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Effect of a combination of gliptin and metformin on serum vitamin B12, folic acid, and ferritin levels

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SUMMARY

OBJECTIVE: The primary objective of this study was to explore the impact of metformin and metformin/gliptin combination therapy on the serum concentrations of vitamin B12, ferritin, and folic acid in individuals diagnosed with type 2 diabetes.

METHODS: This study included 118 patients, classified into two groups: 59 patients using only metformin and 59 patients using a combination of metformin/gliptin. Among the latter group, 35 patients used vildagliptin/metformin, and 24 used sitagliptin/metformin. The study recorded the demographic data such as the age and gender of the patients, as well as their initial and 1-year follow-up blood parameters.

RESULTS: Folic acid decreased significantly in the metformin group but not in the metformin/gliptin group. Vitamin B12 and ferritin decreased significantly in both groups. The decrease in vitamin B12 and ferritin was not significantly different between the two groups. The decrease in fasting plasma glucose was more significant in the metformin/gliptin group than in the metformin group.

CONCLUSION: After 1 year, both groups taking metformin and metformin/gliptin showed low serum ferritin and vitamin B12 levels. Therefore, vitamin B12 levels in patients using these drugs should be closely monitored. Ferritin levels can be used to indicate whether glycemic control has been achieved.

KEYWORDS: Diabetes mellitus. Metformin. Folic acid. Gliptin. Ferritin. Vitamin B12.

INTRODUCTION

Metformin is commonly the first choice in monotherapy if there is no contraindication in cases where hyperglycemia cannot be controlled despite lifestyle changes, which is the first step[1].

Intestinal absorption of vitamin B12 is reduced in approximately 30% of patients taking metformin. According to one view, the reason for this decrease is the antagonism of the calcium-dependent channel of vitamin B12 in the ileum. This situation is likely to improve with vitamin B12 and calcium supplementation[2]. This decrease in vitamin B12 absorption begins 4 months after metformin is started[3]. Since vitamin B12 can be stored in the liver, the clinical manifestation of vitamin B12 deficiency may take 3–10 years, but it may cause megaloblastic anemia beforehand[4].

Gliptins function by inhibiting the DPP-4 enzyme. It is OADs that increase the effect of endogenous incretins by this mechanism. Vildagliptin, saxagliptin, sitagliptin, linagliptin, and alogliptin are in the dipeptidyl peptidase-4 inhibitors (DPP4-I) group. Drugs in this group have a low risk of hypoglycemia, a small weight loss effect, and are well tolerated. It is more expensive than sulfonylureas and metformin and is not recommended for use in patients with a history of liver failure, heart failure, or pancreatitis[5].

Vitamin B12

The most common side effect of metformin is gastrointestinal intolerance, such as diarrhea, nausea, flatulence, and indigestion[6]. Vitamin B12 is essential for many systems, such as the nervous and hematopoietic systems. Intestinal B12 absorption is reduced in approximately 30% of patients taking metformin. Serum vitamin B12 levels decrease in 10% of patients. This decrease in vitamin B12 absorption begins 4 months after starting metformin[3].

Folic acid

Inadequate dietary intake, pregnancy, chronic hemolytic anemia, increased folic acid requirements of the body due to hemodialysis, intestinal malabsorption, antibiotics such as methotrexate and trimethoprim, and antiepileptic drugs such as phenytoin, carbamazepine, and valproate may cause folic acid deficiency. There is no generally accepted judgment that metformin can cause folic acid deficiency[7].

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Ferritin

Ferritin is a positive acute-phase reactant and, along with transferrin and its receptor, is a member of a protein family that modulates cellular defenses against inflammation. Its serum levels increase oxidative stress and inflammation[8,9].

Publications have shown a relationship between serum ferritin levels, excessive iron absorption, and type 2 diabetes, but this relationship has not yet been proven[10]. Although diets with low iron content are recommended, publications show that ferritin levels are high in patients whose glycemic control cannot be achieved[11,12].

Aim

This study examines the effects of serum levels of vitamin B12, ferritin, and folic acid at baseline and after 1 year in patients with type 2 diabetes using a combination of metformin and metformin/gliptin.

