Research Article

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A new approach for the pleiotropic effect of metformin use in type 2 diabetes mellitus

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Abstract

Objectives: Metformin is the first choice for type 2 diabetes mellitus (T2DM) treatment in the guidelines and is used in combination with many drugs. Growth arrest-specific protein 6 (Gas6)/Axl signaling plays a role in many metabolic disorders. This study aims to investigate the effects of metformin and metformin-insulin combination used in patients with T2DM on Gas6, Axl, and soluble Axl (sAxl) levels.

Methods: A total of 71 patients diagnosed with T2DM and 21 healthy subjects were divided into 4 groups control, diet and exercises recommended (DER), metformin, and metformin + insulin. Diabetic patients were treated with metformin only or with a metformin-insulin combination and monitored for six months. Gas6, Axl, and sAxl levels of subjects' sera obtained from their baseline and post-therapeutic sixth month blood samples were measured by ELISA methods.

Results: Compared to baseline, the sixth month Gas6 and Axl levels of metformin and metformin + insulin groups significantly decreased ($p<0.05$). However, there was no statistically significant difference in sAxl values for these two groups of patients.

Conclusions: The use of metformin in diabetic patients may be beneficial for inhibiting the Gas6/Axl pathway. This study presents a new aspect of the pleiotropic effects of metformin. This study will be clinically useful for designing therapeutic approaches targeting Gas6/Axl.

Keywords: axl; Gas6; metformin; sAxl; tip 2 diabetes mellitus.

Introduction

For long-term diabetic patients, insulin and oral antidiabetic drugs may need to be used together in order to control their glycemia. Metformin is one of such drugs belonging to the biguanide class of anti-diabetic compounds. It is the most preferred drug in the treatment of T2DM in combination with many drugs, and hence it is the first choice in the guidelines [1–4].

Metformin appears to have pleiotropic effects in addition to an antihyperglycemic effect. The drug has provided clinical efficacy in conditions associated with hyperinsulinemia such as prediabetes, gestational diabetes, polycystic ovary syndrome, and non-alcoholic steatohepatitis [5–7].

Metformin may also have an antiproliferative effect in various cancer types such as pancreatic, breast, prostate, and colon tumors [8]. Reduced risk of cancer has been reported in T2DM patients treated with metformin compared to patients treated with other antidiabetic drugs [9].

Growth arrest-specific protein 6 (Gas6) protein is the ligand of Tyro3, Axl, and Mer receptors (TAM receptors). Gas6 protein binds and activates the TAM receptors. The binding affinity of Gas6 to TAM family receptors is as follows: Axl ≥ Tyro3 ≥ Mer [10].

Axl, a membrane-bound 140 kDa form, is expressed in many cells and tissues. Gas6 has a function in the pathogenesis of impaired glucose, insulin resistance, and homeostasis [11]. The vitamin K-dependent Gas6 binds Axl and causes autophosphorylation of Axl. This complex activates several downstream signaling pathways, including the phosphoinositide 3-kinase (PI3K)/AKT pathway, one of the important signaling pathways involved in glucose metabolism [12].

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Soluble Axl (sAxl) is an 85 kDa protein and it is produced by the proteolytic cleavage of extracellular domains by metalloproteinases (ADAM10 and ADAM17) and a disintegrin. sAxl is a negative regulator of endogenous Axl signaling [13]. Serum Gas6 circulates in a complex with sAxl and hence Gas6 is present at low concentrations in circulation [14].

The Gas6/Axl signaling has been shown to play an important role in inflammation, homeostasis, autoimmune diseases, nervous system, reproductive system, vascular system, cancer, and associated glucose intolerance with metabolic disorders [15, 16].

Studies have reported that the use of metformin in patients with T2DM regulates blood glucose and reduces the risk of cancer. Metformin treatment improves insulin sensitivity, inhibits hepatic glucose output, and lowers circulating insulin levels [17].

