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ORIGINAL ARTICLE

Effect of Metformin usage on Vitamin B12 deficiency in patients with Type 2 Diabetes Mellitus

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Abstract

Metformin is the most commonly used drug in antihyperglycemic treatment in patients with type 2 diabetes mellitus (T2DM). In recent years, there have been many studies reporting B12 deficiency in diabetic individuals using metformin drug. This study evaluates B12 deficiency in individuals with T2 DM diagnosis using metformin in our hospital. This study is a cross-sectional and descriptive research. In this study, the data of 786 patients who applied to the Muğla Education and Research Hospital Internal Medicine outpatient clinic with T2DM diagnosis and used metformin between January 01, 2018 and March 01, 2019 were evaluated. Demographic data, vitamin B12, HbA1c levels of the patients were obtained retrospectively from the hospital information system records. Patients with missing data were excluded from the study. 347 (44.1%) male and 439 (55.9%) female patients were included in the study. Metformin dose of 248 (31.6%) of the patients was 1000 mg or less, 123 (15.6%) was 1700 mg, 414 (52.8%) was 2000 mg or more. According to the distribution of medication use, 161 (20.5%) patients were receiving metformin only, 322 (41%) patients were receiving metformin + other oral antidiabetic agents (OAD), and 303 (38.5%) patients were receiving metformin + insulin treatment. Vitamin B12 deficiency (57.8%) was higher in patients aged 60 years old and was over who used metformin (p<0.001). 347 (44.1%) of the patients included in the study received vitamin B12 replacement. We detected serious B12 deficiency in patients with T2DM who used metformin, especially in patients aged over 60 years old. Therefore, we emphasize the importance to monitor vitamin B12 levels in patients who are started on metformin and to perform B12 replacement in patients who are found to be deficient.

Keywords: Vitamin B12 deficiency, metformin, type 2 diabetes mellitus

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Introduction

Diabetes mellitus (DM) is a significant chronic metabolic disease with increasing frequency in developed and developing countries [1]. The prevalence of diabetes was estimated as 10.5% worldwide in 2021 by The International Diabetes Federation. It is estimated that it will increase to 11.3% in 2030 [2]. Metformin, a biguanide, has been used in the treatment of type 2 diabetes mellitus (T2DM) in Europe since 1957. According to the results of clinical researches, metformin improves cardiovascular outcomes in individuals with T2DM. Its proven efficacy, safety, and the fact that it can be used with other antidiabetic drugs have made metformin the most frequently used drug in the treatment of diabetes in the world [3]. The most common side effects of metformin are gastrointestinal origin such as loss of appetite, nausea, gas and metallic taste in the mouth. Another known important side effect of metformin is B12 malabsorption. In recent years, there have been many studies taking attention to vitamin B12 deficiency in diabetic individuals using metformin [4]. In 1969, Berchtold and colleagues reported evidence that vitamin B12 malabsorption developed in patients treated with metformin in a short period of 3 months [5]. In 1971, Tomkin and colleagues recommended annual B12 testing in patients using metformin for a long time [6]. Vitamin B12 deficiency itself causes neuropathy and should not be confused with diabetic neuropathy. On the other hand, B12 deficiency can become more pronounced and impair cognitive functions, especially in elderly individuals using metformin, due to the decrease in gastric parietal cells that produce intrinsic factor with aging [4]. Diagnosis and treatment of vitamin B12 deficiency are important, especially in elderly individuals using metformin. The Turkish Endocrine and Metabolism Association 2024 Diabetes Guideline recommends that B12 should be checked periodically in patients using metformin [7]. Although the relationship between metformin usage and vitamin B12 deficiency is known, the prevalence and onset of B12 deficiency among T2DM patients using metformin have not yet been fully elucidated [8]. Vitamin B12 which is also known as cobalamin, is a water-soluble cobalt-containing vitamin and is an important cofactor in many metabolic reactions. Vitamin B12 is found in many different forms such as 5-deoxyadenosyl cobalamin (adenosyl-Cbl), methycobalamin, hydroxocobalamin and cyanocobalamin. All forms of vitamin B12 are converted into methylcobalamin and adenosyl-Cbl within the cell. As a cofactor, it plays critical role in intracellular enzymatic reactions related to fatty acid and amino acid metabolism and DNA synthesis, and these enzymatic reactions are necessary for central nervous system functions and erythropoiesis [4]. In high-income countries, the most common cause of B12 deficiency is pernicious anemia, while in low-income countries, it is more likely due to inadequate B12 intake. Pernicious anemia can affect all ages, but its incidence increases with age. It is seen in 2-3% of individuals over the age of 65. Clinical B12 deficiency with classic hematological and neurological findings is rare. Low or borderline B12 deficiency without hematological and neurological findings is more common [9]. Diagnostic criteria for vitamin B12 deficiency are controversial; there is no full agreement on which biomarkers to use and which cut-off values to use [4]. In research and in the field, the cut-off value for vitamin B12 deficiency is used as < 148 pmol/L (200 pg/mL) and 200 pmol/L. While the 148 pmol/L value cannot find individuals with a deficiency of 3-5%, the 200 pmol/L value can find all individuals with deficiency. The most commonly used low-normal definition for serum vitamin B12 levels varies between 150 and 220 pmol/L [4].

