Evaluation of Serum Vitamin B12 Levels in Type 2 Diabetes Patients Metformin Treated

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Vitamin B_{12} deficiency is a condition characterized by neurological or haematological abnormalities, and may occur after treatment with metformin in patients with type 2 diabetes. We evaluated 119 patients with type 2 diabetes mellitus, treated with oral antidiabetic agents. The parameters evaluated were: vitamin B_{12} intake, anthropometric data, disease history, neuropathic complications evaluation (using MNSI), serum vitamin B_{12}, glycated hemoglobin, erythrocyte indices. Vitamin B_{12} deficiency was defined at values below 194 pg/mL. We have found a prevalence of vitamin B_{12} deficiency of 13.9% in the patients treated with metformin (10.9% in all participants). B_{12} levels were not correlated with age, metformin dose or duration of treatment. Patients using B_{12} supplements had higher B_{12} serum levels (472.50 vs. 329.22, $p \leq 0.05$).

Keywords: B_{12} deficiency, type 2 diabetes mellitus, metformin

According to guidelines [1], metformin is the first-line oral antidiabetic in the treatment of type 2 diabetes mellitus, in the absence of contraindications. Its beneficent effects are a decrease in insulin resistance and a decrease in mortality and cardiovascular risk [2].

Vitamin B_{12} (or cobalamin) is a water-soluble vitamin, an essential micronutrient, with important neuro-cognitive, haemopoietic and vascular roles in serotonin and dopamine synthesis, DNA synthesis and energy metabolism [3].

Several studies have shown a higher frequency of vitamin B_{12} deficiency in patients with type 2 diabetes, one of the factors associated with the deficit being the use of metformin [4,-7]. This risk can be influenced by age, dose and duration of treatment [8].

Metformin could induce vitamin B_{12} deficiency in patients with type 2 diabetes through changes in small intestinal motility which stimulates bacterial over-agglomeration - with B_{12} deficiency, competitive inhibition or inactivation of vitamin B12 absorption, changes in intrinsic factor (IF) levels and interaction with the cubulin endocytary receptor [3,9].

From a clinical point of view, B_{12} deficiency can occur without anemia, in the form of peripheral neuropathy, with the same symptomatology as diabetic neuropathy [10-12]. Often the development of peripheral neuropathy precedes the macrocytic anemia encountered in the deficiency of this vitamin. According to Thomas [13,14], peripheral neuropathy can be divided into generalized symmetric polyneuropathy and asymmetric (focal and multifocal) neuropathy. Neuropathy may also be the only clinical manifestation of vitamin B_{12} deficiency without haematological symptoms and signs [15]. Also, peripheral neuropathy is the most common chronic complication of diabetes [16].

Clinically, peripheral neuropathy caused by B_{12} deficiency is difficult to differentiate from diabetic neuropathy [17], may remain subclinical [18], or interact with diabetic neuropathy [19]. The role of vitamin B_{12} in the etiology of neuropathy is important, as supplementation with B_{12} in these cases may cause reversal of neurological symptoms [20]. Wile and Toth [21] suggested an association between long-term metformin use and peripheral neuropathy associated with B_{12} deficiency. Screening for B_{12} deficiency in patients with type 2 diabetes is similar to that for the general population: serum vitamin B_{12} measurements. There is evidence in literature that emphasizes the lack of vitamin B_{12} sensitivity and that this is not sufficient to reflect the metabolic status of vitamin [22]. Serum measurements of methylmalonic acid and homocysteine are more sensitive and more specific in patients with type 2 diabetes with serum levels of vitamin B_{12} at the lower limit [3,23].

The primary source of vitamin B_{12} is protein of animal origin, and hypovitaminosis may appear by insufficient intake, malabsorption or metabolic mechanisms [24]. Vegetarian diets are associated with low levels of vitamin B_{12} [25]. Diet plays an important role in preventing this nutrient deficiency. Estimating the daily average intake of vitamin B_{12} may identify another factor that could influence the serum levels of this vitamin [26].

There is evidence in the literature that also shows methods for the quantitative determination of metformin [27].

The purpose of this study is to evaluate serum vitamin B_{12} levels, determine the prevalence of B_{12} deficiency, and highlight the risk factors for this deficiency in patients with type 2 diabetes who are receiving metformin.

Experimental part

Material and method

119 patients with type 2 diabetes that were present for their periodical evaluation at the Diabetes Clinical Center at the County Hospital for Emergencies Sf.Spiridon, Iaºi, were included in the study. The inclusion criteria were: over 18 years of age and type 2 diabetes treated with oral antidiabetic agents for at least 6 months. Patients with surgery (gastric, intestinal) and malabsorption syndromes were excluded. The patients signed an informed consent before being included in the study.

