ORIGINAL RESEARCH

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Metformin Therapy Preserves Superoxide Dismutase and Glutathione Peroxidase, and Minimizes the Enlargement of Ascendant Aorta in the Newly Diagnosed Type 2 Diabetes Patients

Metformin Tedavisi: Yeni Tanı Konmuş Tip 2 Diyabet Hastalarında Süperoksit Dismutaz ve Glutatyon Peroksidazı Korur ve Asendan Aort Genişlemesini En Aza İndirir

Sedat Yıldırım[1], Rukiye Çelik[1], Zehra Sucuoğlu İşleyen[1], Ekrem Bilal Karaayvaz[2], Hakan Sarı[1], Mikail Yetmiş[1], Erdal Gündoğan[1], Fatih Pektaş[1], Özcan Sönmez[1], Saadet Pilten Güzel[3], Numan Görgülü[1], Ahmet Engin Atay[1]

[1]University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital, Department of Internal Medicine, İstanbul, Turkey
[2]University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital, Department of Cardiology, İstanbul, Turkey
[3]University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital, Department of Biochemistry, İstanbul, Turkey

Abstract

Objective: To examine the levels of antioxidants including glutathione peroxidase (GPx), superoxide dismutase (SOD) and total antioxidant capacity (TAC), and to compare the effects of oral antidiabetics and insulin therapy on antioxidant molecules and echocardiographic measurements in patients with newly diagnosed type 2 diabetes mellitus (T2DM).

Method: Eighty-one newly diagnosed (diagnosed in the last 4 weeks) T2DM patients were divided into 3 groups according antidiabetic regimen (group 1: Receiving metformin only and HbA1c <8.5, group 2: Receiving metformin plus vildagliptin and HbA1c between 8.5-10 and group 3: Receiving metformin plus vildagliptin plus premixed insulin and HbA1c >10). The serum levels of GPx, SOD, TAC and echocardiographic measurements including ejection fraction (EF), left ventricular end diastolic thickness (LVEDT) and ascendant aorta diameter (AAD) of the patients were examined at the first visit and at the 6th month of the study.

Öz

Amaç: Yeni tanı konmuş tip 2 diabetes mellitus (T2DM) hastalarında glutatyon peroksidaz (GPx), süperoksit dismutaz (SOD) ve total antioksidan kapasite (TAK) gibi antioksidanların düzeylerini incelemek ve oral antidiyabetikler ile insülin tedavisinin antioksidan moleküller ve ekokardiyografik ölçümler üzerindeki etkilerini karşılaştırmaktır.

Yöntem: Yeni tanı konmuş (son 4 hafta içinde tanı konmuş) T2DM 81 hasta antidiyabetik rejime göre 3 gruba ayrılmıştır (grup 1: Sadece metformin alan ve HbA1c <8,5, grup 2: Metformin artı vildagliptin alan ve HbA1c 8.5-10 arasında ve grup 3: Metformin artı vildagliptin artı premiks insülin alan ve HbA1c >10). Hastaların serum GPx, SOD, TAK düzeyleri ve ejeksiyon fraksiyonu (EF), sol ventrikül diyastol sonu kalınlığı (LVEDT) ve asendan aort çapı (AAD) gibi ekokardiyografik ölçümleri ilk vizitte ve çalışmanın 6. ayında incelenmiştir.

Address for Correspondence: Sedat Yıldırım MD, University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital, Department of Internal Medicine, İstanbul, Turkey
E-mail: sedat.yildirim2@saglik.gov.tr **ORCID:** orcid.org/0000-0002-2423-6902
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Abstract

Results: While there was no statistically significant difference between the baseline and the 6th month averages of SOD, GPx and TAC values in 3 groups, the reduction in the mean SOD, GPx and TAC levels throughout the study period were significant in the groups, except group 1. The echocardiographic measurements of the groups were similar but the mean increase in the AAD was significant in all groups, with the lowest enlargement in aortic diameter was also in metformin only group. The changes in EF and LVEDT throughout the study period were non-significant. The correlation between SOD and AAD in the group 1 was significant.

Conclusion: Metformin therapy preserves the levels of SOD and GPx in the newly diagnosed diabetic patients, and the enlargement in the AAD is minimized by single metformin therapy compared to other combinations. Echocardiography parameters revealed that ascendant aorta diameter is a reliable and early marker of cardiovascular complications of DM.