This research was conducted to evaluate B12 deficiency in T2DM patients using metformin and followed up at the Muğla Education and Research Hospital Internal Medicine Polyclinic.

Materials and Methods

Ethics committee approval was accepted by the Muğla Sıtkı Koçman University Presidency Clinical Research Ethics Committee with the decision numbered 05/II dated 28/03/2019. This study is a retrospective cross-sectional and descriptive study. 786 patients who were followed up with a diagnosis of diabetes in the Muğla Education and Research Hospital Internal Medicine outpatient clinic between January 01, 2018 and March 01, 2019. Data required for the

study criteria were registered in the system and who were using metformin were included in the study. The patients' B12 levels and HbA1c levels were obtained by scanning through the Hospital Information Management System (HIMS). According to records, patients with T2 diabetes duration and a duration of metformin use of at least one year were included in the study. Diabetic patients were divided into 3 groups as those using only metformin, those using metformin and oral antidiabetic drugs (OAD) and those using metformin and insulin. According to metformin usage doses, 3 groups was taken as metformin doses of 1000 mg and below, 1700 mg, 2000 mg and above. HIMS records were also examined to see if they had taken vitamin B12 in the last year. According to age, they were divided into two groups as under 60 and over 60. As exclusion criteria, patients under 18 years of age, those with known diseases such as malabsorption that may cause vitamin B12 deficiency were not included in the study. The level for vitamin B12 deficiency was taken as <200 pg/mL (148 pmol/L). Vitamin B12 levels of all patients were studied with the electrochemiluminescence method on cobas e immunological test analyzers. HbA1c levels were also studied with the turbidimetric inhibition immunological test (TINIA) for hemolyzed whole blood on cobas c and COBAS INTEGRA systems.

Statistical Analysis

Data were tested with SPSS 25 package program. Descriptive statistics of the evaluation results will be given as number and percentage for categorical variables, mean and standard deviation for numerical variables. The conformity of the data to normal distribution was checked with *Kolmogorov-Smirnov* Test. Comparisons of numerical variables between two independent groups were made with Student T test when the normal distribution condition was met, and *Mann Whitney U* test when it was not met. Differences between the ratios of categorical variables in independent groups were tested with Chi-Square analysis. Spearman correlation analysis was used to determine the relationship between numerical data. Statistical significance level was accepted as $p<0.05$.

Results

In the study, data of individuals with T2DM who applied to Muğla Education and Research Hospital Internal Medicine outpatient clinics between January 01, 2018 and March 01, 2019, who had been using metformin for at least 1 year and whose B12 and HbA1c levels were examined were obtained retrospectively from the hospital information system. The data of 786 individuals with T2DM were examined within the study. The data of 786 individuals with T2DM were grouped according to the metformin dose they

Table 1. Characteristics of individuals with T2DM in terms of gender, medication use, and B12 replacement.

Gender	***n***	***%***
Male	354	45
Female	432	55
Metformin doses		
1000 mg ≤	248	31.6
1700 mg	123	15.6
≥2000 mg	415	52.8
Drug usage		
Metformin	161	20.5
Metformin + OAD (oral antidiabetic agents)	322	41
Metformin + insülin	303	38.5
B12 replacement		
Administered	347	44.1
Non-administered	439	55.9

used, medication usage and B12 replacement status in the last year. pg/mL was used as the unit for vitamin B12 level. 55.9% (n=439) of the individuals included in the study were female, 44.1% (n=347) were male. The characteristics of individuals with T2DM in terms of gender, medication usage and B12 replacement are given in Table 1.

