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Use of Sodium Glucose Cotransporter-2 Inhibitor and Thiol Disulfide Homeostasis in Type -2 Diabetic Patients

Saliha Yildiz[1*], Mehmet Ali Akgündüz[2], Salim Neselioglu[3], Ozcan Erel[3], Murat Alay[1]

[1]*Department of Endocrinology, Van Yuzuncu Yil University, Van, Turkey*
[2]*Department of Internal Diseases, Van Education and Research Hospital, Van, Turkey*
[3]*Department of Biochemistry, Yıldırım Beyazit University, Ankara, Turkey*

ABSTRACT

To investigate the presence of antioxidant activity in sodium glucose cotransporter-2 inhibitors (SGLT-2I), which have a preventive effect on the progression of complications since reactive oxygen species damage has a major effect on diabetes morbidity and mortality.

Thiol, disulfide and ischemia-modified albumin levels were measured in the blood of type 2 diabetes mellitus (DM) patients who were using SGLT-2I for at least one year and those who were not using this drug.

Data of 53 patients using SGLT-2I and 62 patients not using SGLT-2I were analyzed. Gender and age were similar in the two groups (p: 0.293, 0.772, respectively). DM duration, HbA1c level and number of drug types used in the combination were higher in the SGLT-2I group (p: 0.003, <0.001, and <0.001, respectively). Disulfide/native thiol was significantly higher in the SGLT-2I group (4.29±1.65 vs 4.06±1.99, p: 0.038). In the analyses of the groups under 50 years of age, female gender, HbA1c≥7%, no complications and concomitant use of dipeptidyl peptidase-4 inhibitor (DPP-4I), the disulfide/native thiol ratio was significantly higher in SGLT-2I users than in non-users (p: 0.072, 0.021, 0.040, 0.008 and 0.032, respectively). In the analysis of patients with DM duration of less than 10 years, the disulfide/native thiol ratio in the SGLT-2I group was significantly higher than those who did not use the drug (p:0.048). Native thiols were statistically similar in all group analyses (p>0.05). Again, IMA was similar in all group analyses (p>0.05)

SGLT-2I use affects thiol disulfide homeostasis more especially in those younger than 50 years, with diabetes duration <10 years, without complications, HbA1c≥7% and using DPP-4I together. The fact that no decrease was detected in native thiol suggests that this effect is more in the antioxidant direction.

Keywords: Type-2 Diabetes Mellitus, Native Thiol, Thiol Disulfide Homeostasis, SGLT-2I

Introduction

Diabetes Mellitus is a chronic, insidious, metabolic disease that can be devastating with its macro and microvascular complications. Hyperglycemia causes an imbalance between the bioavailability of Nitric Oxide, which inhibits platelet activation, smooth muscle cell migration and is a powerful vasodilator, and the accumulation of reactive oxygen species (ROS), which leads to the deterioration of vascular health (1-3). In the formation of complications, in addition to platelet dysfunction triggered by hyperglycemia, vascular inflammation associated with an increase in reactive oxygen species (ROS) and oxidative stress due to increased production and metabolism of advanced glycation end products (AGEs) and sorbitol plays a major role (1, 4, 5-10).

Oxidizing agents that occur as a result of any intracellular or extracellular oxidative stress and have a destructive effect are neutralized by binding with thiols. As a result of the reaction, free disulfide bonds (-S-S-) are formed. Afterwards, free disulfide bonds can be reduced back to native thiol by bonding with hydrogen atoms and thus Thiol Disulfide Homeostasis (TDH) is provided (11).

Dynamic TDH has critical roles such as antioxidant protection, detoxification, stabilization of protein structures, signal transduction, receptor structure, regulation of apoptosis, regulation of enzymatic activity, regulation of transcription factors and cellular signal mechanisms (12). Today, TDH, whose levels change in various diseases such as diabetes, can be dynamically

***Corresponding Author:** Saliha Yıldız, Van Yüzüncü Yıl Üniversitesi Dursun Odabaş Tıp Merkezi, Endokrinoloji Bilim Dalı, Van
e-mail: salihacekici_34@hotmail.com
ORCID ID: Saliha Yildiz: 0000-0001-5607-7259, **Mehmet Ali Akgündüz:** 0000-0000-2523-1234, **Salim Neselioglu:** 0000-0002-0974-5717, **Ozcan Erel:** 0000-0002-2996-3236, **Murat Alay:** 0000-0002-5318-9981
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evaluated by measuring Thiol and Disulfide with a special method (13, 14).

