THE EFFECTS OF METFORMIN AND METFORMIN PLUS CALCIUM TREATMENTS ON SERUM VITAMIN B_{12} LEVELS

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ABSTRACT

Objective: *It is well known that patients with impaired fasting glucose (IFG) and type 2 diabetes mellitus (T2DM) on metformin therapy, may be exposed to vitamin B_{12} deficiency. Our study aimed to compare the effects of metformin versus metformin plus calcium treatments on serum vitamin B_{12} levels in newly diagnosed T2DM and IFG patients.*

Material and Methods: *The study patients with a new diagnosis of T2DM and IFG were randomised into two groups; one group received metformin; daily 2x1000 mg (group 1), while the other group received metformin; 2x1000 mg/d plus oral calcium supplements; 1x1000 mg/d (group 2) in a 3 month-period. Fasting blood glucose, vitamin B_{12}, lipid parameters, HbA1c and homocysteine levels were compared before and after the treatment.*

Results*: The study included 48 patients, 22 women (45.8%) and 26 men (54.2%). There were 12 men (46,2%) and 14 women (53.8%) in group 1, 14 men (63.6%) and 8 women (36.4%) in group 2. Mean ages were 54.77±7.59 (36-65) and 53.45±9.15 (35-65) years in group 1 and 2, respectively. When pre and post treatment biochemical parameters in group 1 were compared, significant reductions in serum vitamin B_{12}, lipid parameters, HbA1c and fasting blood glucose levels were found following the treatment. In group 2, there were also significant reductions in serum homocysteine vitamin B_{12}, HDL-chol, triglyceride and fasting glucose levels after the treatment. When serum vitamin B_{12} levels were compared before and after the treatment, although the difference was not statistically significant, the decrease in serum vitamin B_{12} levels in group 2 was found to be 26.60 pg/ml lower than in group 1.*

Conclusions: *We found that vitamin B_{12} levels decreased less with metformin plus calcium therapy compared to only metformin therapy. It may be suggested that additional calcium supplements may prevent B_{12} vitamin deficiency and associated complications in patients on metformin therapy. Further studies regarding this suggestion are needed.*

Key Words: *Metformin, vitamin B_{12} deficiency, calcium, homocysteine* ***Nobel Med 2013; 9(3): 58-63***

METFORMİN VE METFORMİN+KALSİYUM TEDAVİLERİNİN SERUM B_{12} VİTAMİN DÜZEYLERİ ÜZERİNDEKİ ETKİLERİ

ÖZET

Amaç: Tip 2 Diabetes Mellitus (T2DM) ve bozulmuş açlık glukozu olan (BAG) hastalarda kullanılan metforminin B_{12} vitamin eksikliğine neden olabildiği bilinmektedir. Çalışmamızda yeni tanı konmuş T2DM ve BAG hastalarında, metformin ve metformin+kalsiyum tedavilerinin serum B_{12} vitamin düzeyleri üzerindeki etkilerinin karşılaştırılması amaçlanmıştır.

Materyal ve Metod: Tip 2 DM ve BAG tanısı yeni konmuş çalışma hastaları iki gruba ayrıldı. Hastalar randomize edilerek üç ay süreyle bir gruba günde 2x1000 mg metformin (1. grup), diğer gruba günde 2x1000 mg metformin+günde 1x1000 mg kalsiyum tedavisi (2. grup) uygulandı. Her iki grupta tedavi öncesi ve sonrasında açlık serum glukoz, B_{12} vitamini, homosistein, lipid profili ve HbA1c düzeyleri karşılaştırıldı.

Bulgular: Çalışmaya alınan ve yaşları 35-65 arasında değişen 48 hastanın 22'si kadın (%45,8), 26'sı erkekti (%54,2). 1. grupta 12 erkek (%46,2) ve 14 kadın (%53,8), 2. grupta ise 14 erkek (63,6) ve 8 kadın (%36,4) mevcuttu. Ortalama yaş, 1. ve 2. grupta sırasıyla, 54,77±7,59 (36-65) ve 53,45±9,15 (35-65) yıl idi. 1. grupta, tedavi öncesi ve sonrası parametreler karşılaştırıldığında, B_{12} vitamini, lipid profili, HbA1c ve açlık glukoz değerlerinde tedavi öncesine oranla tedavi sonrasında anlamlı düşüşler saptandı. 2. grupta ise serum homosistein, B_{12} vitamini, HDL-chol, trigliserid ve açlık glukoz düzeylerinde, tedavi öncesine oranla tedavi sonrasında anlamlı düşüşler bulundu. Her iki grubun tedavi öncesi ve sonrası B_{12} vitamin düzeylerindeki azalma karşılaştırıldığında, fark istatistiksel anlamlı olmamakla birlikte, 2. gruptaki azalmanın, 1. gruba göre ortalama 26,60 pg/ml daha düşük olduğu saptandı.