The median age, B12 and HbA1c levels, and diabetes duration of individuals with T2DM were found to be 59 years, 181.35 pg/mL, 6.5, and 5 years, respectively.

In our study, it was observed that B12 levels increased as metformin dose increased. It was determined that B12 levels were higher in those using only metformin.

There was a positive correlation between HbA1c level and B12 replacement (p=0.001). HbA1c level was found to be lower in those who underwent B12 replacement. There was a positive correlation between B12 level and B12 replacement (p=0.000). B12 level was found to be

Table 2. B12 levels according to metformin dosage and type of usage.

		B12 level (median)	***Min-max***
Metformin dose	**1000 mg and below**	124	76-928
	1700 mg	134	67-634.8
	2000 mg and above	261	73-986
Metformin usage form	**Only metformin**	207.2	70-928.70
	Metformin + other OAD	181.8	73-934.70
	Metformin + Insulin	167	67-986

Table 3. Relationship between B12 level and age in the last year.

	B12 level (200 pg/mL below)		***B12 level (200 pg/mL and above)***			***p***
Age	**n**	**%**	**n**	**%**	**Total**	
Under 60 years old	207	50	207	50	414	**0.029**
Above 60 years old	215	57.8	157	42.2	372	

B12 levels were lower in patients aged 60 years and older (p=0.029).

Table 4. Relationship between B12 replacement status in the last year and gender, age, DM duration, B12 and HbA1c levels.

		B12 replacement done (n=347)	***No B12 replacement done (n=439)***	***p***
Age	Mean	59.82	57.60	0.032
	Median	60	58	
DM time	Mean	5.68	5.95	0.210
	Median	5	5	
B12 level	Mean	168.25	276.34	**0.000**
	Median	135	249	
HbA1c level	Mean	6.68	7.13	**<0.001**
	Median	6.4	6.6	
Gender		**n**	**%**	0.471
	Male	151	42.7	
	Female	203	57.3	

lower in those who underwent B12 replacement. There was a positive correlation between age and B12 replacement (p=0.032). The mean age of those who underwent B12 replacement was found to be higher. No significant relationship was found between B12 replacement status in the last year and gender and DM duration. The relationship between B12 replacement status in the last year and gender, age, DM duration, B12 and HbA1c levels is shown in Table 4.

The correlation between B12 replacement and metformin dose was found to be statistically significant (p=0.001). While 28.9% of those who received B12 replacement used 2000 mg and above metformin, 71.1% of those who did not receive replacement were using high dose metformin. No significant relationship was found between the type of metformin use and B12 replacement status. The relationship between B12 replacement status in the last year and metformin dose and usage status is shown in Table 5.

When the mean age was examined according to the method of metformin use, the mean age of the group using only metformin was found to be significantly lower than the other groups (p=0.046). No significant relationship was found between B12 levels and the method of metformin use. When the duration of DM was examined according to the method of metformin use, the duration of DM in the group using only metformin was found to be significantly lower than the other groups (p=0.001). When the HbA1c level was examined according to the method of metformin use, the HbA1c level in the group using only metformin was found to be significantly lower than the other groups (p=0.001). The relationship between the method of metformin use and age, duration of DM, B12 and HbA1c levels is given in Table 6.

Table 5. Relationship between B12 replacement status in the last year and metformin dose and method of use.

		B12 replacement done (n)		***No B12 replacement done (n)***			***p***
		n	**%**	**n**	**%**	**Total**	
Metformin dose	**1000 mg and below**	156	62.9	92	37.1	248	**0.001**
	1700 mg	71	57.7	52	42.3	123	
	2000 mg ve üzeri	120	28.9	295	71.1	415	
Metformin usage form	**Only metformin**	61	37.9	100	62.1	161	0.119
	Metformin + other OAD	141	43.8	181	56.2	322	
	Metformin + Insulin	145	47.9	158	52.1	303	

Table 6. Relationship between metformin use and age, DM duration, B12 and HbA1c levels.