Keywords: Ascendant aorta diameter, glutathione peroxidase, metformin, superoxide dismutase, total antioxidant capacity

Öz

Bulgular: SOD, GPx ve TAC değerlerinin başlangıç ve 6. ay ortalamaları arasında 3 grupta istatistiksel olarak anlamlı bir fark bulunmazken, çalışma süresi boyunca ortalama SOD, GPx ve TAC seviyelerindeki azalma grup 1 hariç tüm gruplarda anlamlıydı. Grupların ekokardiyografik ölçümleri benzerdi ancak AAD'deki ortalama artış tüm gruplarda anlamlıydı ve aort çapındaki en düşük genişleme de sadece metformin grubundaydı. Çalışma süresi boyunca EF ve LVEDT'deki değişiklikler anlamlı değildi. Grup 1'de SOD ve AAD arasındaki korelasyon anlamlı bulunmuştur.

Sonuç: Metformin tedavisi, yeni tanı konmuş diyabet hastalarında SOD ve GPx seviyelerini korumakta ve AAD'deki genişleme, diğer kombinasyonlara kıyasla tekli metformin tedavisi ile en aza indirilmektedir. Ekokardiyografi parametreleri, asendan aort çapının DM'nin kardiyovasküler komplikasyonlarının güvenilir ve erken bir belirteci olduğunu ortaya koymuştur.

Anahtar kelimeler: Asendan aort çapı, glutatyon peroksidaz, metformin, süperoksit dismutaz, total antioksidan kapasite

Introduction

The frequency of type 2 diabetes mellitus (T2DM) has been rising alarmingly. According to the Turkey Diabetes, Hypertension, Obesity and Endocrinological Disease Prevalence (TURDEP)-1 Study, the rate of T2DM in the Turkish population was 7% in 1998, almost doubling to 13.7% in the TURDEP-2 study in 2010 (1). Diabetes is recognized as comparable to coronary artery disease, presenting an elevated risk of cardiovascular mortality relative to non-diabetics, with cardiovascular complications being the primary causes of death and morbidity (2,3).

Among different pathogenetic mechanisms, oxidative stress plays a vital role in the development of atherosclerosis and the subsequent emergence of micro and macrovascular complications of T2DM (4,5). Oxidative stress is defined as the impairment of the oxidative balance as a result of the increase in reactive oxygen species (ROS) production during cellular metabolism or insufficiency in antioxidant mechanisms detoxifying ROS, which eventually results in the oxidization of carbohydrates, lipids, proteins, deoxyribonucleic acid and damage on cellular membranes (6,7). Antioxidants with enzymatic activity are capable of neutralizing ROS. Superoxide dismutase (SOD) is an enzymatic antioxidant which removes superoxide anions from tissues (8). Glutathione peroxidase (GPx) is another antioxidant that reverses the oxidation of sulfhydryl groups (9). On the other hand, total antioxidant capacity (TAC) is defined as the combined ability of antioxidant enzymes ranging from classical enzymes like SOD, GPX, and catalases to ancillary enzymes like oxidoreductases and conjugate enzymes (10,11).

This study aimed to assess the impact of various antidiabetic regimens on antioxidant molecule levels and echocardiographic indicators in newly diagnosed T2DM patients with differing HbA1c values at the initial visit and six months post-diagnosis. The secondary objective was to examine the impact of antidiabetic therapy combinations on blood concentrations of SOD, GPx, and TAC, as well as echocardiographic parameters at the initial diagnosis and after 6 months.

Materials and Methods

Study Design and Participants

Eighty-one patients diagnosed with T2DM in the last 4 weeks at the Internal Medicine Outpatient Service of University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital, İstanbul, between 1 February 2017 and 15 February 2018, were enrolled in the study. Diagnosis of T2DM was based on the criteria of 2017 American Diabetes Association guidelines and the guidelines of the Turkish Society of Endocrinology and Metabolism. The participants' antidiabetic medications (metformin only, metformin plus vildagliptin, and metformin plus vildagliptin plus premixed insulin) were recorded after examining medical recordings and/or interviews with the patient. We classified the patients according to the antidiabetic regimen and HbA1c value

(group 1: Receiving metformin only and Hba1c <8.5, group 2: Receiving metformin plus vildagliptin and HbA1c between 8.5-10, and group 3: Receiving metformin plus vildagliptin plus premixed insulin and HbA1c >10). The groups were age, gender, and body mass index (BMI) matched. The patients with hypertension, ischemic heart disease, stroke, thyroidal dysfunction, autoimmune disorders, chronic liver disease, chronic kidney disease, smokers, or the patients receiving any other medication were excluded. The study was approved by the Ethics Committee of University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital (approval number: 2016-507, date: 27.12.2016). Entire patients gave written informed consent. Demographic data of participants were collected from patients themselves or family members.