Sonuç: Çalışmamızda, T2DM veya BAG'li hastalarda metformin tedavisine kalsiyum eklenmesi ile serum B_{12} vitamin düzeylerinin yalnızca metformin alan gruba göre daha az oranda düştüğü saptanmıştır. Metformin tedavisi alan hastalara kalsiyum eklenmesi ile B_{12} vitamin eksikliği ve bununla ilişkili komplikasyonların engellenebileceği önerilebilir. Özellikle bu konuda daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Metformin, B_{12} vitamin eksikliği, kalsiyum, homosistein **Nobel Med 2013; 9(3): 58-63**

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is characterized by chronic hyperglycaemia affecting carbohydrate, protein and fat metabolism.[1] Diabetes Mellitus (DM) has turned into an epidemic, even a pandemic, throughout the whole world. According to TURDEP 1 and TURDEP 2 (Turkish Diabetes, Hypertension, Obesity and Endocrinological Diseases Prevalence Study) studies, the prevalence of diabetes has increased by 90%, from 7.7% to 13.7%, and impaired glucose tolerance (IGT) prevalence from 6.7% to 13.9% in Turkey in the last 12 years.[2] In the studies conducted in the USA, DM prevalence in individuals aged 20 and older was found to be around 8.3% while it can increase to 19.2% in those aged 60 and older.[3] Prospective studies made in diabetic patients revealed a linear relationship between diabetes associated complications and hyperglycaemia level.[4] Metformin is a drug frequently prescribed for T2DM treatment and advised as a first line treatment in the absence of contraindications.[5] Generally known to be a safe drug, some side effects may develop in long term metformin use as vitamin B_{12} deficiency.[6]

Vitamin B_{12}, also known as cobalamin, was identified for the first time in 1948 and proven to be effective in pernicious anaemia (PA).[7] After revealing the fact that vitamin B_{12} plays an important role in DNA synthesis and neurologic function and the deficiency can lead to a wide spectrum of hematologic and neuropsychiatric disorders that can often be reversible by the treatment, this vitamin has been a subject of great interest.[8] In a study conducted in Turkey with outpatient patients, vitamin B_{12} deficiency was reported to be 4% and to be associated with diabetes.[9] Vitamin B_{12} deficiency is a common reason for macrocytic anaemia and is associated with a group of neuropsychiatric diseases. Although vitamin B_{12} deficiency often causes haematological and neuropsychiatric disorders, some disorders related to malfunctioning of the autonomic nervous system -such as orthostatic hypotension, impotence, constipation and urinary retention- are rarely seen as well.[10] Homocysteine, on the other hand, appears as an intermediary metabolite in methionine metabolism. Today, hyperhomocysteinemia is recognized as an independent risk factor for coronary artery disease.[11] Vitamin B_{12} deficiency results in hyperhomocysteinemia and increased serum homocysteine levels are thought to play a role in the development of atherosclerosis.[11] Biochemical effects of homocysteine in vascular diseases has not been fully understood yet. There is only a few study regarding the prevention of vitamin B_{12} deficiency with calcium supplements in patients on metformin. Metformin impairs calcium dependent membrane function. There are some studies reporting that serum total vitamin B_{12} and holotranscobalamin-II levels in patients on metformin therapy decrease due to calcium-dependent ileal membrane antagonism and that oral calcium →

Table 1: The pre and post treatment plasma levels of lipids, vit B_{12}, HbA1c and homocysteine and their statistical associations in the metformin group