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The patients answered a numbered of questions (socio-demographic information, personal pathological history, treatment history, vitamin supplementation). Also, to assess the daily average intake of vitamin B_{12}, they completed a translated and previously validated EPIC-FFQ questionnaire. Anthropometric data (weight, height, abdominal circumference - AC, body mass index - BMI) were obtained, blood pressure was measured. MNSI (Michigan Neuropathy Screening Instrument) was used to assess the presence of clinical neuropathy. MNSI has two parts: a questionnaire related to positive or negative symptoms and examination of the foot. Foot examination included: inspection, Achilles reflex test and vibrational sensitivity with the 128 Hz tuning fork. A score ≥ 2 meant the presence of peripheral neuropathy [27,17]. In addition, tactile sensitivity (with Semmes-Weinstein Monofilament 10 g), thermal sensitivity (with tiptherm), painful sensitivity (with pinprick), and mioartrokinetic sensitivity were also tested.

We used Architect B_{12} assay, witch is a Chemiluminiscent Microparticle Intrinsec Factor assay for the quantative determination of vitamin B_{12} on the Architect iSystems i2000SR. It is a two step assay with an automated sample pretreatment, for determining the presence of B_{12} in human serum using CMIA technology with flexible assay protocols, reffered to as Chemiflex. The B_{12} concentration range was 194-982 pg/ml. Levels below 194 pg/mL defined the B_{12} deficit.

Vitamin B_{12} is synthesized by bacteria, fungi and algae, accumulated in animal tissues, which are consumed later. Vitamin B_{12} or cobalamin (fig. 1) consists of a class of related chemical compounds containing the cobalt biochemical element, molecules that can be converted to methylcobalamin or 5'-deoxyadenosylcobalamin, the two coenzymes (forms of vitamin B_{12}) active in human metabolism.

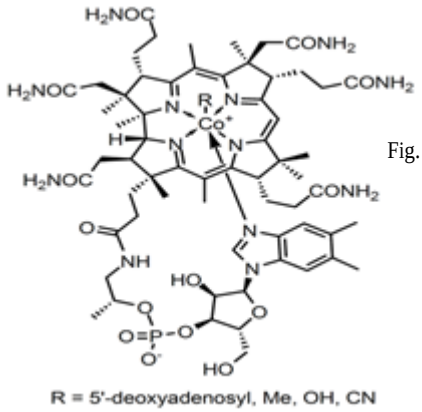

Fig. 1. Vitamin B_{12} formula

The parameters of glycemic control (glycaemia and glycated hemoglobin - HbA1c) and blood count, were also analyzed. Glycated hemoglobin is a form of hemoglobin measured primarily to identify the mean glucose concentration in plasma over the last three months. The lifetime of a red cell is four months (120 days), however, because the red blood cells are not destroyed at the same time, Glycated hemoglobin is taken as an average of three months. Non-enzymatic glycation is formed by attaching plasma glucose to hemoglobin. Anemia was defined at an Hb of 13 g/L for men and ≤12 g/dL for women. Macrocytosis was defined as a MCV> 96 fl.

Statistical data processing was performed with SPSS v17. The verification of the significance of the differences between the mean values was made by the independent t-test by groups, within the descriptive statistics. Significance was considered at $p < 0.05$. Pearson R or Spearman's correlation coefficients were used to investigate relationships between variables.

Rezults and discussions

A total of 119 patients were enrolled in the study. Of these, 86 underwent metformin treatment. In the entire group, mean serum vitamin B_{12} levels were 383.6 ± 202.95 pg/mL, with 19.35% standard error. In the group of 12 subjects with deficiency, the mean was 170.25 ± 15.88 pg/mL, with a standard error of 4.58. In the non-deficient subjects, the mean value was 409.83 ± 199.83 pg/mL, with a standard error of 20.18. Patients on metformin had statistically lower B_{12} values than those without metformin (354.91 pg/mL vs 457.03 pg/mL, $p = 0.017$). There were also statistically significant differences in mean values of BMI and glycated hemoglobin, which were significantly higher in the metformin-treated subjects than in the others (table 1).

The daily average intake of vitamin B_{12} was consistent with the recommendations of dietary guidelines, with no significant difference between groups. Only 3 patients (3.5%) had insufficient intake. There are no significant correlations between vitamin B_{12} and intake ($r = 0.026$, $p = 0.079$) (fig. 2).