Clinical and Laboratory Assessments

Patients aged >18 years without a comorbidity underwent a detailed physical examination. The demographic characteristics, including age, gender, and BMI were recorded. Blood samples were collected after an 8-hour fasting period. Biochemical analysis including glucose, urea creatinine, uric acid, aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase, calcium, sodium, potassium, total protein, albumin, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, thyroid-stimulating hormone (TSH), and free thyroxine was performed by using photometric method in Siemens Advia 1800 device. Blood samples for analyzing SOD, GPx, and TAC were obtained at the first and second visits and preserved at -80 °C. All blood samples were centrifugated for 5 minutes at 2000 turn before analysis.

Antioxidant Enzyme Measurements

ELISA method with the commercially available kits was used to analyze SOD, GPx, and TAC after dissolving the portions of the serum samples on the same day as instructed by the company [Abbkine kit (Wuhan China) as follows; human SOD KTE62765, Abbkine, INC Bldg 1, No.35, Optical Valley Ave, Wuhan, China]. The SODELISA Kit employs a two-site sandwich ELISA to quantitate SOD in samples (Human SOD Elisa Kit). An antibody specific to SOD has been pre-coated onto a microplate. Standards and samples are pipetted into the wells, and any SOD present is bound by the immobilized antibody. After removing any unbound substances, HRP-Conjugate Human SOD detection antibody is added to the wells. Following a wash to remove any unbound HRP reagent, a Chromogen solution is added to the wells, and color develops in proportion to the amount of SOD bound in the initial step. This inhibition activity of SOD is measured by the colorimetric method at OD 450 nm.

The analysis of GPx (Human GSH-PX Elisa KiT, KTE62766 Abbkine, INC Bldg 1, No.35, Optical Valley Ave, Wuhan, China) was done by mixing 0.2 mL of 0.8 Mm EDTA, 0.2 mL of reduced glutathione and 0.4 mL of phosphate buffer (pH 7.0) at 37.8 °C for 10 minutes and centrifugated at 2000 rpm. After adding disodium hydrogen phosphate, the activity of GPx was read photometrically at 420 nm and expressed as nanomoles of glutathione oxidized per minute per milligram.

For the TAC analysis (Human TAC Elisa KiT, KTE62767 Abbkine, INC Bldg 1, No.35, Optical Valley Ave, Wuhan, China), plasma samples were collected by centrifugation for 1.5 minutes at 2000 rpm. DPPT was dissolved in methanol (200 mL 80%) and centrifugated to obtain the supernatant. The absorbance of TAC was measured after the reaction between the supernatant and DPPH solution at 517 nm.

Additional Clinical Measurements

Urine analysis was done using the spectrophotometric method on the Siemens Advia 1800 device. The height and weight of participants were measured in the Tanita Body Composition Analyzer (Tanita Corporation of America, Illinois, USA). BMI was calculated as weight/height (kg/m^2). Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or receiving any antihypertensive agent. Urine albumin excretion rate exceeding 20 µg/min (30 mg/day), in the absence of uncontrolled hypertension or urinary tract infection, was defined as microalbuminuria.

Echocardiographic Examination

An echocardiographic examination was performed by the same cardiologist at the Cardiology Department of University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital. All cases were examined by iE33 echocardiography system to detect LV end-diastolic dimension, left atrial diameter, left ventricular (LV) posterior wall thickness in diastole, interventricular septum thickness in diastole, LV ejection fraction (LVEF). The echocardiographic examination included measurements of LV end-diastolic thickness and ascending aorta diameter (AAD). LV mass was calculated according to the American Society of Echocardiography formula. Body surface area (BSA) was calculated as [(height(cm) x weight (kg)/3600)]

1/2, and LV mass index was calculated by dividing LV mass into the BSA. LV hypertrophy was defined as LV mass index >115 g/m^2 (for men) and >95 g/m^2 (for women).