Parameters		Mean	SD	Min.	Max.	R	p
Homocysteine	BT	12.33	3.65	6.8	26.1	0.44	<0.001
	AT	12.24	2.57	7.7	19.9		
HDL-chol	BT	46	11.1	31	69	0.901	<0.001
	AT	45.5	11.7	31	75		
LDL-chol	BT	138	26.25	105	227	0.677	<0.001
	AT	124	29.47	64	213		
Triglyceride	BT	175	61.31	80	313	0.525	0.006
	AT	158	67.63	80	387		
Total cholesterol	BT	220	30.46	174	319	0.813	<0.001
	AT	203	37.67	117	308		
HbA1c	BT	6.8	1.88	4.9	12.9	0.783	<0.001
	AT	6.3	1.36	5.0	12.3		
Vit B_{12}	BT	367.63	192.33	202.8	1002	0.873	<0.001
	AT	305.18	108.65	132.5	535.9		

BT: Before treatment, AT: After treatment, SD: Standart Deviation

supplementation can reverse this effect.[12] Present study evaluated the effects of metformin and metformin plus calcium treatments on vitamin B_{12}, homocysteine, lipid profile [Triglyceride (TG), LDL-chol and HDL-chol, T. cholesterol], HbA1c and fasting blood glucose (FPG) levels in newly diagnosed T2DM and impaired fasting glucose (IFG) patients.

MATERIAL and METHOD

The study was conducted with patients applied to outpatient clinics of Internal Diseases and/or Endocrinology and Metabolism between 2009 and 2010. Totally 48 patients were included in the study; 22 of them were women (45.8%), 26 of them were men (54.2%). Mean ages were 54.77±7.59 (36-65) and 53.45±9.15 (35-65) years in group 1 and 2, respectively. Study subjects were classified as newly diagnosed with IFG or T2DM by the American Diabetes Association (ADA) proposed diagnostic criteria [for IFG: fasting plasma glucose level between 100-125 mg/dl, for diabetes mellitus: blood glucose level of 126 mg/dl at 0 min and/or 200 mg/dl at 120 minute following the 75 g oral glucose tolerance test (OGTT)]. The patients were randomised into two groups: one group received only metformin (2x1000 mg/d) as group 1 (n=26), while the other group received metformin plus oral calcium supplementation (2x1000 mg/d metformin, 1x1000 mg calcium supplement) as group 2 (n=22) in a 3 month treatment period.

Fasting blood glucose (FBG), creatinine, liver transaminases (AST and ALT), calcium, phosphorus, vitamin B_{12}, lipid parameters, HbA1c and homocysteine levels were measured before and after the treatment period. Blood samples were collected at 09.00 a.m.-11.00 a.m. after a minimum 12 h fast. FBG, lipid profile, vitamin B_{12}, homocysteine and HbA1c parameters were analyzed in the biochemistry laboratories of Gulhane Military Medical Academy (GATA). Patients with renal or liver failure, pregnancy, previously known DM or prediabetes, cancer and inflammatory bowel disease, using any antidiabetic or systemic drug, and whom metformin treatment is contraindicated were excluded from the study. Written consents were taken from all patients. The study was approved by the GATA local ethic committee (4/17/2009, n:1491-641-09/1539). For serum levels of homocysteine, lower limit levels were considered as "<10 micromol/L" whereas for vitamin B_{12}, "<200 pg/ml" were considered as low, "200-300 pg/ml" as limit and ">300 pg/ml" as optimal levels. Plasma samples collected for homocysteine measurement were stored at -80°C. Patients were classified into "low", "limit" and "optimal" groups according to their vitamin B_{12} levels. Same blood parameters were repeated, following a night-fast, after three months from the beginning of the treatment. These samples were analyzed in the biochemistry laboratories. Serum homocysteine levels were measured via HPLC (Shimadzu RF-10AXL, Duisburg, Germany) device and Immuchrom Homocysteine Kit (Immuchrom, Heppenheim Hessen, Germany). Intra-assay and inter-assay coefficients of variation were 1.97% and 2.83%, respectively. Serum HbA1c levels were detected using ion exchange chromatography method by HPLC (Shimadzu SPD-20A UV, Duisburg, Germany) device and Recipe HbA1c Kit (Recipe Chemicals-Instruments GmbH, Munich, Germany). Intra-assay and inter-assay coefficients of variation were 1.6% and 2.4%, respectively. Serum vitamin B_{12} levels were measured with immunoassay method by Siemens Advia Centaur XP (Siemens Healthcare Diagnostics Inc. Tarrytown, NY, USA) device and Advia Centaur VB12 Kit (ADVIA Centaur, Siemens, Tarrytown, NY, USA). Intra-assay and inter-assay coefficients of variation changed between 2.4%-5.0% and 3.0%-9.2%, respectively. Serum total cholesterol levels were measured using cholesterol oxidase/esterase enzyme method via Beckman Coulter AU 600 (CA, USA) device and Beckman Cholesterol Kit (CA, USA). The within-run and between-run coefficients of variation changed between 0.71%-0.91% and 1.06%-1.13%, respectively.

