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SPECIAL REPORT

Vitamin B_{12} deficiency and the lack of its consequences in type 2 diabetes patients using metformin

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ABSTRACT

Objectives: To study vitamin B_{12} concentrations in patients with type 2 diabetes with and without metformin use and to identify risk factors and consequences of low vitamin B_{12} concentrations.

Research design and methods: This study had a cross-sectional design. During eight weeks all patients with type 2 diabetes visiting the diabetic outpatient clinic of the Isala Clinics in Zwolle were approached for participation. Participation included measurement of haemoglobin, mean corpuscular volume and vitamin B_{12} levels. Data on neuropathy were retrospectively searched for in the patient records. Vitamin B_{12} deficiency was defined as serum B_{12} concentrations <150 pmol/l.

Results: In the total cohort (n=298), the overall prevalence of vitamin B_{12} concentrations <150 pml/l was 9.7% (95% CI 6.6-13.7%). In type 2 diabetes patients not taking metformin (n=134), the prevalence was 4.4% (95% CI 1.6-9.4%) compared with 14.1% in metformin users (n=164) (95% CI 9.2-20.4%; p=0.006). Each 100 mg step in metformin dose increased (OR=1.081, p=0.014), whereas PPI use lowered (OR=0.322, p=0.037) the odds of having a vitamin B_{12} deficiency in logistic regression. Nevertheless, metformin use did not predict the chance on having anaemia or neuropathy.

Conclusion: Among patients with type 2 diabetes using metformin, the prevalence of vitamin B_{12} deficiency is higher than compared with patients not using metformin. However, metformin use did not predict the chance of having anaemia or neuropathy.

KEYWORDS

Anaemia, metformin, neuropathy, type 2 diabetes mellitus, vitamin B_{12} deficiency

INTRODUCTION

Metformin is the drug of choice in the treatment of type 2 diabetes.[1] One of the possible side effects of metformin that has gained attention over the last few years is a decreased concentration of vitamin B_{12} due to decreased absorption.[2] It is estimated that this problem occurs in approximately 10-30% of patients using metformin[2] and is associated with a 4-24% decrease of vitamin B_{12} (B_{12}) concentrations.[3,4] Although it may take up to five years before a deficiency manifests itself,[5] it potentially has serious consequences, with an increased risk of macrocytic anaemia and neurological disturbances. However, when acknowledged and treated in time, these consequences can be prevented.[6]

In one of the earliest studies on metformin and B_{12} deficiency, published in 1972, a prevalence of B_{12} deficiency of 5.6% among type 2 diabetes patients using metformin was found.[2] Subsequent studies showed a prevalence of 5.8-22.6%.[7,8] In a recent randomised, placebo-controlled trial in type 2 diabetes patients using insulin, 196 patients were given metformin for 4.3 years while 194 patients received a placebo. The mean duration of diabetes was 14 (SD=9) years in the metformin group and 12 (SD=8) years in the placebo group. At baseline, 1.6% of patients using metformin and 2.2% patients on placebo had a B_{12} deficiency. The average daily metformin dose in the metformin group was 2050 mg. Of the participants treated with metformin, 9.9% had a vitamin B_{12} deficiency (defined as a concentration <150 pmol/l) at the end of the trial compared with 2.7% in placebo-treated patients (p=0.004). Incidences of anaemia or neuropathy were not reported.[4]

Since these studies do not describe what the actual prevalence of B_{12} deficiency is among metformin-treated type 2 diabetes patients, the aim of this study was to

determine its prevalence in a secondary care setting and to compare it with the prevalence of B_{12} deficiency in non-metformin treated type 2 diabetes patients, treated in the same setting. In addition, the relationship between metformin use and presence or absence of anaemia and neuropathy was investigated.

PATIENTS AND METHODS

Study design, participants and procedures

This study had a cross-sectional design to describe the prevalence of vitamin B_{12} deficiency among a sample of individuals with type 2 diabetes at the outpatient clinic of the Isala Clinics, Zwolle, the Netherlands.

All patients aged 18 years or older who were being treated for type 2 diabetes at the outpatient clinic were eligible for inclusion, regardless of metformin use. Participants with diabetes after necrotic pancreatitis, late-onset autoimmune diabetes of adults (LADA) or use of cobalamin injections or tablets were excluded.

Prior to 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, information about metformin use and dosage, supplemental vitamin use, proton pump inhibitor (PPI) use, height and weight was gathered. Electronic patient records were consulted for additional patient characteristics and diabetes complications. Finally, blood samples were collected directly following this visit.