The clinical role of the Gas6/Axl pathway in the molecular mechanism of complications associated with T2DM is not fully understood. In addition, studies related to impaired glucose metabolism are controversial and insufficient. Therefore, we aimed to investigate the effect of metformin and metformin-insulin combination on serum Gas6, Axl, and sAxl parameters in patients with T2DM. We also wanted to see how the effect changed when insulin was used in combination with metformin, and to determine whether sAxl levels were associated with transmembrane Axl levels.

Materials and methods

Subjects

This study was conducted with the approval of Erciyes University Clinical Research Ethics Committee (2018/370). Seventy-one patients diagnosed with T2DM and 21 healthy subjects admitted to Erciyes University Health Application and Research Center between 1 August 2018 and 1 March 2020 were included in the study. T2DM was diagnosed based on the American Diabetes Association diagnostic criteria. Newly diagnosed T2DM patients and those previously diagnosed with T2DM were prospectively evaluated. The subjects were divided into 4 groups, control (11 females and 10 males), diet and exercise recommended (DER) (14 females and 8 males), metformin (Met) (16 females and 8 males), and metformin + insulin (Met + Ins) (11 females and 14 males). Diabetic patients were medicated with metformin only or with a metformin-insulin combination and monitored for 6 months.

Inclusion Criteria for the Control Group were as follows: Male and female individuals aged 20–65 years, BMI between 18 and 30 kg/m^2, HbA_{1c}<5.7%, non-smoking and non-alcohol consuming, and not using statin group drugs.

Inclusion criteria for the patient group: Male and female patients aged 20–65 years, BMI between 18 and 30 kg/m^2, diabetes duration does not exceed 15 years (HbA_{1c}≥5.7%), non-alcohol consuming and non-smoking patients, not using statin group drugs.

Exclusion criteria: Male and female patients who are not between the ages of 20 and 65 years, BMI not between 18 and 30 kg/m^2, diabetes duration exceeds 15 years (HbA_{1c}≥5.7%), alcohol-consuming and smoking patients, using statin group drugs.

The groups were determined according to the HbA_{1c} levels of the patients. Treatments were determined by considering the lifestyles (diet and exercise) and age of the patients. Group 1 was the control group consisting of volunteers who were admitted to the outpatient clinic for routine control purposes and did not have any chronic diseases. Group 2 was the DER group consisting of volunteers newly diagnosed with T2DM, not using drugs but recommended diet and exercise. Group 3 was the Met group consisting of volunteers newly diagnosed with T2DM and planned to be treated with metformin. Group 4 was the Met + Ins group consisting of volunteers who were previously diagnosed with T2DM and treated with metformin but this treatment was not sufficient, and therefore they started insulin in combination with metformin. In this group, two patients used basal insulin, one used Basal + Bolus insulin, and nineteen used 2 or 3 mixed insulin.

Patients were prospectively treated for six months. Blood samples were collected from volunteers in the groups at the start of the study and six months after the start of treatment. All measurements were performed after the study.

Specimens

Fasting blood specimens were collected in evacuated blood collection tubes (SST, Dickinson & Company, USA) between 9:00 a.m. and 11:00 a.m., and the specimens were centrifuged at 1300 *g* for 10 min. Then serums were removed and collected in Eppendorf tubes. Serum samples were kept frozen at −80 °C until the measurements.

Procedures

The study was carried out with materials obtained during routine examination and treatment procedures. During these studies, underwent routine treatment deemed appropriate by the physician to the patient. The study was conducted using the study groups' volunteered individuals' blood samples obtained during the routine examination and treatment procedures at the beginning of the study and then six months later.

