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Is there a relationship between metformin-related gastrointestinal symptoms and vitamin B12 deficiency in patients with type 2 diabetes mellitus?

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Abstract

Metformin (MTF) associated gastrointestinal symptoms are fairly common side effects that adversely affect patients' treatment adherence. However, the variability of gastrointestinal symptoms in MTF-using patients has not been fully explained. In our study, we aimed to investigate the relationship between vitamin B12 deficiency with MTF-related gastrointestinal symptoms. Patients with type 2 diabetes mellitus (T2DM) using MTF were included in the study sequentially. Demographic characteristics of the patients, duration of diabetes, MTF dose and duration used, and gastrointestinal symptoms were recorded. Afterward, a hemogram, HgbA1c, and vitamin B12 measurements were performed. Patients with and without vitamin B12 deficiency were divided into two groups. The two groups were compared with statistical methods. Twenty-five percent of patients had low serum vitamin B12 levels. Patients with vitamin B12 deficiency had a longer diabetes duration, and a longer MTF usage duration ($p<0.001$, $p<0.001$) than the patients without vitamin B12 deficiency. There was no correlation between B12 deficiency and MTF dosage ($p=0.590$). Gastrointestinal symptoms were seen more frequently in the B12 deficiency group ($p=0.025$). Bloating and constipation, nausea, abdominal pain, and vomiting were seen commonly in the B12 deficiency group ($p=0.002$, $p<0.001$, $p=0.014$, $p=0.004$, respectively). Three or more symptoms were frequently seen in B12-deficient patients ($p<0.001$). Patients with both a MTF usage duration of 10 years or higher and vitamin B12 deficiency are found to be 434% more likely to have active gastrointestinal symptoms than all other patient groups (OR:5.343, 95%CI (2.173-13.140), $p<0.001$). Study results have shown that gastrointestinal symptoms seen in patients with T2DM taking MTF may be associated with vitamin B12 deficiency. MTF-related gut microbiome changes may play a role in this relationship. In particular, we recommend that patients who have been using MTF for ≥10 years and have gastrointestinal complaints should be followed more closely for vitamin B12 deficiency.

Keywords: Diabetes mellitus, gastrointestinal symptoms, metformin, vitamin B12 deficiency

Introduction

Type 2 diabetes mellitus (T2DM) constitutes the vast majority of all diabetes mellitus (DM) patients. The prevalence and incidence of T2DM are increasing rapidly all over the world. T2DM and its comorbidities have now reached epidemic proportions [1]. Metformin (MTF), a biguanide derivative, is the most widely used therapeutic agent in the treatment of patients with T2DM and has been used for nearly a century [2]. The European Association for the Study of Diabetes (EASD) and the American Diabetes Association (ADA) recommended the use of MTF in pharmacological treatment in addition to exercise and

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diet practices in T2DM [3]. MTF also has several other non-Food and Drug Administration (FDA)-approved indications, including gestational DM, management of antipsychotic-induced weight gain, prevention of T2DM, and treatment and prevention of polycystic ovarian syndrome. Currently, MTF is the only antidiabetic recommended by the ADA for pre-diabetes [4].

Some side effects may also develop due to the use of MTF. The most common unpleasant side effects are symptoms related to the stomach and intestinal tract, such as diarrhea, nausea, intestinal gas, abdominal pain, constipation and vomiting [5]. Gastrointestinal side effects occur in 20-30% of patients using MTF, of which approximately 5% are severe adverse events resulting in discontinuation of MTF. Although research has been done on how MTF causes gastrointestinal side effects, it is still not clear [6]. However, altered histamine or serotonin transport, localized MTF concentrations in the intestinal cell, effects due to increased bile acidity in the colon, or changes in the gut microbiome are thought to be responsible for these side effects [7]. Recent research has suggested that one of the target sites of MTF may be the gut microbiota [8].

In conclusion, MTF has a rather mixed relationship with the intestinal system in terms of both drug action and gastrointestinal side effects [7]. In addition, long-term use of MTFs can interfere with the absorption of vitamin B12 in the terminal ileum region of the intestine, resulting in a deficiency of this vitamin. The clinical significance of vitamin B12 deficiency is that it can cause megaloblastic anemia, mental status changes, and some neurological defects [9].