ROS produced during various metabolic and biochemical reactions have multifaceted effects that also cause deoxyribonucleic acid (DNA) damage, leading to various degenerative and autoimmune diseases (15). Ischemia-modified albumin (IMA) is formed as a result of hypoxia reducing the binding ability of the N-terminal region of albumin for copper, cobalt and nickel. This parameter, which increases in most acute ischemic conditions, can be measured to indicate the hypoxic effect (16).

Luminal Sodium Glucose Transporter-2 Inhibitors (SGLT2I) are the primarily recommended drug group in diabetes treatment in patients with concomitant nephropathy, congestive heart failure, atherosclerotic diseases or in patients at very high risk in this respect (17). The main antidiabetic mechanism of action of SGLT-2I is the filtration of excess glucose from the blood from the proximal tubule in the kidneys. Since reactive oxygen species damage has a significant effect on diabetes morbidity and mortality, our study aimed to investigate the presence of antioxidant activity in the mechanism of action of this group of drugs, which prevents the progression of complications, by measuring TDH and IMA.

Materials and Methods

Study Design and Patients: The study was initiated after the approval numbered 05 received from the Ethics Committee of Van Yuzuncu Yil University Dursun Odabas Faculty of Medicine on 18.01.2023. The study was conducted on a total of 115 patients aged between 18-65 years who were diagnosed with Type 2 DM and followed up at the Department of Internal Medicine, Endocrine and Metabolic Diseases Diabetes Polyclinic of Yuzuncu Yil University Faculty of Medicine.

53 patients with Type 2 DM who had been using any SGLT-2 inhibitor (dapagliflozin or empagliflozin) regularly for at least 1 year were included as the case group, and 62 patients with Type 2 DM who had not used SGLT-2 inhibitors were included as the control group.

1 ml of peripheral blood was taken from individuals in both study groups into biochemistry tubes. The blood samples were stored at -80°C on the day they were taken. When the number of stored blood samples reached 43 in the case group, the biochemistry study process was started.

With the new automatic measurement method developed by Erel and Neşelioğlu;

• Native thiol (-SH)

• Dynamic Disulfide (-S-S-) these two parameters are measured,

• Total Thiol: NativeThiol (-SH) + Dynamic Disulfide (-S-S-)

• Dynamic Homeostasis: Dynamic Disulfide (-S-S-) / Native thiol (-SH) levels were calculated (14).

Measurement methods;

Thiol level measurement: While the primary reagents to be used in the TT and -SH measurements [(reagent 1 (R1) for TT calculation, reagent 1' (R1') for -SH calculation] were different, the other two reagents were similar. R1 was freshly prepared by dissolving 378 mg of sodium borohydrate (NaBH4) in 1000 ml of water-methanol solution (1/1 volume ratio) to achieve a final level of 10 mM. R1´ was prepared by dissolving 585 mg of sodium chloride (NaCl) in 1000 ml of water-methanol solution (1/1 volume ratio) to achieve a final level of 10 mM. Reagent 2 (R2) was used in both TT and -SH calculations. It was prepared by dissolving 0.5 ml of formaldehyde (final level 6.715 mM) and 3.8 g of EDTA (final level 10 mM) in 1000 ml of Tris buffer, 100 mM and pH 8.2. Reagent 3 (R3) was used in both TT and -SH calculations and 3.963 g of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) was prepared by dissolving 3.963 g of 5.5'-dithiobis-2-nitrobenzoic acid (DTNB) in 1000 ml of methanol and used as is so that the final level of DTNB would be 10 mM.

Total and free thiol calculation criteria: For the TT calculation, 10 µl of R1 (10 µl of R1 is used for the -SH calculation) was mixed with 10 µl of sample. After the process, R2 and R3 were added and the first absorbance (A1) reading was made spectrophotometrically at a wavelength of 415 nm. (The second absorbance (A2) reading was made at the same wavelength at the 10th minute when the reaction plateaued. The measurement was completed by obtaining the absorbance difference between A2 and A1. The molar extinction coefficient of 5-thio-2-nitrobenzoic acid (TNB) of 14,100 mol/L-1 cm-1 was used to calculate TT and -SH levels. The -S-S level was measured from the formula (total disulfide-free disulfide)/2. All results were documented in micromoles per liter (µmol/L) (18,19).