Serum vitamin B_{12} deficiency was found in 10.9% of the whole group and 13.9% in the metformin-treated group. There are no significant differences in clinical and biological parameters between the B_{12}-deficient and the normal group in patients receiving metformin.

Regarding the metformin dose, the patients were divided into two categories: <1000mg/day and >1000mg/day. There are no statistically significant differences, clinical

Table 1

CHARACTERISTICS OF THE PATIENTS BASED ON TREATMENT AGENT

	Metformin treated (n=86)			Without metformin (n=33)			p value
	Means±SD	95%CI	Min-max	Means±SD	95%CI	Min-max	
Age (years)	60.42±10.38	58.19-62.65	33-86	61.94±9.01	58.74-65.14	40-76	0.461
BMI (kg/m²)	32.97±5.97	31.69-34.25	21.7-52.8	29.38±4.13	27.92-30.85	20.9-40.6	**<0.001**
Duration of diabetes	5.83±4.54	4.85-6.80	0.5-19	4.39±6.17	2.20-6.58	0.5-20	0.230
Glycated Hb (%)	7.24±1.18	6.99-7.49	5.15-11.3	6.21±0.99	5.85-6.56	5-10	**<0.001**
VitB12 (pg/ml)	354.91±187.89	312.83-397.0	150-863	457.03±223.90	374.9-539.16	179-934	**0.017**
Hb (g/dl)	13.65±1.29	13.37-13.93	11-16.2	14.11±1.21	13.68-14.54	12.1-16.6	0.076
MCV (fl)	88.86±5.16	87.75-89.97	57.9-98.5	91.40±4.17	89.92-92.88	82.8-99.5	**0.013**
n(%)							
Anemia	12(14)			0(0)			**<0.001**
Macrocytosis	4(4.7)			5(15.2)			0.127
Neuropathy	25(29.1)			5(15.2)			0.087
Supplim B12	14(16.3)			7(21.2)			0.531

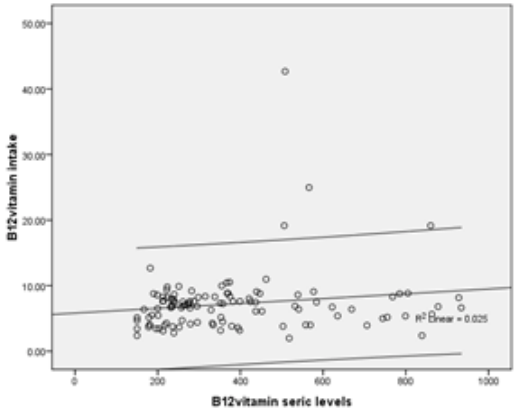

Fig. 2. Correlation between B12 serum levels and B_{12} intake

Table 2

CHARACTERISTICS OF THE PATIENTS BASED ON B_{12} CATEGORIES

	B_{12} deficiency (n=11)			B_{12} normal (n=68)			p value
	Means±SD	95%CI	Min-max	Means±SD	95%CI	Min-max	
Age (years)	63.18±5.89	59.22-67.14	54-72	60.43±10.7	57.82-63.03	33-86	0.411
BMI (kg/m^2)	35.20±4.17	32.39-38.00	28.2-42.5	32.67±6.29	31.15-34.20	21.7-52.8	0.204
Duration metf (yrs)	6.18±4.7	3.02-9.34	0.5-16	6.06±4.63	4.94-7.18	0.5-19	0.939
Glycated Hb (%)	7.11±1.24	6.27-7.94	5.40-10.00	7.30±1.20	7.00-7.59	5.15-11.30	0.638
Hb (g/dl)	13.54±1.29	13.67-14.41	11.0-15.0	13.67±1.33	13.35-13.99	11.4-16.2	0.768
MCV (fl)	90.22±4.39	87.27-93.18	82.2-96.1	88.67±5.35	87.37-89.96	57.9-98.5	0.364
	n(%)						
Anemia	2(18.2)			10(14.7)			0.769
Macrocytosis	1(9.1)			2(2.9)			0.328
Neuropathy	4(36.4)			17(25)			0.435
SupplimB_{12}	2(18.2)			9(13.2)			0.665

