Vitamin B12 levels in type 2 diabetic patients on metformin compared to those never on metformin: a cross-sectional study in tunisia

Statut en vitamine B12 cez les diabétiques type 2 traités par metformine par rapport à ceux sans metformine : une étude transversale en Tunisie

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Abstract

Introduction : Recent studies suggest that long-term use of metformin may decrease the plasma level of Vitamin B12.

Aim: To assess the Vitamin B12 status in Tunisian patients treated with metformin and to study its association with the dose, the duration of metformin use, and the clinical and biological parameters.

Methods: It was a cross-sectional, comparative study on 200 type 2 diabetic (T2D) patients. A vitamin B12 assay was performed with a neurological examination and a Complete blood count.

Results: The mean level of Vitamin B12 assayed in our population was 398.5 ± 188.3 pg/ml. The serum Vitamin B12 levels were 356.9 ± 153.5 pg/ml in the metformin group and 460.9 ± 218.6 pg/ml in the no metformin group (p <0.01). Metformin intake was associated with an increased prevalence of Vitamin B12 deficiency and borderline level. The level of Vitamin B12 was correlated with the duration and the dose of metformin.

Vitamin B12 deficiency was significantly associated with anemia, macrocytosis, and diabetic neuropathy.

The multivariate analysis concluded that Vitamin B12 deficiency was significantly associated with the duration, cumulative metformin dose, clinical neuropathy, anemia, and macrocytosis.

Conclusion: Our study showed an association of Vitamin B12 deficiency with the dose and duration of metformin intake in Tunisian T2D patients, with hematological and neurological repercussions.

Keywords: Vitamin B12, Diabetes mellitus type 2, Metformin, Anemia, Diabetic neuropathy

Résumé

Introduction : Des études récentes suggèrent que la prise au long cours de la Metformine peut diminuer le taux plasmatique de la Vitamine B12.

Objectif : Evaluer le statut en Vit B12 dans une population tunisienne des diabétiques de type 2 (DT2) traités par Metformine et étudier l'association de leur statut vitaminique avec la dose, la durée de prise de Metformine, ainsi qu'avec les différents paramètres cliniques et biologiques.

Méthodes : Cette étude transversale et comparative a inclus 200 patients DT2. Un dosage de la Vit B12 était réalisé chez tous les patients avec un examen neurologique et une numération formule sanguine.

Résultats : Le taux moyen de Vit B12 dans notre population était de 398.5 ± 188.3 pg/ml. Le taux moyen de la Vit B12 était significativement plus bas chez les patients traités par Metformine (356,9 pg/ml versus 460,9 pg/ml, p<0,01). Le déficit en Vit B12 était significativement plus fréquent dans le groupe sous Metformine et de même pour l'insuffisance en Vit B12. Le statut en Vit B12 était significativement associé à la durée, la dose journalière et la dose cumulative de Metformine. Le déficit en Vit B12 était associé à l'anémie, la macrocytose et la neuropathie diabétique. En analyse multi variée, le déficit en Vit B12 dans notre population était associé avec la durée de prise, la dose journalière et cumulative de metformine, la neuropathie diabétique, l'anémie et la macrocytose.

Conclusion : Notre travail a montré une association de la Vitamine B12 avec la dose et la durée de prise de Metformine chez les DT2, avec des répercussions hématologiques et neurologiques.

Mots-clés : Vitamine B12, Diabète type2, Metformine, Anémie, Neuropathie diabétique.

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INTRODUCTION

Metformin, discovered in 1922, is one of the first antihyperglycemic molecules used in the treatment of type 2 diabetes (1). Several studies, such as that of the UKPDS, have shown the benefits of metformin on improving glycemic control, reducing the micro and macrovascular complications of diabetes with a low risk of hypoglycemic accidents (2). Consequently, metformin currently represents the first line treatment for T2D by several international recommendations (2–4). In addition to its anti-diabetic role, metformin has beneficial effects on improving the metabolic profile of patients, reducing cardiovascular morbidity and mortality from all causes (5–7). However, some recent studies have shown that long-term use of metformin by diabetic patients may decrease the absorption of Vitamin B12 and increase the risk of vitamin B12 deficiency. Vitamin B12 deficiency could have some clinical and biological repercussions, such as diabetic neuropathy (8–11) , elevated homocysteine levels and hematologic abnormalities (12–16).