Blood glucose levels were detected in serum by using hexocinase method and Beckman Coulter AU 600 (CA, USA) device and Beckman Glucose Kit (CA, USA). The within-run and between-run coefficients of variation changed between 0.67%-2.30% and 1.90%-4.15%, respectively. Serum triglyceride levels were measured enzymatically with glycerol-phosphate oxidase method and via Beckman Coulter AU 600 →

(CA, USA) device and Beckman Triglyceride Kit (CA, USA). The within-run and between-run coefficients of variation changed between 0.53%-1.37% and 1.13%-2.80%, respectively. Serum HDL-cholesterol levels were measured enzymatically using cholesterol oxidase /esterase method by Beckman Coulter AU 600 (CA, USA) device and Beckman HDL-Cholesterol Kit (CA, USA). The within-run and between-run coefficients of variation changed between 0.61%-0.85% and 1.32%-1.92%, respectively. Serum LDL-cholesterol levels were calculated using Friedewald formula.

SPSS 15.0 programme was used for the statistical analysis of study data. Descriptive statistical data were expressed in mean±standard deviation for continuous variables, discrete data and %. For continuous variable analyses, the analyses were tested via Kolmogrov Smirnov goodness-of-fit test (K-S test). In comparison of the blood parameters of the study groups; independent t-test was used for the parametric, Mann Whitney U test was used for nonparametric tests. Inter-group comparisons of the blood parameters were made via paired sample t test. Statistical significance was set at $p<0.05$. Pearson correlation test was used for the relationship among the variables.

RESULTS

In the metformin group, all lipid parameters improved after the treatment (Table 1). At the end of the three-month follow up, metformin treatment was observed to reduce HbA1c levels by 0.5%; a statistically significant correlation (r=0.783) was found between the pre and post-treatment data of this group ($p<0.001$). However, although minimum decreases were recorded at homocysteine levels, no statistically significant difference was recorded between the pre and post-treatment comparisons between the groups ($p=0.832$) (Table 1).

In the metformin plus calcium group, the pre and post-treatment levels of vitamin B_{12}, TG, HDL-chol revealed a significant correlation (r=0.908, r=0.817, r=0.998, respectively) ($p<0.001$) among the groups. A statistically significant difference was found (r=0.558) between the pre and post-treatment homocysteine levels ($p=0.008$). On the other hand, no significant difference was found among LDL-chol, total cholesterol and HbA1c parameters of the groups ($p>0.05$) (Table 2).

In metformin group, 25% (n=12) of the patients had T2DM, 29% (n=14) had IFG while these rates were 27% (n=13) and 19% (n=9), respectively in the metformin plus calcium group. In group 1, there were reductions in serum homocysteine, B_{12}, HDL-chol, LDL-chol, triglyceride, total cholesterol, HbA1c and APG levels by 0.088 micromol/L, 62.46pg/ml, 0.58 mg/dl, 13.92 mg/dl, 17.08 mg/dl, 16.92 mg/dl, 0.50% and 22.77 mg/dl, respectively following the treatment. In group 2, after the treatment, serum levels of B_{12}, HDL-chol, LDL-chol, triglyceride, T-chol, HbA1c and FBG reduced by 36.86 pg/ml, 0.23 mg/dl, 30.36 mg/dl, 32.00 mg/dl, 41.91 mg/dl, 0.47% and 23.50 mg/dl, respectively while homocysteine levels increased by 0.38 micromol/L. In this study, a significant difference was found only in serum total cholesterol levels. Although no significant decrease was recorded in other biochemical parameters, vitamin B_{12} levels were found to decrease by 26.60 pg/ml. Mean change in pre and post-treatment blood parameters and statistical differences between the groups are presented in Table 3.