Table 3

CHARACTERISTICS OF THE PATIENTS BASED ON METFORMIN DOSE

	≤1000mg/day (n=26)			>1000mg/day B12 (n=60)			p val
	Means±SD	95%CI	Min-max	Means±SD	95%CI	Min-max	
Age (yrs)	62.88±9.63	58.99-66.78	39-86	59.35±10.59	56.61-62.09	33-79	0.148
BMI (kg/m^2)	32.48±6.57	29.83-35.14	21.7-52.8	33.18±5.73	31.70-34.66	21.7-47.6	0.622
Duration metf	4.42±4.07	2.77-6.06	0.5-16.0	6.44±4.63	5.24-7.63	0.5-19.0	0.058
Glycated Hb (%)	7.15±1.17	6.68-7.63	5.15-10.30	7.28±1.19	6.97-7.59	5.40-11.30	0.650
VitB_{12} (pg/ml)	357.08±202.07	275.46-438.70	150-839	353.85±182.53	303.54-404.16	150-863	0.943
Hb (g/dl)	13.56±1.12	13.11-14.02	11.8-16.0	13.69±1.36	13.38-14.04	11.0-16.2	0.693
MCV (fl)	87.54±7.30	84.59-90.49	57.9-95.0	89.43±3.84	88.44-90.42	80.5-98.5	0.119
	n(%)						
Anemia	4(15.4)			8(13.3)			0.804
Macrocytosis	0(0)			4(6.7)			0.045
Neuropathy	5(19.2)			20(33.3)			0.164
Supplim B_{12}	5(19.2)			9(15)			0.630

or biological, between the two groups. The mean serum B_{12} was 357.08 pg/dL in those on a dose ≤1000 mg/day and 353.85 pg/dL in those on a dose >1000mg/day, with no statistical significance between the groups (p = 0.943) (table 3).

Regarding the duration of treatment with metformin, lower mean serum B_{12} values were observed in patients who had metformin treatment for more than 7 years, 4 of whom (36.4%) had a B_{12} deficiency (table 4).

There are differences in mean values of serum B_{12} depending on consumers of oral B_{12} supplements in those in the metformin group with statistical significance, the average of B_{12} levels being higher in those who received such supplements (table 5).

Table 4.
CHARACTERISTICS OF THE PATIENTS BASED ON DURATION OF METFORMIN TREATMENT

	duration≤7yrs (n=57)			duration>7yrs B12 (n=29)			p val
	Means±SD	95%CI	Min-max	Means±SD	95%CI	Min-max	
BMI (kg/m^2)	31.93±5.44	30.49-33.38	21.7-47.6	35.01±6.51	32.54-37.49	25.6-52.8	0.023
Glycated Hb (%)	7.29±1.11	7.00-7.59	5.40-11.30	7.14±1.31	6.64-7.64	5.15-10.00	0.570
VitB12 (pg/ml)	369.72±195.01	314.30-425.14	150-863	329.38±175.28	262.70-396.05	150-799	0.361
Hb (g/dl)	13.78±1.26	13.45-14.12	11.0-16.2	13.39±1.32	12.88-13.89	11.5-16.1	0.178
MCV (fl)	88.34±5.80	86.80-89.88	57.9-97.4	89.88±3.46	88.56-91.20	83.0-98.5	0.194
	n(%)						
Anemia	7(12.3)			5(17.2)			0.536
Macrocytosis	3(5.3)			1(3.4)			0.710
Neuropathy	16(28.1)			9(31)			0.778
Supplim B_{12}	11(19.3)			3(10.3)			0.255

Table 5
VITAMIN B_{12} SUPPLEMENT USE AND SERUM VALUES

	B12 Supplements	No	Mean B_{12} value	Std. Deviation	p
Subjects with metformin	yes	14	472.50	253.394	0.006
	no	72	329.22	154.885	
Subjects without metformin	yes	7	434.43	247.334	0.838
	no	26	454.48	224.048	

29.1% of subjects in the metformin group show clinical neuropathy vs 15.2% of the other group, with no statistical significance between groups (p = 0.118). Four patients (36.4%) of the low serum vitamin B_{12} category had clinical neuropathy, but no statistically significant difference was found (p = 0.429) (table 6).

There was no statistically significant association between B_{12} deficiency and age (r = 0.001, p = 0.411), metformin dose (r = 0.001, p = 0.922), or neuropathy (r = 0.089, p = 0.435). The presence of macrocytic anemia in patients with low vitamin B_{12} levels has not been demonstrated. 2 patients in the B_{12}-deficient group and 6 in the B_{12}-normal group had normocytic anemia. 4 patients in the B_{12}-normal group had macrocytosis with normal red blood cells. There are no correlations between B_{12} deficiency and erythrocyte counts (r = 0.008, p = 0.424).