Determination of ischemia modified albumin: 50 µl of 1 g/l cobalt chloride solution was added to 200 µl of serum, mixed and incubated for 10

min at room temperature. Then 50 µl of 1.5 g/l DTT solution was added and mixed. Incubated for another 2 min at room temperature. 1 ml of 9.0 g/l NaCl solution was added. Sample blanks were prepared in the same way without adding DTT. The absorbance of the test mixtures was reported at 470 nm. The results were examined in absorbance units. The Corrected-IMA (D-IMA) levels of each patient and healthy control groups according to IMA and albumin were calculated with the formula [D-IMA = IMA x (Albumin / Median Albumin of the Group)] and documented as absorbance unit (ABSU).

Determination of Total Sulphydryl Groups (TSH): The method is based on the principle of reduction with sulfhydryl groups of ELLMAN reagent (DTNB) to form 2-nitro-5-mercaptobenzoic acid. The nitro mercaptobenzoic acid anion is pale yellow and is based on the spectrophotometric measurement of sulfhydryl groups in plasma. Reduced glutathione is used as standard.

To this end;

1-0.5 ml serum was placed in centrifuge tubes.

2-1.5 ml of 0.2 M Tris buffer (pH: 8.2) was added.

3-0.1 ml of 0.01 M DTNB was added.

4-The tubes were then vortexed to ensure thorough mixing.

5-7.9 ml of methanol was added.

6-The solution was allowed to rest for 15 minutes to allow color to develop. The solution was centrifuged at 4500 rpm for 10 minutes at room temperature.

7- It was read against the blank at 412 nm.

8-Disodium EDTA was added to the blank instead of serum, and distilled water was added instead of DTNB.

Statistical Analysis of Data

Statistical Method: In this prospective study, primary characteristic was considered native thiol. According to previous studies (20); the standard deviation (σ) for native thiol was considered as 62 µmol/L. In the study, sample size was determined by considering %80 power ($\alpha = 0.05$ and $\beta = 0.20$) value, 16 effect size (d), and 1.96 Z value. Thus minimum sample size was found 58 by using the "$n = Z^2\ \sigma^2\ /d^2$" equation for sample size calculation.

In the descriptive statistics of the data, mean, standard deviation, median lowest, highest, frequency and ratio values were used. The distribution of variables was tested with the Kolmogorov-Simirnov test. After checking the homogeneity of the variances for analysis of quantitative independent data Independent t test was used for those that were homogeneous and Mann-Whitney U test was used for those that were not. In the analysis of qualitative data, the chi-square test was used, and when the chi-square test conditions were not met, the Fischer test was used. Statistical significance level was considered as 5% and SPSS (ver: 28) statistical program was used for all statistical computations.

Result

The mean age of the participants in the study was 57. There were 56 female and 59 male patients. The gender distribution rates of our patients were similar (p: 0.772). Diabetes duration was determined between 1 and 23 years. HbA1c levels were between 5.4% and 11.5%. The patients were using 1 to 5 different class oral antidiabetic drugs.

When we divided the group into SGLT-2I users (n: 53) and non-users (n: 62) and analyzed them, age was found to be similar in both groups (54.47±7.67 vs 54.66±8.22 respectively; p: 0,772). There were 23 female (43.4%) and 30 male (56.6%) patients in the SGLT-2I user group, and 33 female (53.2%) and 29 male (46.8%) patients in the non-user group, and statistically, gender was similar in both groups (p: 0,293).

In the statistical analysis, diabetes duration, HbA1c and the amount of different class diabetes medication used in the SGLT-2 inhibitor group were significantly higher than the group not using SGLT-2I (9.32±6.22 years vs 6.39±4.73 years, 7.95±1.31% vs 7.02±1.15%, 2.85±0,86 vs 1,60±0,64 (median: 3 vs 2) respectively; p: 0.009, <0.001, <0.001).

Complication rate, sulfonylurea use and DPP4I use in the SGLT-2 inhibitor group were significantly higher than in the group not using SGLT-2 inhibitors (56.6 vs 37.1%, 39.6 vs 16.1%, 62.3 vs 43.5 %; p: 0.036, 0.005, 0.045, respectively). Metformin use rate in the SGLT-2 inhibitor group was significantly lower than in the group not using SGLT-2 inhibitors (73.6 vs 88.7% respectively, p: 0.036). Pioglitazone use was similar in both groups (7.5 vs 9.7%, p: 0,686).