Our main aim in this study was to investigate whether vitamin B12 deficiency has an effect on MTF-related gastrointestinal symptoms. Our secondary aim is to determine the prevalence of vitamin B12 deficiency in our T2DM patients using MTF therapy and to compare the demographic/clinical characteristics and laboratory results of our patients with and without vitamin B12 deficiency.

Material and Methods

Study design

Ethics committee approval was obtained for the study from the Hitit University Faculty of Medicine Clinical Research Ethics Committee (Date: 20.04.2022, No: 2022-40). After obtaining approval from the institution where the research would be conducted, the research was conducted. It was planned as a observational and cross-sectional study. The research was carried out with patients who applied to the internal medicine outpatient clinic. The research population consisted of T2DM patients using MTF. With the "patient follow-up form" we prepared, demographic characteristics of the patients such as gender, age, body mass index (BMI), duration of DM, duration of MTF use, daily dose of MTF and whether they had diabetes were questioned. Neuropathy was noted. Patients meeting the inclusion criteria were included in the research consecutively. Gastrointestinal system symptoms (diarrhea, nausea, abdominal pain, vomiting, bloating, constipation and metallic taste in the mouth) of the patients were evaluated. Gastrointestinal symptoms were evaluated according to the following criteria, and the symptoms of patients who met all criteria were considered MTF-related gastrointestinal symptoms.

MTF-related gastrointestinal symptom criteria

The onset of symptoms after MTF use, the symptom has been persistent or frequent in the last month, answering the presence of symptoms by the patients considering the last month, absence of any other medication that may cause these symptoms in the last month, and absence of acute gastroenteritis and biliary, urinary system and perineal region infections in the last month.

After the patients' data were recorded, routine evaluation tests for DM were performed. Hemogram parameters, HbA1c, and vitamin B12 values were recorded. Serum vitamin B12 measurements were made with the chemiluminescence method using Roche cobas e601 analyzers. Vitamin B12 measurements below 200 pg/mL were defined as deficiency. Patients participating in the study were divided into two groups as patients with vitamin B12 deficiency and patients without vitamin B12 deficiency. These two groups were compared in terms of demographic characteristics, clinical features, gastrointestinal symptoms and laboratory data using statistical methods.

Inclusion criteria in the study

T2DM patients who have been using MTF for at least three months, adults aged 18-80, and patients who voluntarily want to participate in the research

Exclusion criteria from the study

Patients who do not want to participate in the research voluntarily, individuals under the age of 18 and over the age of 80, patients with previous gastrointestinal complaints, patients with renal, hepatic, and chronic pancreatic insufficiency, patients with intestinal malabsorption, patients with known hematological disease or malignancy, patients with gastrointestinal malignancies, individuals with pernicious anemia, ulcerative colitis, and Crohn's disease, individuals with gastric and intestinal operations, those with cholelithiasis and those with cholecystectomy, those who are pregnant, breastfeeding, those who use alcohol, those who eat vegan and vegetarian, those who use proton pump inhibitor, H2 receptor antagonist, antacid, calcium preparation, colchicine, vitamin B12, and multivitamin drugs and patients using the extended-release oral formulation of MTF.

Statistical Analysis

All statistical analysis was performed using IBM SPSS Statistics for Windows software (version 26; IBM Corp., Armonk, N.Y., USA). Distribution of data evaluated by Shapiro Wilks test.

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Descriptive statistics were reported as number and percentage for categorical variables, mean ± standard deviation for normally distributed numerical variables, and median value and minimum and maximum values in parentheses for non-parametric distributed variables. The relationships between the variables were investigated with Spearman or Pearson correlation coefficient in accordance with the data distribution. Comparison of numerical measurements according to research groups for two independent groups were evaluated with two sample t-tests for age only and were evaluated with Mann Whitney U test for height, weight, BMI, diabetes duration, MTF use duration, serum white blood cell (WBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), Platelet (Plt), HbA1c, vitamin B12 levels in accordance with the data distribution. Categorical variables such as gender, MTF dosage, presence of gastrointestinal symptoms, bloating and constipation, nausea, vomiting, abdominal pain, metallic taste, diarrhea, number of gastrointestinal symptoms, presence of neuropathy, vitamin B12 deficiency ratio comparisons according to research groups and subgroups were evaluated by Chi-square test. A p value of <0.05 was accepted for statistical significance.