Table 2: The pre and post treatment plasma levels of lipids, B_{12}, HbA1c and homocysteine and their statistical associations in metformin+calcium treatment group

Parameters		Mean	SD	Min.	Max.	R	p
Homocysteine	BT	11.79	±3.45	8.4	19.1	0.548	0.008
	AT	12.17	± 3.57	7.2	24.1		
HDL-chol	BT	45.13	±12.03	25	72	0.908	<0.001
	AT	44.91	±11.31	25	74		
LDL-chol	BT	141.5	±24.82	101	186	0.352	0.108
	AT	11.1	±35.92	52	167		
Triglyceride	BT	168.77	±72.15	59	350	0.817	<0.001
	AT	136.77	±64.16	57	267		
Total Cholesterol	BT	225.36	±42	162	350	0.413	0.056
	AT	183.45	±44	111	255		
HbA1c	BT	6.44	±1.22	4.9	10.8	0.328	0.136
	AT	5.97	±0.58	4.9	7		
Vit B_{12}	BT	374.57	±346.67	129.3	1739	0.998	<0.001
	AT	337.71	±314.55	127.6	1577		

BT: Before treatment, AT: After treatment , SD: Standart Deviation

DISCUSSION

Metformin is used as a safe drug for many decades in diabetes treatment, but its side effect on vitamin B_{12} metabolism with long term use is under discussion. Therefore it may be necessary to frequently and strictly monitorize vitamin B_{12} levels of the patients on metformin therapy.[13] Since vitamin B_{12} measurement is less sensitive than holotranscobalamin-II, it is stipulated that "so-called" normal vitamin B_{12} levels may be misleading. Vitamin B_{12} and holotranscobalamin-II levels decrease and homocysteine levels increase as a result of metformin use.[14] Metformin impairs calcium-dependent membrane function. There are some studies reporting that serum total vitamin B_{12} and holotranscobalamin-II levels in patients on metformin therapy decrease due to calcium-dependent ileal membrane antagonism and that oral calcium supplementation can reverse this effect.[12] Calcium, when taken orally, may act as a cofactor enzyme on →

Table 3: The reductions in blood parameters before and after treatment

Parameters	Metformin	Metformin+Calcium	p
Homocysteine	0.088	-0.38	>0.05
Vit B_{12}	62.46	36.86	>0.05
HDL-chol	0.58	0.23	>0.05
LDL-chol	13.92	30.36	>0.05
Triglyceride	17.08	32.00	>0.05
Total Cholesterol	16.92	41.91	0.028
HbA1c	0.50	0.47	>0.05
FBG	22.77	23.50	>0.05

FBG: Fasting blood glucose

ileal membrane and compensate the metformin-associated decrease. B_{12} vitamin testing is not sensible as holotranscobalamine test. The concentrations of holotranscobalamine are reduced by metformin in an unknown mechanism. This effect might be reduced by an enzyme sensitive to calcium following its ingestion on ileal membrane. The mechanism is still unrevealed totally.

In the study by Bauman et al., 14 T2DM patients was on sulfonylurea treatment switched to metformin treatment and remaining 7 patients stayed on sulfonylurea as control group.[12] While the pre-treatment serum vitamin B_{12} levels of all patients were normal; vitamin B_{12} and holotranscobalamin-II levels of metformin group were reported to decrease while it remained unchanged in the sulfonylurea group. Daily 1.2 g calcium carbonate supplements were prescribed to patients on metformin. These measurements were repeated one month later showed that the decrease in their serum vitamin B_{12} levels continued in metformin group but their holotranscobalamin-II levels increased. This study suggested that since holotranscobalamin-II is the precursor of vitamin B_{12} and sensitive to the treatment, it responded more rapidly to calcium supplementation. The result of our study was similar to those of the previous literature studies. However, since we could not measure serum holotranscobalamin-II levels, due to financial limitations, our study may not have fully presented the results of calcium supplementation. Scientific weaknesses of the present study can be listed as the failure to measure the differences in serum homocysteine levels and to investigate the presence of Methylenetetrahydrofolate Reductase (MTHR) enzyme mutation.

In a cohort study by Ting et al., use of metformin at a dosage higher than 1000 mg/day for more than 3 years was stated to be a risk factor for vitamin B_{12} deficiency.[15] Comparison of 155 patients on metformin therapy with vitamin B_{12} deficiency and 310 patients as control with serum normal vitamin B_{12} levels proved a strong relationship of vitamin B_{12} deficiency with the dosage and duration of metformin use. Close monitoring of the complications that can develop due to long-term metformin use as well as treatment of these complications will enable utilization of metformin by avoiding its rare disadvantages. The advantages of metformin can be listed as blood glucose control, improvement in lipid profile and weight control.[16] These improvements with metformin were also observed in our study. Lin et al. reported that long-term metformin use results in vitamin B_{12} deficiency, which, in turn, leads to hyperhomocysteinemia.[17] They even stated that metformin patients with MTHR enzyme mutation are exposed to vascular thrombosis risk increased by hyperhomocysteinemia.