Statistical Analysis

Data were analyzed using the NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA). Data distribution was assessed using Shapiro-Wilk test before applying parametric tests. Parametric tests were applied for normally distributed data, while non-parametric tests were used for data that did not meet normality assumptions. All data is expressed as the mean ± standard deviation. Categorical variables were compared using chi-square test. For normally distributed continuous variables, One-Way ANOVA was used for between-group comparisons and paired t-test for within-group comparisons. For non-normally distributed data, Kruskal-Wallis test was used for between-group comparisons and Wilcoxon signed-rank test for within-group comparisons. Correlations were assessed using Pearson correlation for normally distributed data and Spearman's rank correlation for non-normally distributed data. A p-value <0.05 was considered to be statistically significant.

Results

The mean ages of the three groups participating in the study were 51.83±11.71, 53.01±9.83, and 49.63±12.06 years, respectively, and there was no significant difference between the groups. Similarly, BMI values were also similar between groups. There was no statistically significant difference between the groups in terms of gender distribution. Significant weight loss was observed in all groups during the follow-up period (Table 1).

The HbA1c measurements from biochemical tests verified the correct patient distribution based on the established criteria because group 1 started with HbA1c levels at 8.1±0.4% while group 2 began at 9.2±0.5% and group 3 started at 11.8±1.2%. All three groups achieved substantial HbA1c reductions during their follow-up period (p=0.0001 for all groups). The initial glucose measurements in group 1 exceeded those of the other two groups because HbA1c levels directly correlated with these measurements. The baseline measurements of creatinine, urea, LDL cholesterol and triglycerides showed no significant differences between the groups but all groups demonstrated a significant decrease in LDL cholesterol levels during follow-up.

The metformin group demonstrated the most substantial rise in HDL cholesterol levels among all groups (p=0.0001). The liver enzyme values of ALT and AST together with thyroid function tests TSH and free T4 remained constant throughout the study period with no differences between groups (Table 2). The initial measurements of SOD, GPx and TAC antioxidant molecules showed no significant differences between the groups.

However, during follow-up, while no decrease in SOD and GPx levels was observed in the group receiving only metformin, a significant decrease in these parameters was detected in the other two groups. A significant decrease in TAC levels was observed in all three groups during follow-up (Table 3).

In terms of echocardiographic measurements, the ejection fraction and LV end-diastolic thickness values of the groups were similar, and no changes in these parameters were observed during the follow-up period. In the measurements of the aortic outflow tract diameter, there was no difference between the groups at baseline, but a significant increase in the aortic outflow tract diameter occurred in all three groups during the follow-up period. This increase was observed at least in the group receiving metformin alone (Table 4).

Table 1. Demographic characteristics of groups

	Metformin group n=30	Metformin+sOAD group n=32	Metformin+sOAD+insulin group n=19	p
Age	51.83±11.71	53±9.83	49.63±12.06	0.579*
Gender				0.230†
Male	13 (43.33%)	17 (53.13%)	13 (68.42%)	
Female	17 (56.67%)	15 (46.88%)	6 (31.58%)	
BMI				
1st visit	34.13±5.87	32.92±4.77	31.49±5.1	0.238*
2nd visit	31.89±4.88	30.91±4.74	29.75±4.54	0.310*
p	0.0001‡	0.0001‡	0.041‡	

*: One-Way ANOVA, †: Chi-square test, ‡: Paired t-test, sOAD: Second oral antidiabetic (vildagliptin), BMI: Body mass index