		Only metformin	***Metformin + other OAD***	***Metformin + Insulin***	***p***
Age	Mean	56.73	59.57	58.51	**0.046**
	Median	57	60	59	
DM time	Mean	4.78	5.53	6.70	**0.001**
	Median	5	5	6	
B12 level	Mean	243.12	221.29	228.67	0,187
	Median	207.20	181.80	167.00	
HbA1c level	Mean	6.28	6.85	7.37	**0.001**
	Median	6.1	6.5	7	

Discussion

In our study of 786 patients with T2DM using metformin, the B12 level of 422 (53.69%) patients was found to be below 200 pg/mL (148 pmol/L). 347 (44.1%) of the patients were replaced because they had vitamin B12 deficiency. B12 levels were lower in patients who received replacement. Studies in the literature have shown that there are different rates of B12 deficiency in people using metformin. Our study found a high rate. It varies between 5.8% and 52% in the literature [4,10]. Randomized clinical studies have shown that metformin significantly reduces vitamin B12 levels after several months of use [3]. In cross-sectional studies, the decrease in vitamin B12 levels due to metformin use was found to be between 17.8% and 26.8%, while this decrease was found to be 6.3% and 18.7% in clinical researches lasting 6-16 weeks [11]. A randomized controlled study conducted by De Jager et al. showed that the decrease in vitamin B12 due to 4.3 years of metformin use was 19%. This study is the first to show a progressive decrease in vitamin B12 over the years in patients using metformin [12]. In the study conducted by Raizada et al., B12 deficiency was found to be 35.5% [13].

Many studies have shown that long-term and/or high-dose metformin use affects serum vitamin B12 levels [12,14,15]. In our study, B12 levels were found to be higher in patients using high-dose metformin than in those who did not use it. This situation, which is different from the literature, is thought to be due to the long duration of diabetes in patients using high-dose metformin and since our study did not examine replacement levels before 1 year, the patients may have previously received B12 replacement. In our study, when the groups using only metformin and those using metformin and other antidiabetic drugs were compared, the B12 level was found to be higher in the group using only metformin. The mean age of this group was lower than in the other group. 'Reinstatler et al.' In their study on patients with T2DM, B12 levels were found to be higher in patients with T2DM who used metformin than in those who did not use it [16].

In our study, B12 deficiency was more common in patients aged 60 and over who used metformin. There is limited research on the prevalence and determinants of vitamin B12 monitoring in elderly patients using metformin. In a study conducted in the United States, it was estimated that 6% of individuals under the age of 60 and 20% of individuals over the age of 60 had B12 deficiency (serum B12<148 pmol/L) [17].

B12 intake is low in individuals over the age of 60, and B12 malabsorption may occur due to atrophic gastritis [18]. In addition, drugs such as proton pump inhibitors, H2 receptor antagonists, and metformin also inhibit B12 absorption [19,20]. It is estimated that nearly 92 million metformin prescriptions were written in 2021 alone, a more than 2-fold increase since 2004 [21]. Despite the increase in metformin prescriptions, a single-center chart review study from the Veterans Affairs Medical Center (VAMC) found that only 40% of patients on high-dose metformin (≥2000 mg/day) had their serum vitamin B12 levels checked, and 50% of those treated with metformin for more than 10 years had never had their vitamin B12 levels checked [22]. Elderly diabetic patients with B12 deficiency are at higher risk for peripheral neuropathy, neuropathic pain, and related mobility limitations than younger patients and those without diabetes [19]. These patients have a variety of risk factors for peripheral neuropathy. Since these patients have a history of T2DM, their complaints may be directly attributed to diabetic neuropathy, and a simple treatable cause such as B12 deficiency may be overlooked. This may lead to polypharmacy and inappropriate medication prescribing [14]. Therefore, screening and treatment for B12 deficiency should be recommended for all patients, especially elderly patients, due to possible effects. The limitations of our study include not looking at the patients' B12 replacement before 1 year and the duration of Metformin use, and not including comorbidities in the study.

Conclusion

T2DM is increasing day by day in the world and in our country. Metformin is the most commonly used drug in treatment. Side effects such as B12 deficiency are emphasized in the literature. As

a result of our 1-year retrospective follow-up in the Muğla Education and Research Hospital Internal Medicine outpatient clinic, we detected serious B12 deficiency in T2DM patients using metformin, especially in patients over the age of 60. Therefore, we emphasize the importance to monitor vitamin B12 levels in patients who are started on metformin and to perform replacement in patients with deficiency.

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Conflict of interest

There is no conflict between the authors.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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