Gas6, Axl, and sAxl serum protein concentrations were measured by sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA) (Biotek, Beijing, China) in accordance with the manufacturer's instructions. Serum Gas6, serum Axl and serum sAxl were detected using a human ELISA kit (YL Biotech, Shanghai, China), and the inter-assay and intra-assay values were <8% and <10%, respectively, for each of Gas6, Axl and sAxl. Fasting blood glucose (FBG) and HbA_{1c} values were measured to better evaluate the glycemic status of the patients in the study groups. Serum glucose levels were enzymatically measured using the hexokinase method in a Roche Cobas c701 analyzer (Roche Diagnostics, Germany), while HbA_{1c} measurements of the full blood samples were collected in EDTA-containing tubes were conducted immunoturbidimetrically in a Roche Cobas c501 immune analyzer (Roche Diagnostics, Germany).

Statistical analysis

The data obtained in this study were statistically analyzed using SPSS statistical software version 27 (IBM Corp., Armonk, NY, USA) and Microsoft Excel® 365. Pearson's chi-square test was used for categorical variables. Numerical variables were compared with tests suitable for dependent and independent samples (Wilcoxon test, Kruskal–Wallis H test, or Mann-Whitney U test) after the normality distribution was checked with the Kolmogorov–Smirnov test. Results are given as medians (interquartile ranges). Two-sided p<0.05 were considered statistically significant. After the Kruskal–Wallis H test, Bonferroni correction of p-value was made for post-hoc analysis when necessary.

Results

Demographic information of T2DM groups and control group

Demographic information (gender, age, and body mass index) of the groups is presented in Table 1. The individuals in the control group and other groups were identical in terms of age (p=0.245) and gender (p=0.362). Also, body mass index (BMI) data did not differ statistically among the groups (p=0.132).

Comparison of the 0th and 6th month values of Gas6, Axl, and sAxl parameters of the groups

The comparison of the 0th and 6th month values of the Gas6, Axl, and sAxl parameters of the study groups is given in Table 2.

There were no statistically significant changes in Gas6, Axl, and sAxl parameters in the groups without drug treatment (Control and DER groups). However, a statistically significant decrease in Gas6 and Axl values was detected in the Met and the Met + Ins groups. There were no statistically significant changes in sAxl values.

When the parameters (Gas6, Axl, and sAxl) of the study groups investigated in the 0th and 6th months were evaluated, there were no significant differences among the groups in the 0th month. In the 6th month, there were no significant differences in Gas6 and sAxl parameters between the groups, but only a significant decrease in Axl value was observed.

Table 1: Demographic information of the study groups.

	Control (n=21)	DER (n=22)	Met (n=24)	Met + Ins (n= 25)	p-Value[c]
Gender (M/F)[a]	10/11	8/14	8/16	14/11	0.362
Age (Year)[b]	49 (10)	53 (13)	54 (12)	53 (18)	0.245
BMI (kg/m^2)[b]	26.1 (5.0)	28.3 (5.5)	28.7 (5.5)	26.7 (3.8)	0.132

DER, diet and exercise recommended group; Met, metformin group; Met + Ins, Metformin + Insulin group; M, male; F, female; BMI, body mass index. [a]The data were given as rates. [b]The data were given as medians (interquartile ranges-IQR). [c]Kruskal–Wallis H and Pearson chi-square tests were used.

Table 2: Comparison of Gas6, Axl, and sAxl parameters for Baseline and 6th month.

Groups	Parameters	0 Month	6 Month	p-Value[a]
Control	Gas6 (ng/mL)	10.8 (15)	9.1 (17)	0.547
	Axl (ng/mL)	145.1 (207)	160.0 (429)	0.916
	sAxl (pg/mL)	3.0 (4)	3.7 (14)	0.109
DER	Gas6 (ng/mL)	10.5 (7)	9.5 (8)	0.137
	Axl (ng/mL)	162.7 (99)	167.6 (117)	0.380
	sAxl (pg/mL)	4.0 (3)	4.2 (3)	0.581
Met	Gas6 (ng/mL)	10.7 (5)	7.4 (3)	**0.028**
	Axl (ng/mL)	147.7 (88)	107.5 (53)	**0.003**
	sAxl (pg/mL)	3.2 (2)	3.1 (1)	0.424
Met + Ins	Gas6 (ng/mL)	10.6 (2)	8.1 (2)	**0.009**
	Axl (ng/mL)	170.2 (49)	95.0 (43)	**0.005**
	sAxl (pg/mL)	2.9 (1)	2.5 (1)	0.382
p-value[b]	Gas6 (ng/mL)	0.823	0.107	–
	Axl (ng/mL)	0.360	**0.001**[c,d,e]	–
	sAxl (pg/mL)	0.222	0.240	–