Measurements

Haemoglobin (Hb) and mean corpuscular volume (MCV) were measured with the SE9000 Haematology Analyser (Sysmex); total vitamin B_{12} was measured with the Modular Analyser (Roche Diagnostics). Anaemia was defined as Hb <8.5 mmol/l in men and <7.5 mmol/l in women. Macrocytosis was defined as MCV >100 fl. Vitamin B_{12} levels <150 pmol/l were considered to indicate deficiency. Vitamin B_{12} levels were grouped as follows: deficiency (<150 pmol/l), low-normal (150-220 pmol/l) and normal (>220 pmol/l). Patient record was searched to determine the presence of neuropathy. Neuropathy was considered present when the treating internist recorded this in the patient records.

Statistical analysis

Assuming a 10% prevalence of B_{12} deficiency in metformin-using patients in the study by De Jager *et al.*,[4] power analysis showed that 158 patients were needed to estimate the prevalence of B_{12} deficiency in type 2 diabetes patients using metformin with a 95% confidence interval width of 10%. Investigated categorical variables included sex, insulin use, use of other DM medication, proton pump inhibitor use, use of vitamin B containing supplements, nephropathy, retinopathy, neuropathy and macrovascular complications. Continuous variables included age, body mass index, duration of type 2 diabetes, duration of metformin use, duration of insulin use, Hb and MCV levels. Associations between B_{12} deficiency and categorical variables were determined with Fisher's exact test. Associations between continuous variables and vitamin B_{12} deficiency were determined with Student's *t* test or the non-parametric Mann-Whitney U test, depending on the distribution of the continuous variables. A multivariate analysis, using binary logistic regression, was used to estimate the influence of factors associated with a B_{12} deficiency. A multiple linear regression analysis was performed to estimate the impact of factors influencing B_{12} levels. The same procedures were performed to estimate the impact of variables influencing Hb levels and predicting anaemia or neuropathy. A (two-sided) p-value of less than 0.05 was considered statistically significant. All analysis were performed using SPSS version 18.0, Inc, Chicago, Il, USA.

The study was performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients, and the protocol was approved by the medical ethics committee of the Isala Clinics in Zwolle.

RESULTS

Patient characteristics

Patients were included between 1 April and 27 July 2012. A total number of 426 patients were eligible for participation in the study of whom 114 (27%) patients declined participation. In addition, eight patients were excluded because they used intramuscular cobalamin injections and six patients gave informed consent but did not have their blood drawn. Subsequently 298 (70%) patients were included in the present study. Of these patients, 164 (55%) had used metformin for at least two weeks, with a maximum of 31.9 years. For two patients these records were not available. For 38 patients using metformin, there were no data on the exact duration of metformin use.

Patient characteristics of all patients classified by metformin use are shown in *table 1*. Patients using metformin were younger, had a shorter median diabetes duration, showed neuropathy less frequently and less often used insulin.

Vitamin B_{12} deficiency

Serum vitamin B_{12} levels ranged from 72-873 pmol/l among all patients. There were 29 (9.7%, 95% confidence interval

Table 1. *Characteristics of type 2 diabetes patients*

	All patients (n=298)	Metformin use (n=164)	No metformin use (n=134)	p-value
Age (years) (mean, SD)[a]	64.8 (11.6)	62.6 (11,9)	67.2 (10.8)	0.001*
Sex (male) (n, %)[c]	158 (52.8)	90 (55.2)	67 (49.6)	0.353
Body mass index (kg/m²) (median (P25-P75)[b]	31.1 (27.6-35.7)	31.6 (27.6-35.9)	30.8 (27.5-35.4)	0.400
Type 2 diabetes (years) (median (P25-P75)[b]	14.9 (8.9-20.9)	12.1 (7.6-17.9)	17.9 (12.1-23.9)	<0.001*
Metformin use (years) (median (P25, P75, min, max)[b]	-	4.9 (1.7-8.1-0.04-31.9)	-	-
Insulin use (n, %)[c]	256 (86.2)	128 (79.0)	128 (94.8)	<0.001*
Duration of insulin use (median (P25-P75)[b]	8.3 (4.9-13.9)	7.1 (3.9-10.7)	10.5 (6.8-15.9)	<0.001*
Use of other DM medication[d] (n, %)[c]	64 (21.7)	50 (31.3)	14 (10.4)	<0.001*
Proton pump inhibitor use (n, %)[c]	130 (43.6)	74 (45.4)	56 (41.5)	0.558
Use of vitamin B containing supplements (n, %)[c]	25 (9.4)	15 (9.2)	10 (7.4)	0.677
Microvascular complications (n, %)[c]	148 (50.0)	72 (45.0)	75 (55.6)	0.080
Nephropathy (n, %)[c]	60 (20.0)	30 (18.6)	29 (21.5)	0.562
Retinopathy (n, %)[c]	70 (23.6)	41 (25.5)	29 (21.5)	0.493
Neuropathy (n, %)[c]	66 (22.3)	28 (17.4)	38 (28.1)	0.035*
Macrovascular complications (n, %)[c]	105 (35.7)	51 (32.1)	54 (40.0)	0.180

[a]Student's *t* test; [b]Mann Whitney U test; [c] Fisher's exact test; [d]diabetes medication other than metformin or insulin; *difference between metformin users and non-metformin users.