Disulfide/native thiol level and Disulfide/Total Thiol ratios were significantly higher in the SGLT-2 inhibitor group than in the non-user group (4.76±1.97 vs 4.06±1.99, 4.29±1.65 vs 3.70±1.66; p: 0.038, 0.038, respectively). Native Thiol/Total Thiol ratio was significantly lower in the SGLT-2

Table 1: Statistical Comparison of Serum Dynamic Thiol Disulfide Parameters and Ischemia Modified Albumin Levels In The Groups Using and Not Using SGLT-2I In Patients Aged ≥50 Years and Under

	SGLT-2I (-)				SGLT-2I (+)				p	
	Mean±sd			Median	Mean±sd			Median		
Age ≤49	N:17				N:17					
Native Thiol (µmol/L)	512.1	±	49.3	504.7	470.8	±	82.5	477.7	0.121	m
Total Thiol (µmol/L)	545.8	±	52.6	537.0	512.5	±	81.8	527.6	0.296	m
Disulfide (µmol/L)	16.9	±	8.9	16.7	20.8	±	8.3	21.4	0.155	m
Disulfide / Native Thiol	3.3	±	1.8	3.1	4.6	±	2.0	4.8	0.072	m
Disulfide / Total Thiol	3.1	±	1.6	2.9	4.2	±	1.7	4.4	0.072	m
Native Thiol / Total Thiol	93.9	±	3.2	94.2	91.7	±	3.4	91.3	0.072	m
IMA (ABSU)	0.80	±	0.23	0.83	0.73	±	0.25	0.69	0.313	m
Age ≥50	N:45				N:36					
Native Thiol (µmol/L)	436.4	±	55.9	438.6	443.4	±	72.1	442.1	0.611	m
Total Thiol (µmol/L)	473.3	±	57.2	473.8	484.8	±	71.3	481.4	0.385	m
Disulfide (µmol/L)	18.4	±	7.6	15.6	20.7	±	7.4	21.1	0.148	m
Disulfide / Native Thiol	4.3	±	2.0	3.6	4.8	±	2.0	4.5	0.188	m
Disulfide / Total Thiol	3.9	±	1.6	3.3	4.4	±	1.6	4.1	0.188	m
Native Thiol / Total Thiol	92.2	±	3.3	93.3	91.3	±	3.3	91.8	0.188	m
IMA (ABSU)	0.69	±	0.21	0.71	0.76	±	0.27	0.74	0.178	m

[m] Mann-whitney U test

Table 2: Statistical Comparison of Serum Dynamic Thiol Disulfide Parameters and Ischemia Modified Albumin Levels Between Genders of Groups Using and Not Using SGLT-2I

	SGLT-2I (-)				SGLT-2I (+)				p	
	Mean±sd			Median	Mean±sd			Median		
Female	N:33				N:23					
Native Thiol (µmol/L)	466.9	±	58.4	473.4	447.9	±	71.5	450.0	0.532	m
Total Thiol (µmol/L)	500.9	±	59.2	495.7	492.2	±	70.2	494.7	0.954	m
Disulfide (µmol/L)	17.0	±	8.4	15.4	22.1	±	7.0	21.4	0.021	m
Disulfide / Native Thiol	3.7	±	2.0	3.4	5.1	±	1.9	4.8	0.011	m
Disulfide / Total Thiol	3.4	±	1.7	3.2	4.6	±	1.6	4.4	0.011	m
Native Thiol / Total Thiol	93.2	±	3.4	93.6	90.8	±	3.2	91.3	0.011	m
IMA (ABSU)	0.74	±	0.23	0.78	0.81	±	0.26	0.72	0.419	m
Male	N:29				N:30					
Native Thiol (µmol/L)	443.4	±	67.4	436.1	455.4	±	80.1	448.4	0.570	m
Total Thiol (µmol/L)	481.9	±	69.0	473.8	494.8	±	80.0	492.7	0.495	m
Disulfide (µmol/L)	19.3	±	7.2	19.1	19.7	±	7.9	20.5	0.750	m
Disulfide / Native Thiol	4.5	±	2.0	3.9	4.5	±	2.0	4.2	0.739	m
Disulfide / Total Thiol	4.0	±	1.6	3.6	4.1	±	1.7	3.9	0.739	m
Native Thiol / Total Thiol	91.9	±	3.2	92.8	91.9	±	3.4	92.3	0.739	m
IMA (ABSU)	0.70	±	0.22	0.67	0.71	±	0.26	0.74	0.688	m