DER, diet and exercise recommended group; Met, Metformin group; Met + Ins, metformin + insulin group; Gas6, Growth Arrest Specific Protein 6; Axl, Axl; sAxl, Soluble Axl. The data were given as median (interquartile range-IQR). [a]The comparison of the 0th and 6th month values of the Gas6, Axl, and sAxl parameters. [b]The comparison of the parameters between all groups. Bold p-values indicate statistically significant differences. In the intergroup analysis: [c]p<0.05 for the difference between the control group and Met + Ins group; [d]p<0.05 for the difference between DER group and Met group; [e]p<0.05 for the difference between DER group and Met + Ins group.

Comparison of FBG and HbA_{1c} parameters for baseline and 6th month

The comparison of the baseline and 6th month values of the FBG and HbA_{1c} parameters of the study groups is given in Table 3. When the FBG and HbA_{1c} parameters of the study groups investigated in the 0th and 6th months were evaluated, there where a significant decrease was observed in the FBG and HbA_{1c} values of the study groups, especially in the groups using drugs (Met group and Met + Ins group) (Table 3).

Table 3: Comparison of FBG and HbA_{1c} parameters for baseline and 6th month.

Parameters	Control (n=21)	DER (n=22)	Met (n=24)	Met + Ins (n=25)	p-Value[g]
FBG, mg/dL (0th month)	93 (13)	98 (15)	129 (39)	226 (141)	**<0.05**[b,c,d,e]
FBG, mg/dL (6th month)	95 (12)	102 (14)	113 (31)	137 (169)	**<0.05**[b,c,e]
HbA_{1c}, % (0th month)	5.4 (0.4)	5.9 (0.1)	7.2 (1.1)	10.0 (2.8)	**<0.05**[a,b,c,e,f]
HbA_{1c}, % (6th month)	5.4 (0.4)	6.0 (0.2)	6.5 (0.7)	8.0 (2.0)	**<0.05**[a,b,c,e]

FBG, fasting blood glucose; DER, diet and exercise recommended group; Met, metformin group; Met + Ins, metformin + insulin group. [a]p<0.05 for the difference between the control group and DER group; [b]p<0.05 for the difference between the control group and Met group; [c]p<0.05 for the difference between the control group and Met + Ins group; [d]p<0.05 for the difference between DER group and Met group; [e]p<0.05 for the difference between DER group and Met + Ins group; [f]p<0.05 for the difference between Met group and Met + Ins group; [g]The comparison of the parameters between all groups. Bold p-values indicate statistically significant differences. In the intergroup analysis.

Correlation of FBG and HbA_{1c} levels with Gas6, Axl and sAxl parameters

A significant decrease was observed in the FBG and HbA_{1c} values of the study groups, especially in the groups using drugs (Met group and Met + Ins group). There was also a decrease in Gas6 and Axl values. However, it was found that the 0th and 6th months FBG and HbA_{1c} levels of the study groups did not significantly correlate with the corresponding Gas6, Axl, and sAxl parameters (Table 4).