Results

The median age of 312 patients was 59 years, and 195 patients (62.5%) were female. The clinical features of these patients are given in Table 1. Of the patients in the entire group, 138 (44.23%) had no gastrointestinal symptoms during the period of MTF use, while 174 (55.77%) had one or more symptoms. Vitamin B12 levels were normal in 234 (75%) of the patients, while vitamin B12 deficiency was seen in 78 (25%) patients.

The median age of vitamin B12 deficient and non-deficient individuals was 58 and 64 years, respectively, a statistical significance was seen (p<0.001). There were no significant differences in gender, height, BMI, WBC, Hb, Hct, or Plt between patients with and without vitamin B12 deficiency (Table 1).

The median weight of patients without vitamin B12 deficiency was found lower than vitamin B12 deficient patients (80 kg vs 84.5 kg, p=0.017). HbA1c levels were higher in vitamin B12 deficient patients than in other groups (7.1 vs 7.6, p=0.015) (Table 1).

The duration of T2DM (p<0.001) and the duration of MTF use (p<0.001) were longer in the patient group with vitamin B12 deficiency compared to the patient group without vitamin B12 deficiency. There were no differences in MTF dosages between the two groups (p=0.590). The incidence of diabetic neuropathy was higher in patients with vitamin B12 deficiency (20.51%) than in patients with adequate vitamin B12 (7.26%) (p=0.001) (Table 1).

In the adequate vitamin B12 group, 52.14% of the patients had one or more gastrointestinal symptoms, this ratio was statistically higher in the deficiency group with a 66.67% symptom rate (p=0.025). Bloating and constipation, nausea, abdominal pain, and vomiting were seen more frequently in the vitamin B12 deficiency group (p=0.002, p<0.001, p=0.014, p=0.004, respectively). There were no differences in metallic taste and diarrhea between the groups (p=0.220 and p=0.078). Three or more symptoms were frequently seen in vitamin B12-deficient patients (32.05% vs 11.11%, p<0.001) (Table 1).

Evaluation of the relationship between Gastrointestinal Side Effects and MTF Dose, Duration of MTF Use, and HbA1c Level

Patients included in the study were further divided into two groups, patients with gastrointestinal side effects, and those without gastrointestinal side effects. When the MTF doses were compared, it was found that there was no statistically significant difference between the groups (p=0.753). In the subgroup analysis according to the presence of vitamin B12 deficiency, there was no significant relationship found between gastrointestinal side effects and MTF dosage in patients with normal serum vitamin B12 levels or in patients with vitamin B12 deficiency (p=0.664 and p=0.772, respectively) (Table 2).

Patients with and without gastrointestinal symptoms were compared according to the duration of MTF use. A statistically significant difference was found between these two groups (p=0.038). In the subgroup analyses, this difference couldn't be observed in patients with adequate vitamin B12 levels (p=0.595) and was much more pronounced in vitamin B12 deficient patients (p=0.005). In patients with vitamin B12 deficiency, the rate of patients who have been using MTF for more than 10 years and who do not have gastrointestinal symptoms is 23.08%, while this rate is observed as 65.38% in patients with gastrointestinal symptoms (Table 3). A patient with a MTF usage duration of 10 years or more is found to be 100.8% more likely to have active gastrointestinal symptoms than a patient with a lesser usage duration (OR:2.008, 95%CI (1.212-3.327), p=0.007). This ratio is much higher in patients with both a MTF usage duration of 10 years or higher and vitamin B12 deficiency, these group of patients are found to be 434% more likely to have active gastrointestinal symptoms than all other patient groups (OR:5.343, 95%CI (2.173-13.140), p<0.001). These significant increases in the incidence of symptoms are especially noteworthy in patients with vitamin B12 deficiency who have been taking MTF for a long time (Table 3).