Table 2. Biochemical and hormonal parameters of the groups

	Metformin group n=30	Metformin+sOAD group n=32	Metformin+sOAD+insulin group n=19	p
HbA1c (%)				
1st visit	8.1±0.4	9.2±0.5	11.8±1.2	0.0001§
2nd visit	6.8±0.3	7.5±0.4	8.7±0.8	0.0001§
p	0.0001‡	0.0001‡	0.0001‡	
Glucose				
1st visit	131.96±30.73	174.58±73.12	295.74±90.94	0.0001§
2nd visit	118.33±23.66	152.54±53.33	120.63±21.64	0.001§
p	0.031‡	0.884‡	0.0001‡	
Creatinine				
1st visit	0.88±0.59	0.83±0.16	0.80±0.2	0.58*
2nd visit	0.82±0.18	0.79±0.12	0.79±0.23	0.195*
p	0.490‡	0.530‡	0.327‡	
Urea				
1st visit	35.42±37.35	30.27±7.35	30.42±8.5	0.645§
2nd visit	31.12±9.56	31.93±6.53	31.03±9.5	0.907*
p	0.512¶	0.277‡	0.765‡	
LDL				
1st visit	127.29±30.86	134.59±28.48	133.47±28.14	0.591*
2nd visit	117.14±28.61	121.47±35.78	102.41±31.63	0.124*
p	0.042‡	0.015‡	0.005‡	
Triglyceride				
1st visit	203.57±146.71	218.09±126.66	347.46±407.61	0.081§
2nd visit	182.21±166.79	225.01±209.73	171.11±81.82	0.473§
p	0.122¶	0.833¶	0.082¶	
HDL				
1st visit	42.7±13.53	44.27±11.16	38.5±8.34	0.228*
2nd visit	52.01±11.96	48.38±14.34	47.1±14.29	0.400*
p	0.0001‡	0.079‡	0.002‡	
Total cholesterol				
1st visit	207.46±33.85	220.16±33.58	227±47.41	0.176*
2nd visit	201.99±33.47	212.57±38.61	182.39±34.56	0.058*
p	0.301‡	0.295‡	0.001‡	
ALT				
1st visit	27.67±18.93	22.91±11.24	28.74±22.77	0.421§
2nd visit	23.26±11.99	20.43±7.55	29.33±23.43	0.103§
p	0.086¶	0.235¶	0.930¶	
AST				
1st visit	23.29±9.9	21.26±11.26	21.16±18.94	0.789§
2nd visit	21.67±6.44	18.69±4.3	23.02±10.46	0.073§
p	0.346¶	0.210¶	0.706¶	
TSH				
1st visit	2.04±1.37	2.82±2.7	2.46±0.97	0.297§
2nd visit	2.36±1.34	2.72±1.89	2.34±1.19	0.584§
p	0.197¶	0.661¶	0.546¶	
fT4				
1st visit	1.15±0.28	1.06±0.24	1.22±0.18	0.068*
2nd visit	1.25±0.27	1.2±0.2	1.18±0.2	0.491*
p	0.056‡	0.002‡	0.479‡	

*: One-Way ANOVA, ‡: Paired t-test, §: Kruskal-Wallis test, ¶: Wilcoxon test, sOAD: Second oral antidiabetic (vildagliptin), LDL: Low-density lipoprotein, HDL: High-density lipoprotein, ALT: Alanine transaminase, AST: Aspartate transaminase, fT4: Free thyroxine, TSH: Thyroid-stimulating hormone

Table 3. The serum levels of antioxidant molecules of groups

		Metformin group n=0	Metformin+sOAD group n=32	Metformin+sOAD+insulin group n=19	p§
SOD	1st visit	36.3±64.12	87.94±150.7	54.79±105.16	0.221
	2nd visit	32.92±69.12	55.44±105.78	22.07±23.16	0.370
	p	0.304¶	0.005¶	0.008¶	
GPx	1st visit	5.22±12.57	9.68±18.59	5.17±10.66	0.501
	2nd visit	4.27±9.69	4.94±10.22	2.61±2.71	0.661
	p	0.600¶	0.008¶	0.007¶	
TAC	1st visit	1.57±2.34	3.28±5.18	1.99±3.33	0.492
	2nd visit	0.81±1.49	0.95±1.76	0.54±0.59	0.656
	p	0.0001¶	0.0001¶	0.0001¶	

§: Kruskal-Wallis test, ¶: Wilcoxon test, SOD: Superoxide dismutase, sOAD: Second oral antidiabetic, TAC: Total antioxidant capacity

Table 4. The echocardiographic measurements of the groups

		Metformin group n=30	Metformin+sOAD group n=32	Metformin+sOAD+insulin group n=19	p*
EF (%)	1st visit	60.77±2.43	60.78±1.85	61.05±2.09	0.884
	2nd visit	59.77±2.4	60.41±1.32	60.11±0.46	0.338
	p	0.114‡	0.385‡	0.077‡	
LVEDT	1st visit	4.27±0.48	4.32±0.56	4.29±0.46	0.941
	2nd visit	4.35±0.66	4.37±0.5	4.43±0.61	0.893
	p	0.328‡	0.601‡	0.457‡	
AAD	1st visit	3.17±0.43	3.18±0.31	3.31±0.26	0.331
	2nd visit	3.29±0.45	3.42±0.31	3.47±0.31	0.210
	p	0.019‡	0.0001‡	0.004‡	