(CI) 6.6-13.7%) subjects with B_{12} deficiency (<150 pmol/l), 65 (21.8%, 95% CI 17.3-26.9%)) patients with low-normal B_{12} levels (150-220 pmol/l) and 204 (68.5%, 95% CI 62.8-73.7%) patients with normal B_{12} levels (>220 pmol/l). In metformin users, B_{12} deficiency was present in 14.1% (95% CI 9.2-20.4) and in non-metformin users 4.4% (95% CI 1.6-9.4%). The absolute difference in prevalence was 9.7% (95% CI 3.2-6.1) (p =0.003). Furthermore, as depicted in *table 2*, metformin users more often had low-normal vitamin B_{12} levels (p<0.001). Only five patients (1.7%, 95% CI 0.5-3.9%) had B_{12} levels below 100 pmol/l, all using metformin.

Prediction of vitamin B_{12} deficiency

In linear regression, metformin dosage was the only variable able to predict vitamin B_{12} levels significantly. When correlating dose increases of metformin with B_{12} concentrations, each incremental increase of 100 mg in metformin dose was associated with an incremental decrease in vitamin B_{12} levels of 3.77 pmol/l (95% CI -6.79 to -0.81). In logistic regression, each dose increase of 100 mg metformin increased odds for B_{12} deficiency by 8% (OR 1.08; 95% CI 1.02-0.15). PPI use reduced odds for B_{12} deficiency by 68% (OR 0.32; 95% CI 0.11-0.94). In this study, duration of metformin use did not have a significant effect in this model. Results of this regression analysis are shown in *table 3*.

Consequences of vitamin B_{12} deficiency

In the metformin group 23.3% showed anaemia, compared with 22.3% in the group not using metformin (95% CI of the difference -1.1 to 8.6; p=0.82). Sex was the only factor influencing odds for anaemia (OR 2.26; 95% CI 1.04-4.93 when male).

Of all individuals with type 2 diabetes, 68 (22.8%) were anaemic of whom 14 (4.7%) also had a vitamin B_{12} deficiency. Of the 29 patients with B_{12} deficiency 14 (48%) were anaemic. Among 269 patients without B_{12} deficiency 44 (20%) were anaemic (p=0.002). In the metformin group, patients with B_{12} deficiency were also more likely to have anaemia (43.5%), compared with metformin-using patients without B_{12} deficiency (20.0%) (p=0.03). There was only one patient with a macrocytic anaemia in both the metformin and the non-metformin group. In both the metformin and non-metformin group three patients had a microcytic anaemia. Mean MCV in the metformin group was 89.1 fl compared with 90.2 in the non-metformin group (p=0.08).

Table 2. *Metformin use and vitamin B_{12} levels*

		Vitamin B_{12} levels			
		Deficient(<150 pmol/l)	Low-normal(150-220 pmol/l)	Normal(>220 pmol/l)	Total
Patients not on metformin	n (%)	6 (4.4)	18 (13.3)	111 (82.2)	135 (45.3)
Patients on metformin	n (%)	23 (14.1)	47 (28.8)	93 (57.1)	163 (54.7)
Patients (total)	n (%)	29 (9.7%)	65 (21.8%)	204 (68.5%)	298 (100.0%)

Table 3. *Logistic regression analysis for possible predicting factors for B_{12} deficiency*

	Odds ratio (95% CI)	p-value
Sex (if male)	0.745 (0.277, 2.006)	0.560
Age	0.998(0.955, 1.043)	0.931
BMI	0.971 (0.892, 1.056)	0.490
Type 2 diabetes duration	1.005 (0.902, 1.119)	0.930
Metformin 100 mg	1.081 (1.016, 1.150)	0.014*
Metformin duration	0.983 (0.869, 1.111)	0.780
Insulin use	0.581 (0.106, 3.178)	0.531
Insulin duration	1.018 (0.898, 1.153)	0.781
PPI	0.322 (0.111, 0.936)	0.037*
Vitamin B-containing supplements	0.993 (0.187, 5.276)	0.993
Other medication[a]	0.562 (0.121, 2.608)	0.462
Constant	0.389	0.654

[a]**Diabetes medication other than metformin or insulin.**

Anaemic patients had mean vitamin B_{12} levels of 294.3 pmol/l compared with 323.1 pmol/l in patients without anaemia (p= 0.44). Patients with metformin use less often had a neuropathy (17.4%, 95% CI 11.9-24.1%) compared with patients without metformin use (28.1%, 95% CI 20.8-36.5%) (p=0.04). In logistic regression, only duration of diabetes was found to predict chances of neuropathy (OR 1.078; 95% CI 1.043-1.114).