When gastrointestinal symptoms were evaluated in terms of HbA1c level, no significant difference was observed in both patients with normal vitamin B12 levels and in patients with deficiency (Table 4).

The distribution of other therapeutic agents used by the patients for the treatment of diabetes mellitus, other than metformin, is given in table 5.

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Table 1. Comparison between vitamin B12 deficient and adequate patients

Variables		All patients (n=312)	Adequate vitamin B12 (n=234)	Vitamin B12 deficient (n=78)	p
Age		58.95±9.65 (59)	57.89±9.53 (58)	62.14±9.38 (64)	0.001
Gender	Male	117 (37.50%)	82 (35.04%)	35 (44.87%)	0.12
	Female	195 (62.50%)	152 (64.96%)	43 (55.13%)	
Height		164 (145-187)	163 (145-187)	165 (150-187)	0.398
Weight		80 (56-125)	80 (56-125)	84.5 (63-124)	0.017
BMI (kg/m2)		30.44 (18.52-46.22)	30.18 (18.52-46.22)	31.33 (23.31-45.18)	0.096
WBC count (109/L)		7.8 (3.5-16.6)	7.8 (3.5-16.6)	7.95 (3.8-12.5)	0.896
Hb (g/dL)		13.65 (7.3-17.4)	13.8 (7.3-17.3)	13.2 (9.5-17.4)	0.177
Hct (%)		41 (19.4-84.8)	41 (19.4-50.5)	41.25 (32-84.8)	0.716
MCV (fL)		86.8 (27-115)	86 (66-115)	87 (27-110)	0.039
Plt count (109/L)		271 (80-553)	273.5 (80-553)	268 (85-440)	0.126
HbA1c (%)		7.4 (5-13.7)	7.1 (5-13.7)	7.6 (5.1-12.8)	0.015
DM duration (months)		90 (1-360)	72 (1-360)	120 (3-360)	0.001
Metformin usage duration (months)		84 (1-360)	72 (1-360)	120 (3-300)	0.001
Metformin dosage	500mg	6 (1.92%)	4 (1.71%)	2 (2.56%)	0.590
	850mg	5 (1.60%)	5 (2.14%)	0 (0.00%)	
	1000mg	41 (13.14%)	31 (13.25%)	10 (12.82%)	
	2000mg	260 (83.33%)	194 (82.91%)	66 (84.62%)	
Vitamin B12 value		280 (97-1500)	309.5 (200-1500)	164.5 (97-198)	<0.001
Known diabetic neuropathy		33 (10.58%)	17 (7.26%)	16 (20.51%)	0.001
Gastrointestinal symptoms	No symptoms	138 (44.23%)	112 (47.86%)	26 (33.33%)	0.025
	One or more symptoms	174 (55.77%)	122 (52.14%)	52 (66.67%)	
Bloating and constipation		137 (43.91%)	91 (38.89%)	46 (58.97%)	0.002
Nausea		70 (22.44%)	40 (17.09%)	30 (38.46%)	<0.001
Abdominal pain		62 (19.87%)	39 (16.67%)	23 (29.49%)	0.014
Metallic taste		36 (11.54%)	24 (10.26%)	12 (15.38%)	0.220
Diarrhea		35 (11.22%)	22 (9.40%)	13 (16.67%)	0.078
Vomiting		19 (6.09%)	9 (3.85%)	10 (12.82%)	0.004
Gastrointestinal symptoms count	No symptoms	138 (44.23%)	112 (47.86%)	26 (33.33%)	<0.001
	One symptom	79 (25.32%)	66 (28.21%)	13 (16.67%)	
	Two symptoms	44 (14.10%)	30 (12.82%)	14 (17.95%)	
	Three or more symptoms	51 (16.35%)	26 (11.11%)	25 (32.05%)	
Vitamin B12 deficiency	Normal	234 (75%)			
	Deficient	78 (25%)			

BMI: Body-mass index, WBC: White blood cell, Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean corpuscular volume, Plt: Platelet, DM: Diabetes mellitus