A randomized, placebo-controlled study by Wulffele et al. analyzed the effects of short-term (16 weeks) metformin treatment on serum homocysteine and vitamin B_{12} levels of T2DM patients.[18] T2DM patients receiving insulin treatment constituted the control group and 390 of total 745 DM patients were included in the concerned study. Metformin and placebo treatments were given to 196 patients and 194 patients, respectively. Serum homocysteine and vitamin B_{12} levels of the patients were measured at the end of 16-week follow-up period. Homocysteine levels increased by 4% while vitamin B_{12} levels decreased by 14% in the metformin group. In conclusion, the study suggested that vitamin B_{12} levels might be increased by calcium supplementation.

A study by Hermann et al. compared vitamin B_{12} levels of the patients who were on metformin treatment at least for one year. The number of subjects in case and control group was 53 and 31, respectively.[19] Vitamin B_{12} and holotranscobalamin-II levels were found to decrease and homocysteine levels to increase in the metformin group. Serum vitamin B_{12} levels decreased by 26.7% and holotranscobalamin-II levels by 21.6% while the homocysteine levels increased by 9.7%. Although metformin is a safe drug, it is impossible to foresee possible future side effects of vitamin B_{12} deficiency induced by its long-term use. For this reason, it would be an appropriate approach to strictly monitor vitamin B_{12} levels during metformin therapy. In a randomized and placebo controlled study by Jager et al., metformin (3x850 mg/day) and placebo treatment started in 194 and 196 type 2 diabetic patients respectively who were on insulin treatment.[20] Serum vitamin B_{12} and homocysteine levels were measured at the end of 16-week of metformin treatment. When the same parameters were repeated on the 17th, 30th, 43rd and 52nd weeks; vitamin B_{12} levels were recorded to decrease by 19% and homocysteine levels to increase by 5%. In conclusion, vitamin B_{12} levels reduced and homocysteine levels increased→

by metformin treatment. However, the present study did not reveal a significant increase in homocysteine levels. Indicators that rapidly detect deficiencies and requirements during should be used to follow-up of metformin-induced side effects.

A cross-sectional study by Pongchaidecha et al. searched for vitamin B_{12} and homocysteine levels of 152 T2DM patients in 35-65 years range.[14] Of these patients, 88 were on metformin therapy and the remaining 86 on non-metformin therapy. The study was designed as minimum metformin use duration as 6 months. No difference was detected between the plasma homocysteine levels of the study groups. However, a statistically significant decrease was found in serum vitamin B_{12} levels of metformin group. The study showed vitamin B_{12} deficiency and unchanged homocysteine levels, which comply with the results of the present study.

In a case report published by Lin et al.; it was hypothesized that vitamin B_{12} deficiency induced by metformin causes hyperhomocysteinemia in patients with homozygote MTHR enzyme mutation.[21] It was also reported that metformin treatment did not cause hyperhomocysteinemia in patients without enzyme mutation. Present study found no change in plasma homocysteine levels of either group as well. Since it is a quite rare enzyme mutation, it may not be cost-effective to ask genetic consultation for each patient whom will be started on metformin. Physicians should pay attention to this problem and it may be appropriate to demand this consultation when long-term, high-dosage metformin treatment is planned.

CONCLUSION

Since metformin treatment in diabetic patients may decrease vitamin B_{12} levels resulting in increased neurological complications; vitamin B_{12} levels should be monitorized during all the treatment period. It should be noted that metformin treatment in patients with MTHR enzyme mutation may induce abnormal increases in plasma homocysteine level. For this reason, the possibility of MTHR enzyme mutation may be taken into consideration, and genetic analysis may be suggested for those patients whom will be started on long-term metformin therapy. As the present study showed that calcium supplementation during metformin treatment resulted in less decrease in vitamin B_{12} levels of diabetic patients, it can be suggested that neurological complications that may result from vitamin B_{12} deficiency may be prevented by calcium supplementation. This suggestion may need further studies with long term treatment period and with more study subjects.

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