METHODS

A total of 2,459 patients who applied to our center were examined. According to the ADA guidelines, these patients were diagnosed with type 2 DM, had regular follow-ups, and were using metformin alone or in combination with gliptin. As a result of a retrospective file review, patients who were newly diagnosed with type 2 DM, had complete vitamin B12, folic acid, ferritin, and HbA1c values before and 1 year after medication prescription, had not been prescribed any medication, including proton pump inhibitor for 1 year, did not receive insulin or other oral antidiabetic treatment, did not use alcohol, did not have megaloblastic anemia, did not undergo stomach surgery, did not have gastrointestinal system diseases such as celiac, Crohn's disease, *Helicobacter pylori*, chronic pancreatitis, malabsorption, and did not have any chronic systemic disease other than a new diagnosis of type 2 DM were included in the study.

A total of 118 patients were included in the study, with 59 using only metformin and 59 using metformin/gliptin. Of those who used metformin/gliptin, 35 used metformin/vildagliptin, and 24 used metformin/sitagliptin. Demographics such as patient age and sex, baseline, and 1-year follow-up blood parameters were recorded.

Statistical analysis method

A descriptive analysis was conducted to elucidate the fundamental characteristics of the populations encompassed within our study. In this cross-sectional retrospective study, distribution tests, namely, normality tests, skewness kurtosis assessments, and histogram plots, were employed to scrutinize the distribution of the collected data. Nonparametric tests were used for data sets exhibiting abnormal distributions. Specifically, the Mann-Whitney U test was employed to examine independent numeric variables, while the chi-square test was used for categorical variables. Numeric variables were reported as the median value accompanied by the interquartile range, whereas categorical variables were presented as numerical values and percentages. To compare medians between two related groups, the Wilcoxon's test was employed, and cases with a significance level of $p<0.05$ were deemed statistically significant. All statistical analyses were conducted using IBM SPSS 23. The present study received ethical approval from the Ethics Committee of Sakarya University School of Medicine on October 12, 2020, with the decision number 71522473/050.01.04/553. This approval confirmed that the study adhered to the established ethical guidelines.

RESULTS

Our study population consisted of 118 patients, including 59 patients who were given metformin as the initial treatment and 59 patients who were given metformin/gliptin as the initial treatment for type 2 DM. Baseline laboratory parameters were compared with laboratory values 1 year later.

The study included 118 patients in total, with 59 patients receiving metformin in their initial treatment and 59 receiving metformin/gliptin. The median age [interquartile range] of all patients was 55 [14] years, with the metformin group having a median age of 51 [17] years and the metformin/gliptin group having a median age of 58 [11] years. The higher age in the metformin/gliptin group was statistically significant (p=0.022). Of the patients in the metformin group, 39 (66.1%) were females, while the number of females in the metformin/gliptin group was 20 (33.9%) (p<0.001). When comparing the baseline HbA1c percentages of the two groups, the metformin group had an HbA1c percentage of 6.5% [1.3], while the metformin/gliptin group had an HbA1c percentage of 8.6% [2.4] (p<0.001).

When comparing initial vitamin B12, ferritin, and folic acid levels in patients receiving metformin alone versus the metformin/gliptin combination, vitamin B12 levels did not significantly differ between the two groups (p=0.122). However, the metformin/gliptin combination group had significantly higher ferritin levels (83 [121.4] µg/L) compared to the metformin group (46 [83] µg/L) (p=0.020). Additionally, folic acid levels were significantly higher in the metformin group (8.9 [4.8]

μg/L) compared to the metformin/gliptin combination group (6.9 [3.2] μg/L) (p<0.001) (Table 1).

The group that initiated metformin exhibited a noteworthy reduction in vitamin B12 levels from 275 [178] to 232 [151] ng/L (p<0.001), while the group that began the metformin/gliptin combination also experienced a substantial decrease from 309 [218] to 260 [160] ng/L (p<0.001). Furthermore, the group that commenced metformin demonstrated a significant reduction in ferritin levels from 46 [83] to 38 [60.3] μg/L (p<0.001), and the group that started metformin/gliptin combination also had a substantial decrease from 83 [121] to 66.2 [90] μg/L (p=0.002) (Table 2).