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Table 2. Crosstabulation of gastrointestinal symptoms, metformin dosage, and vitamin B12 status

Vitamin B12	Metformin dosage	No gastrointestinal symptoms	One or more gastrointestinal symptoms	p	
Adequate	*500mg*	1 (0.89%)	3 (2.46%)	0.664	0.753
	850mg	2 (1.79%)	3 (2.46%)		
	1000mg	17 (15.18%)	14 (11.48%)		
	2000mg	92 (82.14%)	102 (83.61%)		
Deficient	*500mg*	1 (3.85%)	1 (1.92%)	0.772	
	850mg	0 (0.00%)	0 (0.00%)		
	1000mg	4 (15.38%)	6 (11.54%)		
	2000mg	21 (80.77%)	45 (86.54%)		

Table 3. Crosstabulation of gastrointestinal symptoms, metformin usage duration, and vitamin B12 status

Vitamin B12	Metformin usage duration	No gastrointestinal symptoms	One or more gastrointestinal symptoms	p	
Adequate	*Less than one year*	17 (15.18%)	13 (10.66%)	0.595	0.038
	1 to 5 years	37 (33.04%)	36 (29.51%)		
	5 to 10 years	33 (29.46%)	43 (35.25%)		
	10 or more years	25 (22.32%)	30 (24.59%)		
Deficient	*Less than one year*	3 (11.54%)	4 (7.69%)	0.005	
	1 to 5 years	8 (30.77%)	7 (13.46%)		
	5 to 10 years	9 (34.62%)	7 (13.46%)		
	10 or more years	6 (23.08%)	34 (65.38%)		

Table 4. Crosstabulation of gastrointestinal symptoms, HbA1c levels, and vitamin B12 status

Vitamin B12	HbA1c levels	No gastrointestinal symptoms	One or more gastrointestinal symptoms	p	
Adequate	*Less than 6*	13 (11.61%)	13 (10.66%)	0.562	0.283
	Between6 and 8	66 (58.93%)	65 (53.28%)		
	Higher than 8	33 (29.46%)	44 (36.07%)		
Deficient	*Less than 6*	2 (7.69%)	4 (7.69%)	0.604	
	Between6 and 8	15 (57.69%)	24 (46.15%)		
	Higher than 8	9 (34.62%)	24 (46.15%)		

Table 5. Distribution of other therapeutic agents used in the treatment of diabetes mellitus other than metformin.

Treatment agents		n
Insulins	All types	87
Sulfonylureas	Gliclazide	49
	Glimepiride	5
Thiazolidinedione	Pioglitazone	59
Dipeptidyl peptidase 4 (DPP4) inhibitors	Sitagliptin	15
	Vildagliptin	77
	Linagliptin	28
Sodium-glucose cotransporter-2 (SGLT-2) inhibitors	Dapagliflozin	12
	Empagliflozin	24

Discussion

According to our results, we showed that the duration of DM and duration of MTF use are risk factors for vitamin B12 deficiency in patients with T2DM using MTF. More importantly, we found that MTF-related gastrointestinal symptoms were more common in patients with vitamin B12 deficiency. Especially in patients using MTF therapy for ≥10 years, the incidence of gastrointestinal symptoms increased remarkably if vitamin B12 deficiency was also present. Based on our literature review, we noticed that no other study investigated the association of vitamin B12 deficiency with gastrointestinal symptoms in T2DM patients treated with MTF. We think that our research will make an important contribution to the literature since it is the first study to investigate this subject.