Unfortunately, there is a lack of studies in Tunisia on the prevalence of metformin-related vitamin B12 deficiency in T2D individuals. Additionally, there are no guidelines to address how often T2D patients who are being treated with metformin should be screened for vitamin B12 deficiency risk and, if appropriate, prescribed vitamin B12 supplements.

This study was done to assess the vitamin B12 status in Tunisian T2D patients treated with metformin, compared to a control group and to specify the correlation of vitamin B12 deficiency with the dose and duration of metformin intake, as well as with the various clinical and biological parameters.

METHODS

To meet these objectives, we conducted a cross-sectional, comparative and analytical study in our department from September 2019 to June 2020 among type 2 diabetes patients.

Patients with severe renal impairment (chronic kidney disease stage 4 and above), hepatic impairment, untreated thyroid disease, myeloproliferative syndrome, iron deficiency anemia, gastric surgery, a vegetarian diet or vitamin supplementation were excluded from the study.

Finally, 200 patients were divided in two groups: the first group of 120 diabetic patients whose treatment contains metformin for at least 12 months and the second group of 80 patients not treated with metformin. All patients signed an informed consent.

The study was approved by the local Institute Ethical Committee.

For each patient, the following data were determined: The age and sex, physical activity, smoking, the alcoholism, the presence of other pathologies associated with diabetes, duration of diabetes, degenerative complications, the antidiabetic treatment (Metformin: duration of treatment (year), the daily dose (mg), the cumulative dose = the daily dose (g) x duration (months).

We also collected clinical parameters: weight, height, body mass index, waist circumference, blood pressure, evaluation of the superficial sensitivity by the monofilament testing. Biological parameters: fasting glucose, HbA1c, total cholesterol, triglyceride, HDL cholesterol, creatinine, microalbuminuria, Complete blood count (CBC), folate and vitamin B12.

The vitamin B12 status was defined as: normal (> 300 pg/ml), Borderline (200-300 pg/mL) and deficiency (<200 pg/ml) (20).

Clinical neuropathy was defined as a reduction or absence of light touch sensation to monofilament in either foot (< 8 of 10 applications detected). The DN4 (Douleur Neuropathique 4) score was used to define painful neuropathy.

Data entry and statistical analysis was performed using SPSS 20.0 software. Comparisons between qualitative variables were performed by chi-square test and Pearson for non-validity of this test, the Fisher exact test bilaterally. Comparisons between quantitative variables were performed using Student's t test, and in case of invalidity by the nonparametric Mann-Whitney. The study of the correlations between the dose / duration of metformin intake, the levels of Vitamin B12 and the various clinical and biological parameters was carried out using the bivariate correlation test and multivariate analysis by logistic regression method.

RESULTS

Our study population was divided into 100 women and 100 men with a sex ratio of 1. The mean age of the study population was 59.01 ± 9.40 years with a median of 59.5 years and age extremes between 40 and 81 years. The percentage of smoking patients was 30.8% and that of alcoholism was 6.1%. Table 1 shows the baseline characteristics of the metformin users compared to the non-metformin users (Table 1).

Table 1. Comparison of demographic, anthropometric and clinical characteristics between non-metformin users and metformin users.

Variables	Metformin group (n=120)	Non-Metformin group (n=80)	P value
Age (years)	58 ± 8.8	59.9 ± 10.1	0.24
Gender			
Male	49	51	0.77
Female	50.8	48.8	
Smoking	30.8	30	0.90
Ethylism	4.2	8.8	0.18
Strokes	1.7	5	0.17
Coronaropathy	12.5	17.5	0.32
Peripheral arterial disease	21.7	12.5	0.09
Retinopathy	18.3	28.7	0.08
Diabetic Nephropathy	11.7	13.8	0.66
Clinical Neuropathy	20.8	1.2	<0.0001
Blood glucose (g/l)	1.7	1.64	0.7
HbA1c (%)	8.21	8.81	0.4
Vit B12 (pg/ml)	356.9 ± 153.6	460.8 ± 218.6	<0.0001
Vit B12 status			
Normal	60.8	91.2	
Borderline	30	7.5	<0.0001
Deficiency	9.2	1.2	
Folate (µg/l)	6.2 ± 3.3	6.6 ± 3	0.39
Hemoglobin (g/l)	13.34	13.67	0.065
MCV (fl)	86.7	82.6	<0.0001
Leucocytes (/mm3)	7279	7852	0.03
Platelets (/mm3)	250.941	269.485	0.10