Correlations of Gas6, Axl, and sAxl parameters according to use of antidiabetic drugs

Correlations of Gas6, Axl, and sAxl parameters of the study groups according to the use of antidiabetic drugs are given in Table 5. When the groups that did not use drugs were examined, a highly significant positive correlation was found between Gas6 and Axl; and a moderate statistically significant positive correlation was found with sAxl (r=0.725, p<0.001; r=0.541, p<0.001; respectively). A moderate positive correlation was found between Axl and sAxl (r=0.597, p<0.001). A significant moderate positive correlation was found between Gas6 and Axl in the Met group using metformin for 6 months (r=0.664, p<0.05), and between Axl and sAxl (r=0.587, p<0.05) in the Met + Ins group using insulin combined with metformin.

Discussion

In this study, the effects of metformin alone and the effects of insulin used in combination with metformin, in patients with T2DM on Gas6, Axl, and sAxl levels were investigated.

Table 4: Correlation of FBG and HbA_{1c} levels with Gas6, Axl, and sAxl parameters for baseline and 6 months.

Parameters	Groups	n	Gas6		Axl		sAxl	
			r	p-Value	r	p-Value	r	p-Value
FBG, mg/dL (0th month)	Met + Ins[a]	25	0.140	0.078	−0.095	0.650	0.275	0.183
HbA_{1c} (0th month)	Met + Ins[a]	25	0.291	0.158	−0.308	0.134	−0.013	0.950
FBG, mg/dL	Control	13	0.359	0.228	0.337	0.260	0.519	0.069
(6th month)	DER	15	−0.414	0.125	−0.293	0.289	0.104	0.703
	Met	12	0.224	0.484	0.056	0.863	0.392	0.208
	Met + Ins	13	−0.527	0.064	−0.253	0.405	0.138	0.654
HbA_{1c} (6th month)	Control	13	−0.210	0.491	−0.285	0.345	−0.229	0.451
	DER	15	0.114	0.687	−0.234	0.400	−0.279	0.314
	Met	12	−0.359	0.252	−0.085	0.794	0.317	0.316
	Met + Ins	13	−0.352	0.239	−0.357	0.231	−0.146	0.635

FBG, fasting blood glucose; DER, diet and exercise recommended group; Met, metformin group; Met + Ins, metformin + insulin group. Spearman's rho correlation analysis was applied. [a]Caution: at month 0, patients in the Met + Ins group were using only metformin during blood analysis.

Table 5: Correlations of Gas6, Axl, and sAxl parameters according to antidiabetic drug use status.

Groups		n	Axl		sAxl	
			r	p-Value	r	p-Value
0. Month	Gas6	67	0.725	**<0.001**	0.541	**<0.001**
(DER groups)[a]	Axl		–	–	0.597	**<0.001**
0. Month	Gas6	25	0.208	0.319	0.170	0.416
(Met + Ins groups)[b]	Axl		–	–	0.370	0.069
6. Month	Gas6	12	0.664	**0.018**	0.371	0.236
(Met groups)[b]	Axl		–	–	0.587	**0.045**
6. Month	Gas6	13	0.385	0.194	−0.281	0.353
(Met + Ins groups)[c]	Axl		–	–	−0.437	0.135

DER, diet and exercise recommended group; Met, metformin group; Met + ins, metformin + insulin group; Gas6, growth arrest specific protein 6; Axl, Axl; sAxl, Soluble Axl. Spearman's rho correlation analysis was applied. Significant results are written in bold. [a]Patients included in the control, DER and Met groups were not using any antidiabetic agents at the 0th month. [b]The patients in the Met + Ins group were using only metformin during blood analysis at the 0th month and the patients in the Met group at the 6th month. [c]The patients in the Met + Ins group were using insulin therapy for 6 months in addition to metformin. Bold p-values indicate statistically significant differences.

Present studies showed that the effect of metformin on the Gas6/Axl pathway was generally carried out *in vitro*. Therefore, this study is one of the first studies conducted clinically. In this study, a statistically significant decrease was found in Gas6 and Axl levels in patients with T2DM who received metformin treatment. In addition, a statistically significant decrease was observed in Gas6 and Axl levels in the Met + Ins treatment group. sAxl levels were similar in all groups.