*: One-Way ANOVA, ‡: Paired t-test, sOAD: Second oral antidiabetic, LVEDT: Left ventricular end-diastolic thickness, AAD: Ascending aorta diameter, EF: Ejection fraction

According to the results of the correlation analysis, a positive correlation was found between SOD level and aortic outflow tract diameter in the group receiving metformin alone. No significant relationship was found between other antioxidant parameters and echocardiographic indices. No significant correlation was found between biochemical variables, hemogram parameters, thyroid function tests, and antioxidant parameters (Table 5).

Discussion

This investigation revealed that patients with varying glycemic management exhibit comparable antioxidant enzyme levels in the early stages of T2DM; however, the metformin-only therapy strategy emerged as the most favorable alternative, maintaining stable SOD and GPx levels throughout the study duration. All groups showed significant weight loss, but patients receiving an insulin-based regimen had the lowest reduction. Another important finding in our study was that, while all groups experienced an increase in the AAD, the metformin-only group had the least significant increase in the AAD.

Oxidative stress has more severe and destructive effects in both blood and tissue levels in diabetic patients compared to healthy people as a result of the increase of free radicals, especially the oxygen radicals, auto-oxidation of glucose, a shift in redox balances, insufficient activities of antioxidant enzymes such as SOD dismutase, GPx , absence of reduced glutathione, and decrease of tissue concentrations of antioxidants that eventually resulted with the formation and the progression of atherosclerosis (17,18). Kimura et al. (19) showed a linear relationship between SOD activity and the severity of micro and macrovascular diabetic complications, and the authors concluded that serum SOD activity reflects diabetic vascular damage. Gómez-Marcos et al. (20) conducted a study on 307 individuals, including 54 diabetic and hypertensive patients, 16 non-hypertensive diabetic patients, 185 non-diabetic hypertensive patients, and 52 non-hypertensive and non-diabetic healthy

Table 5. The correlation of echocardiographic measurements and antioxidant molecules

		Metformin group			Metformin+sOAD group			Metformin+sOAD+insulin group		
		SOD	GPx	TAC	SOD	GPx	TAC	SOD	GPx	TAC
EF	r	0.166	0.073	0.121	-0.054	-0.03	-0.064	-0.108	-0.142	-0.131
	p	0.381*	0.702*	0.525*	0.769*	0.87*	0.73*	0.659*	0.562*	0.594*
LVEDT	r	-0.242	-0.229	-0.136	0.031	0.003	0.015	0.27	0.22	0.314
	p	0.198†	0.224*	0.473*	0.865*	0.985*	0.936*	0.264†	0.366*	0.191†
AAD	r	**0.387**	0.197	-0.212	-0.027	-0.117	-0.027	0.257	0.282	0.228
	p	**0.034***	0.296*	0.261†	0.882*	0.523*	0.883*	0.288†	0.242*	0.349†

*: Pearson Correlation test; †: Spearman's rank correlation test, sOAD: Second oral antidiabetic, LVEDT: Left ventricular end-diastolic thickness, AAD: Ascending aorta diameter, EF: Ejection fraction, GPx: Glutathione peroxidase, SOD: Superoxide dismutase

individuals, continued for 24 months. They determined a reverse correlation between SOD level and macrovascular complications like carotid intima-media thickness (CIMT) and ambulatory arterial stiffness index (20).

The study design included patients with various HbA1c levels to match real-world clinical practice where treatment decisions depend on glycemic control. The observed differences in antioxidant enzyme preservation could be influenced by baseline disease severity instead of treatment effects alone. The patients who received metformin monotherapy showed better initial blood sugar control with HbA1c levels below 8.5% compared to those who needed combination therapy with HbA1c between 8.5-10% or insulin addition with HbA1c above 10%. The formation of advanced glycation end-products and polyol pathway activation through hyperglycemia leads to oxidative stress according to previous research (21). The scientific literature shows that patients with higher HbA1c levels at baseline tend to have reduced antioxidant capacity because their existing oxidative damage limits their response to antioxidant-preserving treatments (22). The better results in our study might be due to lower baseline oxidative stress levels instead of superior therapeutic effects of metformin monotherapy.