Data presented as % or mean ± SD. Student's t-test and χ2test were applied. P<0.05 considered to be statistically significant.

The mean level of Vitamin B12 assayed in our population was 398.5 ± 188.3 pg/ml with a significant difference between the 2 groups (356.9 vs 460.9 pg / ml; p <0.01). Vitamin B12 deficiency was found in 12 patients (6%) of our population of which 11 patients were on metformin and only one patient was without metformin. Metformin intake was associated with an increase in the prevalence of Vitamin B12 deficiency (9.2% vs. 1.2%; p <0.01) as well as borderline level (30% vs.7.5%; p<0.01).

In the first group, the mean duration of metformin intake was 7.37 ± 5.91 years (range: 1-31 years) with an average daily dose of 1816 ± 681.4 mg / day. An inversely proportional statistical correlation was found between the plasma concentration of Vitamin B12 and the duration of use of metformin (r=-0.606 and p<0.01) as well as the cumulative dose (r=-0.609 and p<0, 01) (Figure 1).

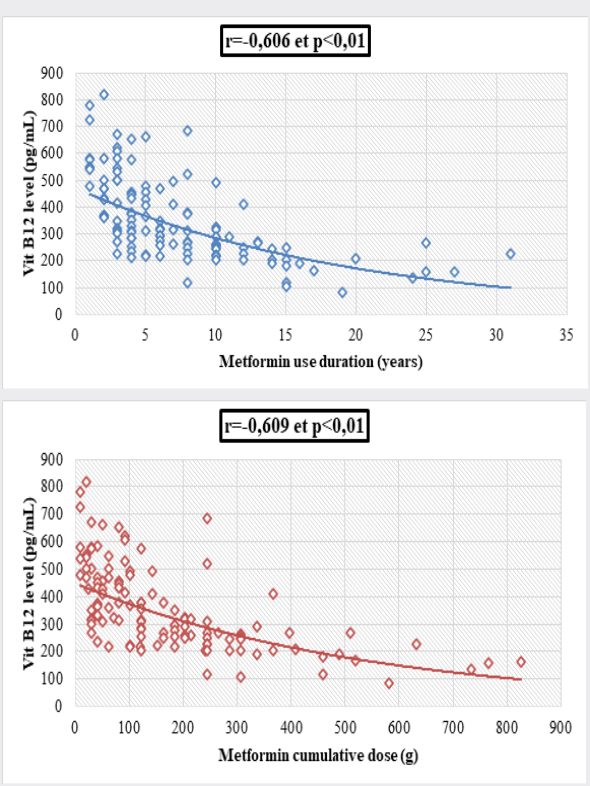

Figure 1. Correlation of Vitamin B12 level with duration and cumulative dose of metformin in metformin users.

The vitamin B 12 status of our patients on metformin was strongly associated with the duration and dose of treatment use: by comparing patients with a Vitamin B12 deficiency to patients with a normal level, the duration of treatment of metformin was significantly higher (17.7 years vs 4.4 years; p<0.01), and the same for the daily dose of metformin (2422 mg vs 1610 mg; p<0.01) and the cumulative dose (520 g vs 93 g; p<0.01).

Mean level of serum Vitamin B12 was found significantly lower in patients with clinical neuropathy (257.8 vs 383 pg/ml; p <0.01) and diabetic painful neuropathy (252 vs 433.6 pg/ml; p <0.01). Additionally, Vitamin B12 deficiency was significantly higher in patients with clinical neuropathy (36% vs 2%; p<0.01).