The global prevalence of DM was reported as 9% in 2019 by the International Diabetes Federation (IDF) [10]. It is estimated that there will be 783 million DM patients by 2045 [11]. According to the ADA, MTF is the agent of choice for the first-line treatment of adults with T2DM [12]. Therefore, the number of patients using MTF worldwide is relatively high. It has been reported that vitamin B12 deficiency due to MTF use can be seen in an average of 6% to 30% of patients [13,14]. Different prevalence values have been reported in different studies. Prevalence of vitamin B12 deficiency was found as %6.9 in a study reported from Brazil [15], as 20.3% after 9.5 years of MTF use in a large prospective study [16], as 22% in the National Health and Nutrition Examination Survey (NHANES) [13] and as 9.5% in a study by College of Medicine of the Catholic University of Korea [9]. In our study, we detected vitamin B12 deficiency in 25% of the patients, and this rate was consistent with other studies. The reasons for the different prevalence values in different studies may be the differences in doses and durations of MTF use, differences in test methods, and differences in alcohol use. Previous studies have shown a clear relationship between the dose or duration of MTF use and vitamin B12 deficiency in patients with T2DM. Sun-Hye Ko et al. showed that patients using MTF at ≥2,000 mg/day and ≥10 years had an approximately 4-fold greater risk of vitamin B12 deficiency than patients using

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MTF at ≤1,000 mg/day and <4 years [9]. Martin et al. reported in a recent study that patients using MTF for more than five years are at bigger risk for vitamin B12 deficiency [17]. Likewise, a study using NHANES data found that patients were more likely to be vitamin B12 deficient as the duration of MTF use (4.1% for 3-10 years of MTF use versus 8.1% for >10 years of use) [13]. Saqer et al. also found that the annual increase in DM duration was associated with lower vitamin B12 levels [18]. According to our study data, the duration of DM and the duration of MTF use of our patients were quite close to each other. The duration of DM and the duration of MTF use were longer in the group with vitamin B12 deficiency compared to the group without vitamin B12 deficiency. These results support other study results. However, unlike other studies, we could not detect a relationship between MTF dose and vitamin B12 deficiency.

Although an increased MCV is expected in megaloblastic anemia, it has been shown that MCV is normal in 30% of cases with a positive response to vitamin B12 therapy [19]. According to our data, although the mean MCV value was within normal limits in the group with vitamin B12 deficiency, it had a higher value than the group without vitamin B12 deficiency. HbA1c value was higher in our patient group with vitamin B12 deficiency. We think this result may be related to the longer duration of DM in patients in the vitamin B12 deficiency group. Another result of our finding was that the detected diabetic neuropathy was higher in the vitamin B12 deficient group. The fact that patients with vitamin B12 deficiency have worse glycemic control (higher HbA1c value) and longer duration of diabetes may explain this result.

Tolerability for any drug is very important in terms of treatment compliance and quality of life of patients. MTF is an antidiabetic agent that improves glycemic control with low cost and also has good safety [20]. The ADA and EASD recommend MTF as initial therapy in patients newly diagnosed with T2DM [21]. However, the majority of patients cannot adequately tolerate this drug due to side effects. Approximately 25% of patients complain of MTF-related gastrointestinal side effects and approximately 5% cannot tolerate MTF at all. [22]. In some studies, gastrointestinal symptoms such as diarrhea, nausea, or abdominal discomfort, usually mild, have been reported to occur in up to 50% of patients using MTF [23]. In our study, our rates of MTF-related gastrointestinal symptoms were found to be higher than in other studies. We think this is because, unlike other studies, a metallic taste in the mouth is also included in the symptoms. However, according to our data, we could not find a significant relationship between the dose of MTF used and gastrointestinal symptoms. Conflicting results were also found in studies evaluating the relationship between increased MTF dose and gastrointestinal symptoms. Although some studies found a positive relationship, the authors did not comment on the importance of this relationship, while some authors reported this relationship as insignificant [24,25]. In a study in which Yuxin et al. examined the safety of different doses of MTF, they found no association between MTF dose and the incidence of gastrointestinal adverse events [26]. However, the ADA and EASD recommend reducing the dose of MTF when gastrointestinal symptoms occur, in the belief that symptoms will improve over time [27].

