients with T2DM using metformin, in a primary care setting [16]. In a study by De Jager et al. found a 9.9% prevalence of B\textsubscript{12} deficiency in patients treated with metformin for 4.3 years [11]. Reinstatler et al. defined B\textsubscript{12} deficiency as B\textsubscript{12} ≤148 pmol/l and found a prevalence of B\textsubscript{12} deficiency of 5.8% in a cohort of patients followed for 6 years [7]. A recent cross-sectional study documented a high prevalence of Vitamin B\textsubscript{12} deficiency of 33% among adult patients with T2DM by Qureshi et al. Vitamin B\textsubscript{12} deficiency was defined as serum Vitamin B\textsubscript{12} concentration <150 pg/mL [17]. It was observed that the prevalence of decreased serum Vitamin B\textsubscript{12} status in metformin-treated patients was 80% in metformin users (median metformin use 3.29 years) and 20% in non-metformin users.

**Table 2: Bivariate associations with Vitamin B\textsubscript{12} deficiency**

| Continuous variables | Vitamin B\textsubscript{12} deficiency Yes (n=96) | Vitamin B\textsubscript{12} deficiency No (n=104) | p value |
|----------------------|---------------------------------------------|-----------------------------------------------|---------|
| Age, years (Mean±SD) | 49.97±7.76                                  | 49.04±7.80                                   | 0.39    |
| Diabetes duration - years (Mean±SD) | 3.63±7.113 | 3.34±7.015 | 0.49 |
| Metformin duration (Mean±SD) | 3.29±2.125 | 3.43±2.84 | 0.66 |
| Body mass index (kg/m\textsuperscript{2}) (Mean±SD) | 28.5±5.21 | 28.6±5.3 | 0.80 |
| Hemoglobin A1c (%) (Mean±SD) | 6.8±0.66 | 6.7±0.58 | 0.24 |
| Categorical variables (%) | | | |
| Gender | | | 0.786 |
| Male | | | |
| Female | | | |
| Metformin use | | | 0.002 |
| Yes | | | |
| No | | | |
| Insulin use | | | 0.022 |
| Yes | | | |
| No | | | |
| Sulfonylurea | | | 0.002 |
| Yes | | | |
| No | | | |
| Retinopathy | | | 0.322 |
| Yes | | | |
| No | | | |
| Neuropathy | | | 0.144 |
| Yes | | | |
| No | | | |
| Nephropathy | | | 0.407 |
| Yes | | | |
| No | | | |
| Hypertension | | | 0.193 |
| Yes | | | |
| No | | | |
| Ischemic heart disease | | | 0.877 |
| Yes | | | |
| No | | | |
| Dyslipidemia | | | 0.489 |
| Yes | | | |
| No | | | |
| Numbness or paresthesia | | | 0.126 |
| Yes | | | |
| No | | | |
| Depression or mood changes | | | 0.644 |
| Yes | | | |
| No | | | |
| Memory changes | | | 0.901 |
| Yes | | | |
| No | | | |

SD: Standard deviation
patients in our study was higher than those in the previous studies. This comparison must be interpreted with caution because there are other factors that may affect the serum Vitamin B₁₂ of these patients which were not addressed in this study (diet, drug interactions, etc.). However, it is interesting to note that our sample population had a better Vitamin B₁₂ status than that of a population without T2DM or metformin treatment.

Ting et al. studied risk factors of B₁₂ deficiency in patients receiving metformin. The dose of metformin was the strongest independent predictor of Vitamin B₁₂ deficiency, and a longer duration of treatment with metformin was associated with a higher prevalence [18]. This study shows an association between the decreasing B₁₂ level and metformin duration. In accordance with the present study, while Reinstatler et al. found no clear increase in the prevalence of deficiency as the duration of metformin use increased [2].

The relatively high prevalence of B₁₂ deficiency found in this study makes it likely that at least a portion of peripheral neuropathy cases in diabetic patients may be attributed to B₁₂ deficiency. Previous studies have demonstrated that supplemental Vitamin B₁₂ improved somatic and autonomic symptoms of diabetic neuropathy [19,20]. Testing for, and treating, B₁₂ deficiency in those patients with neuropathy may lead to improved clinical outcomes. Clinical trials are needed to further evaluate this link.

The present study adds to this discussion by again defining a prevalence, confirms the influence of metformin on a Vitamin B₁₂ deficiency, and shows that although metformin increases B₁₂ deficiency rates, it does not increase odds for neuropathy after 3.29 years treatment with metformin. This finding argues against standard screening and/or supplementation of Vitamin B₁₂ in metformin-treated T2DM patients. We would, therefore, like to plead for more research focusing on the consequences of a metformin-induced B₁₂ deficiency to determine whether screening and supplementation are necessary.

Regarding the relation between the prevalence of B₁₂ deficiency and the age, several studies demonstrated a prevalence of B₁₂ deficiency in the elderly that ranges from 12% to 23% [21,22]. Although the prevalence of B₁₂ deficiency in our diabetic patients was in line with these results, it is important to note that the average age of our population was approximately 10 years younger than the average age of the elderly volunteers enrolled in these other studies. In addition, both bivariate and multivariate analyses demonstrated that age was not significantly associated with B₁₂ deficiency. This suggests that type 2 diabetes, not age, may account for the 48% prevalence of B₁₂ deficiency.

Patients on chronic metformin therapy seem to be at increased risk for B₁₂ deficiency. Its use is associated with lower serum Vitamin B₁₂ level [14,24-26]. Several studies associate metformin use with established clinical B₁₂ deficiency [17,26,27]. It has been reported that higher doses and longer treatment with metformin seem to be risk factors for such deficiency [28]. Although we found that patients using metformin had lower B₁₂ level, we did not find metformin use to be associated with overt B₁₂ deficiency. Our study was not designed nor powered to find these secondary associations. Current metformin use was associated with a significantly higher risk for B₁₂ deficiency when defined as a serum B₁₂ level <150 pmol/L. Patients with B₁₂ level <150 pmol/L may be at risk for B₁₂ deficiency because tissue deficiency may occur despite normal serum B₁₂ level [22,29,30]. Identification of patients “at risk” for B₁₂ deficiency as those with serum B₁₂ <150 pmol/L may help the clinician define a level to test for B₁₂ deficiency using specific tissue markers, especially among diabetics who are using metformin.

Our multivariate analysis looked for specific associations for B₁₂ deficiency. It showed that only metformin duration seemed to be a significant predictor in diabetic patients from B₁₂ deficiency. Other factors known to increase risk for B₁₂ deficiency, such as advanced age and duration of DM, were not significantly associated with B₁₂ deficiency. Further, research needs to be conducted in large and well-designed studies on screening Vitamin B₁₂ deficiency and also to look at the potential risk or protective factors for Vitamin B₁₂ deficiency.

CONCLUSION

The prevalence of B₁₂ deficiency in secondary care T2DM patients using metformin was estimated at 80%. The prevalence is significantly higher in patients treated with metformin compared with non-metformin users. Furthermore, metformin use does predict the deficiency of Vitamin B₁₂ in diabetic patients’ after 3.29 years treatment with metformin. Moreover, metformin use did lead to neuropathy.

AUTHOR’S CONTRIBUTION

Moayad Shahwan drafted and edited the manuscript, Nageeb Hassan interpreted the results, Adel Noshi did the data collection and statistical analysis, and Naheed Banu approved the final version of the manuscript.
CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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