DISCUSSION

The prevalence of B_{12} deficiency among secondary care treated type 2 diabetes patients was 14.1% in metformin users (median metformin use 4.9 years) and 4.4% in non-metformin users. In multivariate models, metformin was a positive, and PPI use a negative predictor of this deficiency. Of all patients (regardless of metformin use), 22.8% were anaemic and 22.3% had neuropathy. Metformin use of 4.9 years was not related to anaemia. It is possible that the chronic disease type 2 diabetes in itself is sufficient to explain the anaemia in this population. Also, 4.9 years of metformin use was related to a lower prevalence of neuropathy than when no metformin was used.

Although the higher prevalence of vitamin B_{12} deficiency among metformin users is in line with previous studies, the magnitude is slightly different. In 1970 in the first described cohort in type 2 diabetes patients using metformin for less than five years, a prevalence of 5.6% among metformin users was found.[2] Pflipsen *et al.* found a prevalence of 22.6% among patients with type 2 diabetes using metformin, in a primary care setting.[8] In this study, vitamin B_{12} concentrations below 100 pg/l (74 pmol/l) or between 100-350 pg/l (74-258 pmol/l) combined with elevated methylmalonic acid or homocysteine levels were defined as deficiency, therefore this study may have well overestimated the prevalence. In a recent study by De Jager *et al.* the same definition of vitamin B_{12} deficiency was used as in the current study. They found a 9.9% prevalence of B_{12} deficiency in patients treated with metformin for 4.3 years.[4] Reinstatler *et al.* defined B_{12} deficiency as B_{12} ≤148 pmol/l and found a prevalence of B_{12} deficiency of 5.8% in a cohort of patients followed for six years.[6]

Ting *et al.* studied risk factors of B_{12} deficiency in patients receiving metformin. The dose of metformin was the strongest independent predictor of vitamin B_{12} deficiency and a longer duration of treatment with metformin was associated with a higher prevalence.[9] In our study we also saw an association between the decreasing B_{12} levels and increasing metformin dose. In accordance with the present study, Reinstatler *et al.* found no clear increase in the prevalence of deficiency as the duration of metformin use increased.[7]

In the current study the decreased occurrence of neuropathy should be interpreted with caution as no objective measures were undertaken to observe the presence of neuropathy. The occurrence of anaemia, however, with actual Hb measurements in every patient, could not be predicted by metformin use after a mean duration of use of 4.9 years. This points toward an important caveat: there is a discrepancy between clinical and biochemical signs of vitamin B_{12} deficiency. Although limited research is available on the question whether biochemical deficiency progresses into clinical deficiency, this does not seem likely.[6]

To identify B_{12} deficiency in metformin-treated type 2 diabetes patients, some authors recommend regular screening of metformin users for B_{12} deficiency or even standard suppletion with, for instance, calcium to reverse the disturbed vitamin B_{12} uptake.[2,4,10,11] There are some important remarks to make in this context. First, it is important to realise that 150 pmol/l as cut-off point for B_{12} deficiency is arbitrarily chosen. Especially with the elderly, B_{12} values below 150 pmol/l are commonly found, and most of them are clinically irrelevant.[5,12] Second, not much is known about consequences of B_{12} deficiency in metformin-using patients. Suppletion is of course not desirable when this does not give any health gain.

The present study adds to this discussion by again defining a prevalence, confirms the influence of metformin on a vitamin B_{12} deficiency and shows that, although metformin increases B_{12} deficiency rates, it does not increase odds for anaemia or neuropathy after 4.9 years treatment with metformin. This last finding argues against standard screening and/or suppletion of vitamin B_{12} in metformin-treated type 2 diabetes patients. We would therefore like to plead for more research focusing on the consequences of a metformin-induced B_{12} deficiency to determine whether screening and suppletion are necessary.

CONCLUSIONS

The prevalence of B_{12} deficiency in secondary care type 2 diabetes patients using metformin was estimated at 14.1%. The prevalence is significantly higher in patients treated with metformin compared with non-metformin users. Metformin use, however, does not predict the odds for anaemia or neuropathy after 4.9 years treatment with metformin.

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