[m] Mann-whitney U test

inhibitor group than in the non-user group (91.42±3.29 vs 92.60±3.33; p: 0.038). Ischemia modified albumin levels were found to be similar in the SGLT-2I user and non-user groups (0.75±0.26 ABSU vs 0.72±0.22 ABSU respectively; p: 0.511). Native thiol, total thiol and disulphide values were also found to be similar in both groups (452.2±75.86 µmol/L vs 455.9±

Table 3: Statistical Comparison of Serum Dynamic Thiol Disulfide Parameters and Ischemia Modified Albumin Levels of The Groups Using and Not Using SGLT-2I, as HbA1c Below 7 and Above 7

	SGLT-2I (-)				SGLT-2I (+)				p	
	Mean±sd			Median	Mean±sd			Median		
HbA1c < 7 (%)				N: 35				N: 15		
Native Thiol (µmol/L)	449.2	±	68.4	450.6	462.1	±	75.5	446.6	0.531	m
Total Thiol (µmol/L)	486.3	±	68.3	484.7	502.6	±	76.8	495.0	0.437	m
Disulfide (µmol/L)	18.6	±	8.0	19.5	20.2	±	8.7	20.7	0.560	m
Disulfide / Native Thiol	4.3	±	2.0	3.8	4.5	±	2.1	4.4	0.730	m
Disulfide / Total Thiol	3.9	±	1.7	3.5	4.1	±	1.8	4.0	0.730	m
Native Thiol / Total Thiol	92.3	±	3.4	93.0	91.9	±	3.5	91.9	0.730	m
IMA (ABSU)	0.69	±	0.23	0.69	0.75	±	0.22	0.75	0.387	m
HbA1c ≥ 7 (%)				N:27				N:38		
Native Thiol (µmol/L)	465.2	±	55.6	471.4	448.6	±	76.7	449.5	0.373	m
Total Thiol (µmol/L)	499.8	±	58.4	495.6	490.5	±	75.4	492.3	0.678	m
Disulfide (µmol/L)	17.3	±	7.8	15.4	20.9	±	7.2	21.4	0.040	m
Disulfide / Native Thiol	3.8	±	1.9	3.4	4.9	±	1.9	4.7	0.015	m
Disulfide / Total Thiol	3.5	±	1.6	3.2	4.4	±	1.6	4.3	0.015	m
Native Thiol / Total Thiol	93.1	±	3.2	93.7	91.3	±	3.2	91.5	0.015	m
IMA (ABSU)	0.77	±	0.21	0.81	0.75	±	0.28	0.72	0.794	m

[m] Mann-whitney U test

63.38 µmol/L, 493.7±75.2 µmol/L vs 492±64.17 µmol/L, 20.76±7.58 µmol/L vs 18.04±7.88 µmol/L; p: 0.775, 0.694, 0.056 respectively).

When we compared those aged 50 and over with those under 50, it was found that the Disulfide / Native Thiol, Disulfide / Total Thiol ratios were higher and Native Thiol / Total Thiol was lower in the group under 50 using SGLT-2 inhibitors, although not significantly (p: 0.072, 0.072, 0.072, respectively). (Table 1)

In female patients, the Disulfide, Disulfide/Native Thiol, Disulfide/Total Thiol values were significantly higher in the group using SGLT-2 inhibitors than in the group not using them (p: 0.021, 0.011, 0.011, respectively). The Native thiol/total thiol value was significantly lower (p: 0.011). (Table 2)

In the group with HbA1c ≥ 7, Disulfide, Disulfide / Native Thiol, Disulfide / Total Thiol values were significantly higher in the group using SGLT-2 inhibitors than in the group not using them (p: 0.040, 0.015, 0.015, respectively). Native thiol/total thiol ratio was significantly lower (p: 0.015) (Table 3).

When the use of SGLT-2I as a single drug or in combination was compared, no significant difference was observed in the parameters ($p<0.05$).