Conflicting findings exist about antioxidant levels in diabetic versus non-diabetic individuals. Markedly reduced levels of antioxidants were observed in conjunction with similar levels of antioxidants disparity was attributed to ethnicity and gender (23,24). It was shown that female gender and African origin positively influence these antioxidants. Another conflict in the literature was observed about the relationship between glycemic regulation and antioxidant enzyme levels (25,26). Similar to our results, Wong et al. (27) found similar SOD and GPx levels between patients with varying glucose control. However, Doddigarla et al. (28) determined an inverse association between HbA1c and SOD and decreased SOD levels among diabetic patients with high HbA1c. Authors emphasized that high antioxidant levels in patients with poor glycemic control are a compensatory response against enhanced oxidative stress burden.

Some researchers examined the role of antidiabetic medications on the serum levels of antioxidants. They demonstrated that rats receiving metformin showed no reduction in heart SOD activity compared to other groups that did not receive metformin in an experimental study (29). Altınkaynak (30) divided 50 diabetic patients into two groups according to their antidiabetic therapy: The metformin-only group and the thiazolidinedione-only group. They determined an increase in the SOD and GPx levels in both groups; however, the rise was significant only in the metformin group. Similarly, Hatipoğlu (31) compared the effects of metformin+DPP4 inhibitor (sitagliptin) combination with metformin plus basal insulin (insulin glargine) on the serum levels of TAC and pointed out significantly higher TAC levels in the former group. In sepsis-induced oxidative stress, SOD and GPx levels of rats remained normal by administration of metformin, but, similar to our study, TAC levels decreased significantly (32). Dogan Turacli et al. (33) proved that metformin enhances SOD and GPx activity in the diabetic population. However, the same positive effect was not seen in the TAC. Some authors raised concerns about the shortcomings of the TAC assay and strictly discouraged the use of hydroxyl radicals as the oxidant because of the molecule's high and non-specific reactivity, resulting in discrepancies in the literature (34). On the other hand, Zhang et al. (35) showed that SOD level is decreased in patients receiving premixed insülin and improved by switching to basal insulin.

The study revealed substantial weight loss in all treatment groups with the metformin-only group achieving the greatest reduction in BMI at 2.24 kg/m² compared to the

insulin-containing regimen with a mean BMI decrease of 1.74 kg/m². Weight reduction leads to better oxidative stress markers and cardiovascular parameters regardless of blood sugar control according to Scioli et al. (36). The study of overweight and obese young adults showed that BMI reductions directly correlated with decreased aortic stiffness measurements but heart rate and C-reactive protein reductions explained the improvements in peripheral arterial stiffness (37). The different weight loss amounts between groups might have protected antioxidant enzyme levels in the metformin group. The better cardiovascular outcomes can be attributed to metformin's weight-neutral or weight-reducing properties compared to insulin's weight gain potential. The study design limitation prevents us from directly evaluating how weight changes affect antioxidant parameters through correlation analysis.

Regarding echocardiographic indices, the only significant change was observed in the ascendant aorta diameter throughout the timescale in our study. The diameter of the ascendant aorta reflects atherosclerosis in the short term and is considered a predictor of aortic aneurysms (16). Several studies mentioned that the diabetic population had smaller arterial diameter and lower frequency of aortic dilatation (38). In contrast, Miao et al. (39) concluded that diabetes induces aortic oxidative stress and increases the aortic wall-thickness and proximal aorta diameter, which can be suppressed by zinc supplementation via antioxidant pathways. Static and dynamic changes in the ascending aorta are the earliest manifestation of vascular aging, and aortic diameter has an important impact on pulse wave velocity and is the most prominent predictor of aortic rupture (40). Jensen et al. (41) concluded that subtle changes occur before the LVEF is impaired and LV mass increases.

The study revealed an interesting positive relationship between SOD levels and AAD that only appeared in the metformin group (r=0.387, p=0.034). The relationship did not exist in patients who received combination therapy or insulin. The unique correlation pattern between metformin-treated patients suggests they have a distinct compensatory response. The research by Buczyńska et al. (42) demonstrates that metformin protects blood cells and vascular endothelial cells from oxidative damage through NADPH oxidase inhibition and SOD upregulation which strengthens cellular resistance to oxidative stress. The research by Buczyńska et al. (42) demonstrates that metformin activates AMPK-dependent pathways which both protect and potentially boost endogenous antioxidant responses when cells experience stress. The lack of this correlation in other treatment groups suggests an impaired adaptive response which could stem from insulin's pro-oxidant effects or the more severe baseline oxidative stress in these patients. The findings confirm that metformin protects blood vessels by maintaining oxidative stress response mechanisms instead of basic antioxidant functions.