We examined the values that showed a statistically significant decrease after 1 year in the metformin and metformin/gliptin groups. Upon comparing the variances between baseline and 1-year measurements in both groups, no statistically significant differences were observed between the two groups regarding the reduction in vitamin B12 (p=0.346) and ferritin levels (p=0.379). While metformin decreased the fasting plasma glucose (FPG) by 8 [23] mg/dL in 1

Table 1. Comparison of baseline values of patients receiving metformin and metformin/gliptin combination.

Variables	Metformin, n=59	Metformin/gliptin, n=59	Total, n=118	p-value
Age, years	52 [17]	58 [11]	55 [14]	**0.022**
Gender				**0.001***
Female, n (%)	39 (66.1%)	20 (33.9%)	59 (50%)	
Male, n (%)	20 (33.9%)	39 (66.1%)	59 (50%)	
Fasting plasma glucose (FPG), mg/dL	131 [27]	183 [84]	146 [80]	**<0.001**
eGFR	99 [21]	100.7 [14.6]	100 [19]	0.651
Triglyceride, mg/dL	152 [127.5]	140 [97.5]	144 [109]	0.481
Total cholesterol, mg/dL	215 [37]	208.5 [53]	212.5 [43]	0.380
HDL, mg/dL	48 [14]	41.5 [12]	43.5 [15]	**0.005**
LDL, mg/dL	142 [44]	133.5 [36]	139.5 [38.5]	0.318
HbA1c, %	6.5 [1.3]	8.6 [2.4]	7.4 [2.8]	**0.001**
Vitamin B12, ng/L	275 [178]	309 [218]	302 [197]	0.122
Ferritin, μg/L	46 [83]	83 [121.4]	60 [94]	**0.020**
Folic acid, μg/L	8.9 [4.8]	6.9 [3.2]	7.95 [3.2]	**0.001**

Median [interquartile range], Mann-Whitney U test was used to compare numerical parameters, and chi-square test (*) was used to compare categorical parameters. Bold values indicate statistical significance at the p<0.05 level.

Table 2. Comparison of changes values after 1 year in patients who received metformin and metformin/gliptin combination.

Variables	Metformin		p-value	Metformin/gliptin		p-value
	n=59	n=59		n=59	n=59	
	Beginning	1 year later		Beginning	1 year later	
Fasting plasma glucose (FPG), mg/dL	131 [27]	116 [30]	**0.001**	183 [84]	143 [57]	**<0.001**
eGFR	99 [21]	104 [24]	0.737	100.7 [16.1]	102.3 [11.05]	0.208
Triglyceride, mg/dL	152 [132.75]	160.5 [118.5]	0.467	140 [98.25]	144 [119]	0.486
Total cholesterol, mg/dL	215 [37.5]	205 [52]	0.564	208.5 [55]	210.5 [76]	0.866
HDL, mg/dL	48 [14.175]	46.5 [17.75]	0.658	41.5 [12.25]	42 [12]	0.611
LDL, mg/dL	142 [44.75]	136 [47.5]	0.362	133.5 [36.75]	138 [61]	0.721
HbA1c, %	6.5 [1.3]	6.4 [1.3]	0.112	8.6 [2.4]	7.3 [1.2]	**0.001**
Vitamin B12, ng/L	275 [178]	232 [151]	**0.001**	309 [218]	260 [160]	**0.001**
Ferritin, μg/L	46 [83]	38 [60.3]	**0.001**	83 [121.4]	66.2 [99]	**0.002**
Folic acid, μg/L	8.9 [4.8]	8.1 [5.1]	**0.001**	6.9 [3.2]	6.4 [3.4]	0.346

Median [interquartile range], Wilcoxon's test was used to compare numerical parameters. Bold values indicate statistical significance at the p<0.05 level.

year, the metformin/gliptin combination decreased it by 37 [76] mg/dL (p=0.001).