The clinical significance of vitamin B12 deficiency is that it can cause reversible bone marrow failure and some neurological disorders. Peripheral neuropathy may develop as a result of MTF-related vitamin B12 deficiency and may be confused with diabetic neuropathy in patients with DM under MTF therapy. It may also contribute to the exacerbation of diabetic neuropathy [28,29]. The important thing is to stop the progression of neurological damage due to vitamin B12 deficiency with early diagnosis and vitamin B12 supplementation [30]. However, permanent neurological damage can occur if neuropathic symptoms are misdiagnosed as diabetic neuropathy [29]. Therefore, detecting vitamin B12 deficiency in DM patients with neuropathic symptoms is extremely important. Already, the 2017 ADA treatment guidelines also recommend that vitamin B12 measurements be performed regularly in patients under MTF treatment [31]. With our results, we showed that the frequency of vitamin B12 deficiency is increased in patients with gastrointestinal symptoms or even three or more symptoms at the same time. We think this finding may provide benefits such as predicting patients with vitamin B12 deficiency by evaluating gastrointestinal symptoms.

The cause of vitamin B12 deficiency in people taking MTF has not been clearly clarified. Patients with T2DM have a different composition of gut microbiota compared to healthy individuals [32]. It has also been shown that daily use of 1 gram of MTF can change the microbiota after as little as three days [33]. It has also been reported that in women with type 2 diabetes, those treated with MTF have a different microbiota than those not treated with the drug [34]. It is known that disruptions in the microbiome can affect the absorption of nutrients, especially B vitamins. Therefore, changes in the gut microbiome are thought to be effective in MTF-related vitamin B12 deficiency [35]. On the other hand, changes in the microbiome by MTF may also have a potential role in gastrointestinal intolerance. Hata et al. reported a significant improvement in gastrointestinal tract symptoms after using the probiotic Bifidobacterium bifidum G9-1 in T2DM patients treated with MTF and supported this hypothesis [36]. As a result, there are highly complex relationships between MTF and the gut. Changes in the microbiome caused by the action of MTF can result in both gastrointestinal symptoms and vitamin B12 deficiency. In our study, we evaluated the relationship between vitamin B12 deficiency and gastrointestinal symptoms, which, to the best of our knowledge, has never been investigated before. Indeed, we found that vitamin B12 deficiency was more common in patients with gastrointestinal symptoms. One of our remarkable findings was that vitamin B12 deficiency was more common in patients with three or more symptoms than in patients with one or two symptoms. When we made a subgroup analysis according to vitamin B12 deficiency, we found that

this difference disappeared in the group without vitamin B12 deficiency. However, in the vitamin B12 deficient group, there was still a significant association between the duration of MTF use and gastrointestinal symptoms. The significant increase in the incidence of gastrointestinal symptoms in patients with vitamin B12 deficiency and using MTF for ≥10 years was the most striking finding of our study. According to our findings, we think that vitamin B12 deficiency may increase gastrointestinal symptoms in patients with T2DM using MTF. When we evaluate it together with the literature information, we think that MTF-related intestinal microbiome changes may be the basis of this.

Limitations of The Study

The fact that the study focused on vitamin B12 deficiency in a specific patient group increases the value of the study. However, it can be said that the study has some limitations. The inclusion of patients of single ethnicity and the fact that it is single-center is a limitation. Due to the study's cross-sectional design, the causal relationship between the variability of vitamin B12 levels over time and gastrointestinal symptoms cannot be clearly explained, but it can be speculated. At the same time, dietary habits may have an effect on gastrointestinal symptoms. However, this situation was not analyzed in the study.

Conclusion

In conclusion, our study showed that gastrointestinal symptoms seen in patients with T2DM using MTF might be related to vitamin B12 deficiency. We think that this relationship may be related to intestinal microbiome changes due to MTF. We recommend that patients who have used MTF for ≥10 years and have gastrointestinal complaints should be evaluated more closely for vitamin B12 deficiency. There is a need for multicenter prospective studies with more patients in which the causal relationship of vitamin B12 on gastrointestinal symptoms can be evaluated more clearly.

Conflict of interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical approval
The study was approved by the Clinical Research Ethics Committee of Medical Faculty of Hitit University (Decision date: 20.04.2022, Decision no: 2022-40).

Author Contributions
F. E. Conception, design, fundings, data collection and procesing, literature review, writting, critical review
D. T. Design, fundings, data collection and procesing, analysis and interpretation, writing

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