Patients with diabetes mellitus are at an increased risk of susceptibility to cancer. It has been suggested that insulin resistance and hyperinsulinemia in T2DM directly or indirectly promote malignant transformation [18, 19]. Several studies have reported an increased incidence of breast, endometrial, and colorectal cancers in the first months after T2DM diagnosis and even in the prediabetes phase [20].

Metformin is a widely used pharmacological agent for the treatment of T2DM. It is the first choice of treatment for complications that are common in long-term T2DM patients and is used in combination with injectable and oral treatments [21, 22]. Studies are showing that the use of metformin in patients with T2DM reduces the risk of cancer [23, 24]. In the study of Evans et al. using metformin has been associated with a reduced prevalence of cancer in patients with T2DM [25].

Axl has emerged as a new biomarker due to its role in biological processes and tumor formation, while Gas6 is a ligand of Axl [26]. Gas6/Axl signaling pathway plays a role in pathological mechanisms for complications such as cardiovascular diseases, kidney dysfunction, amputations, nerve damage, and erectile dysfunction associated with T2DM [27].

Hung et al. showed that plasma Gas6 concentrations were significantly lower among patients with new-onset T2DM and its value was inversely correlated with fasting glucose. Plasma Gas6 is associated with altered glucose tolerance, inflammation, and endothelial dysfunction [28].

In a study, although plasma Gas6 concentrations were higher in women than in men, the difference was not statistically significant. Therefore, both male and female patients were included in this study. They also reported that Gas6 concentrations were negatively correlated with insulin resistance, abdominal obesity, E-selection, and IL-6 [29].

Lee et al. investigated the relationship between Gas6 gene polymorphism (c.834+7G > A) and T2DM pathogenesis. They divided the individuals of the study group into different subgroups according to Gas6 genotypes such as AA, GA, and GG. As a result, they showed that subjects with the AA genotype had higher Gas6 levels and lower levels of glucose, HbA_{1c}, HOMA-IR, and triglycerides compared to subjects with the GG genotype. They reported that the AA genotype of the Gas6c.834+7G>A polymorphism might have a protective role against T2DM [30].

Cavet et al. demonstrated that varying glucose concentration in vascular smooth muscle cells had a significant effect on the Gas6/Axl signal. They reported that Gas6/Axl was stimulated by low glucose levels, caspase 3 expression was decreased, the PI3K/Akt/mTOR pathway was activated, and through this way, Gas6/Axl showed an anti-apoptotic effect and increased cell viability [31].

Axelrod and Pienta have reported that Axl plays a critical role in immune system response and cancer. They have suggested that Axl is generally expressed at higher levels in the disease state compared to normal tissue, and therefore it has an oncogenic role for the receptor [32].

In addition to the Gas6-dependent activation, Axl has been shown to have ligand-independent activation. In breast cancer cells, activation of Axl has been reported to occur via NF-kB of matrix metalloproteinase-9 (MMP9) [33].

In this research, there was a significant decrease in Gas6 and Axl levels after metformin and metformin-insulin combination treatment in Met or Met + Ins groups. When all groups compared each other six months after treatment, only Axl levels were found to be significant. This may be due to the increased expression of Axl independent of its ligand.

The antitumorigenic potential of metformin has been tested in clinical trials in the treatment of several cancer

types [34]. Kim et al. reported that metformin inhibited Axl and Tyro3 expression, resulting in antiproliferative activity in chemotherapy-resistant ovarian cancer cells. They reported that one of the therapeutic effects of metformin was to control the proliferation and chemoresistance of ovarian cancer cells by decreasing Axl and Tyro3 activation [35].

Saito et al. have reported that metformin reduces the proliferation of leukemia cells, inhibits Axl, and downregulates other TAM receptor tyrosine kinase (RTK). They have also shown that metformin enhances the effect of doxorubicin, a chemotherapeutic drug [36].