The level of Vitamin B12 had a statistically positive correlation with hemoglobin (r = 0.46; p<0.01), leukocytes (r = 0.19; p = 0.03) and platelets (r = 0.2; p <0.01), and a negative correlation with MCV (r = -0.59; p <0.01). However, Vitamin B12 deficiency was associated only with anemia (63.3% vs 32%, p<0.01) and macrocytosis (45.5% vs 8.3%; p<0.01).

Table 2 summarizes the association between the Vitamin B12 status and the different clinical and biological parameters in univariate analysis.

Table 2. Univariate associations of Vitamin B12 status and the clinical and biological parameters in the Metformin group.

		Vitamine B12level			P
		Normal (n=73)	**Borderline (n=36)**	**Deficient (n=11)**	
Age (years)		57,2 ± 9,1	59 ± 8,4	63,9 ± 6,1	NS
Gender(%)	Males	81	13	6	NS
	Females	65	12	6	
Metformin daily dose (mg)	Mean	1610 ± 698	2047 ± 542	2422 ± 291	<0,01
	Median	1700	2000	2550	
Metformin use duration (years)	Mean	4,4 ± 2,7	10 ± 6	17,7 ± 5,6	<0,01
	Median	4	10	16	
Metformin cumulative dose (g)		93 ± 80	234 ± 123	520 ± 191	<0,01
BMI (kg/m^2)		29,2 ± 5,3	27,7 ± 5,05	30,6 ± 7,2	NS
Waist circumference (cm)		104,9 ± 18	105,3 ± 14	112,2 ± 16	NS
Fasting glucose (g/l)		1,6 ± 0,55	1,7 ± 0,71	2,2 ± 1,4	0,02
HbA1c (%)		8,1 ± 1,6	8,2 ± 1,9	8,7 ± 2,4	NS
clinical neuropathy (%)		9,6	25	81	<0,01
Painful neurpathy (%)		26,3	74,2	70	NS
Folate (µg/L)		5,7 ± 2,5	6,4 ± 3,8	8,5 ± 5,5	NS
Hemoglobin (g/L)		13,7 ± 0,8	12,9 ± 1,2	12,2 ± 1,3	<0,01
MCV (fl)		83,5 ± 5,3	90,4 ± 5,5	96,4 ± 5,4	<0,01
Leucocytes (/mm^3)		7571 ± 1515	6549 ± 1588	7732 ± 2839	NS
Platelets (10^3/mm3)		269 ± 80,9	218 ± 70	234 ± 55,4	NS

Finally, a multivariate analysis after adjustment for age, sex, weight and diabetes status concluded that B12 deficiency was significantly associated with the duration of metformin use, cumulative dose of metformin, clinical neuropathy, anemia and macrocytosis (Table3).

Table 3. Multivariate analysis of Vitamin B12 deficiency

	B	OR	95%CI	P
Metformin use duration	0,31	1,36	[1,14 – 1,61]	<0,01
Metformin daily dose	0,05	1,005	[1,001-1,008]	0,011
Metformin cumulative dose	0,14	1,014	[1,005-1,081]	<0,01
Clinical neuropathy	2,29	9,93	[1,5 - 65]	0,017
Anemia	1,61	5	[1 - 25]	0,049
Macrocytosis	2,73	15,4	[1,1 - 207]	0,04

DISCUSSION

Recent studies have shown that metformin use is associated with vitamin B12 deficiency. Unfortunately, there is a lack of studies in Tunisia on the prevalence of metformin-related vitamin B12 deficiency in T2D individuals. To our knowledge, our study is the first study in our country.

In our study, the mean level of Vitamin B12 in T2D patients taking metformin was 359.9 (±153.5) pg/ml versus 460.9 (±218.6) pg/ml in patients without metformin. Vitamin B12 deficiency was found in 12 patients in our population of which 11 patients (9.2%) were in the first group versus a single patient (1.2%) in the control group (p <0.01). Borderline Vitamin B12 level was found in 36 patients (30%) in the metformin group versus 6 patients (7.5%) in the control group (p <0.01).