The purpose of this study was to identify an association between metformin treatment and serum vitamin B_{12} deficiency, compared to other treatment groups. Several observational studies [28,29] have described the relationship between metformin and low vitamin B_{12} serum levels and have shown that metformin dose is an important predictor of B_{12} deficiency. Some authors have shown that possible factors for the B_{12} deficiency could be age [30], duration of treatment with metformin [9], or dose [6]. A systematic review in 2014 by Liu Q et al [31] concluded that reduction of serum B_{12} levels may be metformin-induced in a dose-dependent manner. Another review conducted in 2016 by Ahmed MA [32] highlights the controversial results of studies regarding the association of metformin - vitamin B_{12} deficiency. In our group, we identified 13.9% of patients with type 2 diabetes with hypovitaminosis B_{12}, a lower prevalence compared to other studies [20, 33, 34]. Metformin treated patients had an average of B_{12} levels lower than those without metformin, the results being statistically significant (r = 0.227, p = 0.017), consistent with other previous studies [9, 31, 35]. In contrast to other studies [20, 29, 36, 37] no significant association between low B_{12} and age, HbA1c, metformin dose or duration of treatment has been demonstrated. Rodrigues-Gutierrez et al [38] conducted a study on patients with and without diabetes on different metformin-treatment regimens and found no significant difference in the serum levels of vitamin B_{12} between the groups studied.

Peripheral neuropathy is the most common complication of diabetes [17]. As shown by Ahmed et al in a review in 2017 [39] the results on the relation B_{12} deficiency-metformin related and neuropathy in patients with type 2 diabetes, highlighted by the various studies, are controversial, precisely because both diseases are complicated by neuropathy.

Our study did not find significant statistically differences between the normal B_{12} and B_{12} deficiency group (25% vs 36.4%, p=0.429) regarding the presence of peripheral neuropathy, results that are consistent with other studies [40]. Also, in the evaluation of the neuropathy status, we did not found statistically significant differences between the metformin and non-metformin treatment groups

Vitamin B_{12}	Peripheral neuropathie		Total
	Absent	Present	
Normal	51(75%)	17(25%)	68(100%)
Deficiency	7(63.6%)	4(36.4%)	11(100%)
Total	58(73.4%)	21(26.6%)	79(100%)

Table 6
CROSS-TABULATION OF VITAMIN B12 STATUS AND PERIPHERAL NEUROPATHY IN METFORMIN GROUP

(29.1% vs. 15.2%, p=0.118). The findings are consistent with other results [18, 20, 21, 41]. However we did not perform an electrophysiological testing with nerve conduction velocity in our patients, like other investigators [42], so it may be a limitation of the study.

Several studies have demonstrated the beneficial role of B_{12} supplements in patients with peripheral neuropathy [43]. A review from 2017 by Jayabalan and Low [44] found no evidence that the use of oral vitamin B12 supplements is associated with improvement in the clinical symptoms of diabetic neuropathy. In our study, consumption of vitamin B_{12} supplements was not statistically associated with a reduction in B_{12} deficiency however it appears that those who took these supplements had a higher average of B_{12} levels.

The presence of macrocytic anemia in patients with low vitamin B_{12} levels has not been demonstrated. This finding was also highlighted in a study by Gupta et al [42]. The slight B_{12} deficiency observed in our study is not sufficient to cause macrocytic anemia.

Diet plays an important role in preventing nutrient deficiency. Estimating the daily average intake of vitamin B_{12} may be important to identify another factor that could influence the serum levels of this vitamin [25]. The daily average intake of vitamin B_{12} was appropriate, however, in our study there was no correlation between the estimated daily intake of vitamin B_{12} and its plasma levels. Serum levels of vitamin B_{12} can't reliably detect the B_{12} deficiency and measurement of serum homocysteine and / or methylmalonic acid should be used to confirm the B_{12} deficiency in high-risk and asymptomatic patients with normal levels but to the lower limit of vitamin B_{12} [33, 45, 46]. Current recommendations suggest the dosages of methylmalonic acid or homocysteine for a better assessment of intracellular vitamin status [23,47]. This may be a limitation of our study, where we only measured vitamin B_{12}.

Conclusions

Although metformin therapy was associated with lower vitamin B_{12} levels (with a prevalence of 13.9%), there does not appear to be a statistically significant correlation between administration of this drug and B_{12} deficiency or the prevalence of peripheral neuropathy and anemia in those receiving metformin. Using B_{12} supplements appears to have a role in reducing the incidence of B_{12} deficiency.

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