In the group with DM Duration < 10 years, the Disulfide value was significantly higher in the group using SGLT-2 inhibitors than in the group not using SGLT-2 inhibitors (p: 0.048). (Table 4)

In the group without complications, Disulfide, Disulfide/Native Thiol, Disulfide/Total Thiol values were significantly higher in the group using SGLT-2 inhibitors than in the group not using SGLT-2 inhibitors, while Native Thiol/Total Thiol was lower (p: 0.033, 0.008, 0.008, 0.008, respectively). (Table 5)

When we analyzed the effect of SGLT-2I use on the parameters in metformin users and non-users, no significant difference was found ($p<0.05$).

In the DPP4 (+) group, the Disulfide, Disulfide / Native Thiol, Disulfide / Total Thiol values were significantly higher in the group using SGLT-2 inhibitors than in the group not using SGLT-2 inhibitors (p: 0.011, 0.032, 0.032, respectively). The Native Thiol / Total Thiol ratio was significantly lower in the group using SGLT-2 inhibitors than in the group not using SGLT-2 inhibitors (p: 0.032).

Discussion

The stability of thiol disulfide homeostasis is of great importance for humans. It is maintained within a certain range in healthy individuals.

Table 4: Statistical Comparison of Serum Dynamic Thiol Disulfide Parameters And Ischemia Modified Albumin Levels In Groups Using and Not Using SGLT-2I, With Diabetes Durations of Less Than 10 Years and 10 Years and Above

	SGLT-2I (-)				SGLT-2I (+)				p	
	Mean±sd			Median	Mean±sd			Median		
DM Duration < 10 years				N:47				N:29		
Native Thiol (µmol/L)	455.2	±	67.3	464.7	465.5	±	77.2	458.1	0.562	m
Total Thiol (µmol/L)	491.0	±	69.0	493.4	508.3	±	76.2	511.6	0.235	m
Disulfide (µmol/L)	17.9	±	8.3	15.5	21.4	±	6.7	21.5	0.048	m
Disulfide / Native Thiol	4.0	±	2.1	3.4	4.8	±	1.8	4.8	0.054	m
Disulfide / Total Thiol	3.7	±	1.7	3.2	4.3	±	1.5	4.3	0.054	m
Native Thiol / Total Thiol	92.7	±	3.5	93.6	91.4	±	3.0	91.3	0.054	m
IMA (ABSU)	0.71	±	0.22	0.72	0.73	±	0.26	0.71	0.817	m
DM Duration ≥ 10 Years				N:15				N:24		
Native Thiol (µmol/L)	458.5	±	49.5	441.7	431.9	±	70.7	423.7	0.312	m
Total Thiol (µmol/L)	495.5	±	45.6	476.3	471.4	±	69.5	477.8	0.522	m
Disulfide (µmol/L)	18.5	±	6.4	19.6	19.7	±	8.9	20.4	0.590	m
Disulfide / Native Thiol	4.1	±	1.7	3.8	4.7	±	2.3	4.3	0.439	m
Disulfide / Total Thiol	3.8	±	1.4	3.5	4.3	±	1.9	4.0	0.439	m
Native Thiol / Total Thiol	92.4	±	2.9	92.9	91.5	±	3.8	92.1	0.439	m
IMA (ABSU)	0.75	±	0.24	0.76	0.78	±	0.26	0.75	0.637	m

[m] Mann-whitney U test

Table 5: Statistical Comparison of Serum Dynamic Thiol Disulfide Parameters and Ischemia Modified Albumin Levels In Groups With and Without Diabetic Complications Using and Not Using SGLT-2I