Considering the effects of metformin on the cardiovascular system, Kaya et al. (43) determined an improvement in the elastic properties of the aorta by adding metformin to oral contraceptives in women with PCOS. Bjornstad et al. (44) showed that metformin improved both static (intima-media thickness) and dynamic (ascending aorta pulse wave velocity) properties of the aorta in the diabetic population. Dallak et al. (45) demonstrated a protective effect of metformin on type 2-induced aortapathy by suppressing OS. In an experimental animal study, Sena et al. (46) showed that metformin restored endothelial vasodilatation of the aorta via inhibition of OS. In a study from Australia, metformin improved vascular smooth muscle function, and the reducing with metformin vascular adverse lesions in type 1 diabetes mellitus study showed that metformin therapy reduces maximal CIMT (47,48).

Gordin et al. (49) discovered that insulin exposure impairs aortic and brachial pulse wave velocity and enhances arterial stiffness, which is probably related to the mitogenic effect of hormones. Insulin receptors are found on angiogenic structures in human atherosclerotic plaques, and both short and long-acting insulin compounds stimulate angiogenesis (50). Improvement of oxidative stress and microcirculatory damage was significantly better with metformin compared to insulin monotherapy (51). Lloyd et al. (52) showed that insulin administration increased glucose and pyruvate oxidation. Intensification of metformin-based antidiabetic therapy with insulin is associated with a higher risk of cardiovascular disease (53,54). On the other hand, some authors mentioned that insulin infusion may decrease atherosclerosis by improving inflammation in the short term in animal studies (55). However, there is still a lack of available data on the long-term effects of different insulin types on endothelial functions in human studies.

Study Limitations

There are some limitations of our study. First, the number of cases in our study was relatively low due to our strict exclusion criteria. Many patients were excluded from the study due to irregular follow-ups or comorbidities such

as hypertension, heart failure, autoimmune disorder, chronic renal or liver disease, thyroid dysfunction, and prolactinoma. The second limitation may be the shorter follow-up time. We believe that longer follow-ups will impact the significance of our results. This study was performed on the Turkish population, and therefore, it may not be correct to generalize the results of our study to other populations since there may be effects of environmental factors such as diet or lifestyle as well as genetic factors. Planning a study that considers the dietary habits and physical activities of individuals in the future may help produce more meaningful results.

Conclusion

The research demonstrated that metformin monotherapy achieved the best results because it maintained stable SOD levels (p=0.304) and GPx levels (p=0.600) without significant decline while other treatment combinations led to substantial decreases in both antioxidant enzymes. The aortic diameter growth was most limited in patients who received metformin therapy among all treatment groups. The research demonstrates that metformin provides better protection to the antioxidant system than insulin-based regimens thus making it a potential treatment for preventing macrovascular complications in early-stage type 2 diabetes. The baseline glycemic control differences between groups should be taken into account because patients on metformin monotherapy started with less severe disease.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital (approval number: 2016-507, date: 27.12.2016).

Informed Consent: Entire patients gave written informed consent. Demographic data of participants were collected from patients themselves or family members.

Footnotes

The abstract of the study was presented at the 20th Congress of Internal Medicine, Antalya, Turkey in 2018.

Authorship Contributions

Surgical and Medical Practices: S.Y., E.G., A.E.A., Concept: S.Y., Z.S.İ., H.S., M.Y., Ö.S., E.G., F.B., A.E.A., N.G., Design: S.Y., E.B.K., S.P.G., N.G., A.E.A., Data Collection or Processing: S.Y., R.Ç., Z.S.İ., E.B.K., H.S., M.Y., Ö.S., E.G., F.B., Analysis or Interpretation: S.Y., R.Ç., S.P.G., N.G., A.E., Literature Search: S.Y., R.Ç., Z.S.İ., H.S., M.Y., Ö.S., E.G., F.B., N.G., A.E.A., Writing: S.Y., Z.S.İ., E.B.K., S.P.G., A.E.A.

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