DISCUSSION

Wulffelee et al. tested the effect of metformin on serum homocysteine, vitamin B12, and folic acid levels in a placebo-controlled, randomized study in 12 patients who were started on placebo and 25 metformin in a short period of 16 weeks. After 16 weeks, she found a 4% increase in homocysteine, a 7% decrease in folic acid, and a 14% decrease in vitamin B12, which were statistically significant compared to the placebo. The findings of this study indicate that the administration of metformin to individuals with type 2 DM leads to a reduction in folic acid and vitamin B12 levels, resulting in a slight elevation of homocysteine[13].

Indeed, the effects of metformin are not limited to plasma glucose alone. In an androgenized rat model, metformin demonstrates a significant impact on ovarian follicle dynamics by reducing the proliferation of theca cells and suppressing CYP-17 expression[14]. On the contrary, the combination of oral contraceptives and metformin did not improve insulin resistance in women with polycystic ovary syndrome[15]. The combination of metformin and lifestyle changes has the potential to boost the number of menstrual cycles in individuals with polycystic ovary syndrome[16]. Metformin demonstrated positive effects on glucose levels and the homeostasis model assessment-insulin resistance index in female rats androgenized with testosterone. Additionally, it resulted in a partial reversion of ovarian and uterine morphology in these rats[17].

In a prospective study investigating the tolerability of teneligliptin, a DPP4-I, and its effect on peripheral neuropathy in patients with type 2 DM, 20 mg of teneligliptin was given once a day for 3 months. In addition to the study-specific parameters, the vitamin B12 value at baseline and in the third month was also checked to exclude neuropathy due to vitamin B12 deficiency. The initial vitamin B12 value was 594 units/L; 3 months later, it was found to be 457 units/L (p=0.33). No statistically significant difference was found between the vitamin B12 values initially and after 3 months[18]. When examining the effect of metformin on vitamin B12 in 159 patients, they reported no statistically significant difference in the subgroup analysis when metformin was used with DPP4-I[19]. In our literature review for DPP4-I, studies investigating the effects of DPP4-I drugs alone or in combination on serum vitamin B12, ferritin, and folic acid levels were relatively few compared to metformin. We could not find any study on DPP4-I and its combinations of folic acid, ferritin, and vitamin B12 from the time of diagnosis without diabetes.

When we compared the variances between baseline and first-year measurements in both groups, no significant association was found between the groups in which metformin or metformin/gliptin was started in reducing vitamin B12 and ferritin (p=0.346 for vitamin B12; p=0.379 for ferritin).

In a study conducted in 329 type 2 DM patients versus 269 healthy control groups, Canturk et al. found that serum ferritin increased in poorly controlled DM as long as glycemic control was not provided[20]. Chandrashekhar et al. investigated the effect of glycemic control on serum ferritin value in 100 patients, 50 of whom had an HbA1c of 6.5% and below and 50 well-controlled, versus 50 patients with poorly controlled serum Hba1c of 8% and above. While the mean of ferritin in the uncontrolled group was 392 µg/L, it was 91 µg/L in the group with glycemia under control. It was statistically significant that ferritin was high in the poorly controlled group (p<0.001). No statistically significant correlation was found between ferritin and age in either group[21]. Ferritin was 46 [83] µg/L in the metformin-initiated group and 83 [121.4] µg/L in the combination group, and the ferritin value was statistically higher in the metformin/gliptin combination group (p=0.020). This difference may be because women were more common in the metformin group between the two groups, as well as because of statistically higher levels of HbA1c and FPG in the metformin/gliptin group. In both cases, the proportional data of our study on ferritin are compatible with the literature.

CONCLUSION

After 1 year, both metformin and metformin/gliptin groups had low serum ferritin and vitamin B12. Therefore, vitamin B12 levels should be carefully monitored in patients taking these medications. Ferritin levels can be used to indicate whether glycemic control has been achieved. Further randomized, controlled studies are needed for more reliable results.

AUTHORS' CONTRIBUTIONS

FTG: Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **AN:** Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. **ACG:** Resources, Software, Supervision, Validation, Visualization, Writing – original draft. **TK:** Methodology, Supervision, Supervision.

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