The results obtained from our study were in agreement with the majority of studies in the literature (Table 4). In fact, in a recent meta-analysis, including 31 studies and 5500 type 2 diabetic patients (including 2709 patients taking metformin), the average level of Vitamin B12 was significantly lower in patients taking metformin compared to the control group with a higher risk of Vitamin B12 deficiency (17)

The mechanisms responsible for the vitamin B12 deficiency induced by the intake of metformin are not yet well established and remain a subject of controversy. Indeed, several mechanisms are proposed in the literature (15,18,19):

Table 4: Summary table of the various international studies

Name and type of study	Number of patients Met/Ctrl*	Duration of diabetes (years) Met/Ctrl*	Metformin dose (mg/day)	Prevalence of Vitamin B12 deficiency Met/Ctrl*	Mean serum level of Vitamin B12 Met/Ctrl*	P
Baumen[19] Prospective	14 7	6,9 6	1700	14,1% 0%	400 pg/ml 282 pg/ml	<0,05
Liu [10] RCT	62 47	7,7 4,5	1500		Average decrease of -53,9 pmol/L [IC -81,4 à -26,4]	<0,01
Wulffele [25] RCT	171 182	14 12	2163		Average decrease of-14% [IC -4,2% ; -24%]	<0,001
Hermann [26] Cross-sectional	53 31	10,5 15,3	2200	7,55% 3,23%	289,6 pg/ml 395 pg/ml	<0,01
Pongchaidecha[27] Cross-sectional	88 64	58,2 months 26,4 months	1376		318 pg/ml 434 pg/ml	0,011
Sahin [28] RCT	74 36		2550		Average decrease of -20,17 pg/ml	<0,01
Pflipsen [29] Cross-sectional	133 62	8,3	1776/d if Vit B12 deficiency 1602/d if no deficency		425,9 pg/ml 527,4 pg/ml	0,012
De Jager [9] RCT	194 191	14 12	2550	9,79% 2,62%	-19% [IC -24% ; -14%]	<0,001
Wile [15] Prospective	59 63	5,5 4,7			231 pmol/l 486 pmol/l	<0,001
Calvo Romero[30] Cross-sectional	81 28		1779	8,64 % 0 %	395,5 pg/ml 509 pg/ml	0,008
De Groot [13] Cross-sectional	164 134	31,6 30,8	2050	14,02 % 4,48 %		<0,01
Iftikhar [31] Cross-sectional	114 105	8,96 8,82	if Vit B12 deficiency: 2042/d No deficiency: 1607/d	31 % 8,5 %	194 pg/ml 284 pg/ml	0,03
Sato [32] Cross-sectional	46 38	10,7 years 8,3 years	1125	13 % 7,9 %	250 pg/ml 318 pg/ml	0,03
Singh [33] Cross-sectional	84 52			7,14 % 0 %	410 pg/ml 549 pg/ml	0,01
Adaikalakoteswari [34] Cross-sectional	463 198			21,8 % 15 %	290 pg/ml 464 pg/ml	0,001
Niafar [33] Cross-sectional	200 200	7,9 5,2		14,5 % 2 %	320 pmol/l 410 pmol/l	<0,01
Aroda [20] RCT	1073 1082	5 à 13 years of metformin use	1700	At 5 years : 4,3 % 2,3 % At 13 years : 7,4 % 5,4 %	At 5 years : 546 pg/ml 606 pg/ml At 13 years : 615 pg/ml 650 pg/ml	<0,01 0,19
Zbadi [36] Cross-sectional	130 50	3,46	2353	7,6 % 2 %	427,93 pg/mL 570,2 pg/mL	0,001
Our study	120 80	9,16 10,8	1990	9,2 % 1,2 %	356,9 pg/ml 460,9 pg/ml	<0,01

*Met/Ctrl: Metformin group / control group
RCT: randomized controlled trial

competitive inhibition of vitamin B12 absorption, alteration of the structural morphology of enterocytes, alteration of the function of the intrinsic factor and alteration of the bacterial flora. However, another mechanism was recently evoked by certain studies: Metformin can inhibit the calcium-dependent binding of the Vitamin B12 complex and intrinsic factor at

the level of the membrane receptor of the enterocytes (19). Hence, some authors suggest that calcium supplementation can correct this alteration of calcium-dependent absorption of the cobalamin-FI complex at the ileal level. However, this calcium supplementation did not prove its effectiveness and did not increase the plasma concentrations of Vitamin B12 even in this study (19).