In this study, in the groups not receiving metformin treatment (control group and DER group), there were no statistically significant differences in serum Gas6, Axl, and sAxl values at the 6th month compared to the baseline. However, a statistically significant decrease was found in serum Gas6 and Axl values in the Met group and Met + Ins group at 6 months compared to the baseline. According to this result, it was observed that the use of metformin decreased Gas6/Axl levels, while at the same time, this effect continued with the use of insulin.

sAxl, which binds Gas6, functions as a "decoy receptor" that eliminates the Axl signal and the mitogenic effects caused by Gas6. In this study, no statistically significant differences were observed in serum sAxl values. This may be because Gas6 binds at levels similar to sAxl which acts as the decoy receptor. Accordingly, the reason for the significant difference only in Axl levels in the evaluation of parameters between groups may have been caused by the binding of Gas6 to sAxl [12].

Six months after the treatment, a significant decrease was observed in FBG and HbA_{1c} values in the Met and Met + Ins groups, but there was no statistical difference in the control and DER groups. There was also a decrease in Gas6 and Axl values in the Met and Met + Ins groups. However, it was found that the 0th and 6th months FBG and HbA_{1c} levels of the study groups did not significantly correlate with the corresponding Gas6, Axl, and sAxl parameters.

When the groups that did not use drugs were examined, a highly significant positive correlation was found between Gas6 and Axl, and a moderate statistically significant positive correlation was found for sAxl. A moderate positive correlation was found between Axl and sAxl, while a significant moderate positive correlation was found between Gas6 and Axl in the Met group using metformin for six months. In the Met + Ins group, which used insulin combined with metformin, a significant moderate positive correlation was found between Axl and sAxl. Within the groups, Gas6 and Axl levels were found to be decreased in Met and Met + Ins groups due to drug use. However, the decrease in Axl levels in the groups may be due to the small sample size and the individual differences (AA, AG, GG polymorphism) of our sample group. In this study, the possible mechanisms of glucose intolerance and changes in the Gas6/Axl pathway with the use of metformin in patients with T2DM were investigated. As a result of this study, blood glucose levels were found to be regulated with the use of metformin, and the Gas6/Axl pathway was shown to be inhibited. In addition, while metformin regulates insulin resistance, hypertension and hyperlipidemia, a new function has been added to its pleiotropic effects: Inhibitory effect on the Gas6/Axl pathway. In conclusion, these results suggest that the use of metformin can be effective in reducing cancer cases or potential cancer cases in people with T2DM, and it can be used for a better prognosis. Considering the increasing rate of diabetic cases and the cancer incidence in these cases, documenting the importance of metformin will be very useful for investigations involving diabetic cases associated with various cancer types.

This study has some limitations: Patients with T2DM who had chronic diseases (hypertension, cardiovascular disease, dyslipidemia, chronic kidney disease) other than T2DM, diabetes duration exceeds 15 years (HbA_{1c}≥5.7%), who smoked, and who used alcohol were not included in the study. In particular, drug-using groups were formed from patients who would just start the drug for treatment. Patients who refused treatment sometime after the onset of treatment were excluded from the study. In addition, T2DM patients treated with other drugs (SGLT/Incretin-based therapies) were also excluded. These exclusions caused the sample size to be small.

Conclusions

Metformin is a widely used pharmacological agent for the treatment of T2DM. Patients with diabetes mellitus are at an increased risk of susceptibility to cancer. Blood glucose levels were found to be regulated with the use of metformin, and the Gas6/Axl pathway was shown to be inhibited. While metformin regulates insulin resistance, hypertension, and hyperlipidemia, a new function has been added to its pleiotropic effects: inhibitory effect on the Gas6/Axl pathway. The use of metformin can be effective in reducing cancer cases or potential cancer cases in people with T2DM, and it can be used for a better prognosis.

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