In our study we found an inversely proportional correlation between the plasma concentration of Vitamin B12 and the duration of metformin use ($r = -0.606$ and $p < 0.01$) as well as the metformin dose ($r = -0.609$ and $p < 0.01$). In multivariate analysis, and after adjustment for age, sex, weight, seniority and balance of diabetes, we found a statistically significant correlation between the prevalence of Vitamin B12 deficiency and the daily dose and the duration of metformin use. Additionally, Vitamin B12 deficiency was observed in our population from a minimum daily dose of 1700 mg / day and a minimum duration of 8 years of taking metformin. While Vitamin B12 borderline level was noted as early as 3 years after treatment.

In their meta-analysis, Yang et al showed that Vitamin B12 deficiency was statistically more frequent in the group taking metformin for a duration of treatment greater than 3 years (OR = 2.88 and CI (confidence interval) [1.95,4.26]; P <0.01), whereas this was not the case for a period of less than 3 years (17). In addition, for daily doses of metformin greater than 2000 mg, the risk of vitamin B12 deficiency was statistically greater (OR = 3.26 and CI [1.78, 5.97]; P = 0.001).

Our statistical study showed a significant association between the mean level of Vitamin B12 with clinical neuropathy ($p < 0.01$) and diabetic painful neuropathy ($p < 0.01$). In addition, the prevalence of Vitamin B12 deficiency was statistically higher for patients with clinical neuropathy (36% vs. 2%; $p < 0.01$). Our results are consistent with those of Aroda et al in the DPPOS study which showed that the long-term use of metformin gives an increase in clinical neuropathy, defined by the alteration of the Monofilament test, at 5 years (9.7%) and at 13 years (13.3%) of follow-up, especially if a vitamin B12 deficiency is associated ($p = 0.03$) (20). Likewise, Gupta et al have shown that the severity of neuropathy, assessed by the Toronto score, increases with the duration of metformin use and Vitamin B12 deficiency, which is consistent with the results of many other studies (15, 20–22). Indeed, the severe deficiency in Vitamin B12 can give irreversible neurological lesions and of different gravity. Peripheral polyneuropathy, induced by Vitamin B12 deficiency, in T2D patients treated with metformin frequently appears before the hematological abnormalities but is often mistakenly confused as diabetic neuropathy.

Our study showed that the level of Vitamin B12 was correlated with the various hematological parameters. This correlation was statistically positive with hemoglobin ($r = 0.46$; $p < 0.01$), leukocytes ($r = 0.19$; $p = 0.03$) and platelets ($r = 0.2$; $p < 0.01$), and statistically negative with the MCV ($r = -0.59$; $p < 0.01$). However, Vitamin B12 deficiency was associated only with anemia (OR = 5; $p = 0.04$) and macrocytosis (OR = 15.4; $p=0.4$). The first case of megaloblastic anemia induced by vitamin B12 deficiency secondary to metformin intake was reported in 1980. In this article, the duration of metformin use was 8 years (23). Since then, a few cases have been reported in the same direction (10,11,24). However, to date the number of studies on the association of Vitamin B12 with hematologic parameters in T2D treated with metformin remains low, and more research is needed to substantiate this link.

Our study has certain limitations: the relatively small number of patients included and the cross-sectional design of the study makes it impossible to draw conclusions on cause and consequence.

CONCLUSIONS

Despite the limitations of this study (relatively small number, lack of follow-up), it highlights the importance of looking for a vitamin B12 deficiency in type 2 diabetic patients treated with metformin. The dosage of vitamin B12 in these patients is more interesting with the increase in the duration and dose of metformin as well as the presence of diabetic neuropathy, anemia or macrocytosis. More large-scale studies are needed to clarify this link and to specify the methods of screening and vitamin B12 supplementation in diabetic patients treated with metformin.

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