	SGLT-2I (-)				SGLT-2I (+)				p	
	Mean±sd			Median	Mean±sd			Median		
Complication (-)				N:39				N:23		
Native Thiol (µmol/L)	463.8	±	61.4	473.4	436.3	±	79.8	450.0	0.221	m
Total Thiol (µmol/L)	499.7	±	63.0	505.9	481.4	±	82.4	495.3	0.555	m
Disulfide (µmol/L)	17.9	±	8.7	15.1	22.6	±	7.2	23.1	***0.033***	m
Disulfide / Native Thiol	4.0	±	2.2	3.4	5.3	±	1.8	5.7	***0.008***	m
Disulfide / Total Thiol	3.6	±	1.8	3.2	4.8	±	1.5	5.1	***0.008***	m
Native Thiol / Total Thiol	92.8	±	3.6	93.6	90.5	±	3.0	89.7	***0.008***	m
IMA (ABSU)	0.73	±	0.21	0.78	0.77	±	0.29	0.75	0.630	m
Complication (+)				N:23				N:30		
Native Thiol (µmol/L)	442.5	±	65.8	436.1	464.4	±	71.6	445.3	0.243	m
Total Thiol (µmol/L)	479.0	±	65.4	478.7	503.1	±	69.2	492.7	0.243	m
Disulfide (µmol/L)	18.3	±	6.5	20.2	19.4	±	7.7	19.3	0.693	m
Disulfide / Native Thiol	4.2	±	1.7	4.5	4.3	±	2.0	3.9	0.971	m
Disulfide / Total Thiol	3.9	±	1.4	4.1	3.9	±	1.7	3.6	0.971	m
Native Thiol / Total Thiol	92.3	±	2.8	91.7	92.1	±	3.4	92.8	0.971	m
IMA (ABSU)	0.71	±	0.25	0.67	0.73	±	0.24	0.73	0.495	m

[m] Mann-whitney U test

Dynamic TDH levels outside the range have been associated with various disorders of unknown etiology. There is an increasing number of studies demonstrating the presence of TDH disorders in various diseases such as diabetes mellitus, hypertension, solid organ cancers, rheumatological

diseases, hematological diseases, occupational diseases and obstetric diseases (21).

In this study, we investigated whether the use of SGLT-2I in patients with type 2 Diabetes Mellitus caused a change in the oxidant-antioxidant balance by measuring TDH and the presence of hypoxic damage by measuring ischemia-modified albumin level. We did not find any significant change in thiol, disulfide and IMA values in type 2 Diabetes mellitus patients using SGLT-2 inhibitors. However, we found that disulfide-native thiol ratios, which are indicators of dynamic homeostasis, increased significantly. We did not find a study in the literature that included the evaluation of TDH after a therapeutic intervention for the disease. Our study is the first in this respect.

In a study comparing patients with type 1 DM with healthy controls, disulfide/native thiol and disulfide/total thiol ratios were significantly higher in patients, while native thiol level and native thiol/total thiol ratio were significantly lower (22). In a study comparing pregnant women with gestational diabetes with healthy pregnant women, disulfide levels, disulfide/native thiol and disulfide/total thiol ratios were found to be significantly higher in the patient group, and native thiol and total thiol levels were found to be significantly lower (23). In another study conducted on patients with diabetic macular edema and no ocular involvement, native thiol was found to be lower in patients with macular edema. In the case group, total antioxidant level (TAL) was found to be decreased, while total oxidant level (TOL) and IMA were found to be increased compared to the control group (20). In another study comparing diabetic ketoacidosis patients with healthy individuals, total thiol, native thiol and disulfide levels were found to be decreased in favor of oxidative stress (24). In the TDH and IMA study in diabetic microvascular complications, a decrease in native thiol levels in retinopathy and an increase in the disulfide/thiol (native, total) ratio compared to healthy and uncomplicated diabetes were found. An increase in IMA levels was also detected in diabetic retinopathy and neuropathy (25). In another study evaluating diabetic nephropathy, it was observed that as the degree of nephropathy increased in patients with Type 2 diabetes, native and total thiol levels decreased significantly and the balance was disrupted in favor of disulfide and therefore in the direction of oxidative stress (26). In all these studies, native thiol levels have been shown to be low, indicating oxidative stress. The oxidant effects of the disease have been demonstrated in many ways by showing the low native thiol levels. In our study, although diabetic complications were higher, diabetes duration was longer and HbA1c levels were higher in SGLT-2I users compared to those who did not use this drug, we did not detect a significant native thiol decrease. These parameters show that patients using SGLT-2I have a higher disease burden. According to the results of the above study, impaired blood sugar regulation, long duration of diabetes and development of complications increase the oxidative stress and therefore the need for antioxidant activity in patients. The significant increase in the disulfide/native thiol ratio, which is an indicator of dynamic homeostasis in the SGLT-2I group, and the stability and non-decrease of thiol levels can be interpreted as the use of SGLT-2I in such patients supports antioxidant activity and increases thiol regeneration.

In the subgroup analysis of our study, among those who did not develop complications, disulfide, disulfide/native thiol, disulfide/total thiol values were found to be significantly higher in those who used SGLT-2 inhibitors than in those who did not take this drug group. The result of this analysis can be interpreted as the use of SGLT-2 inhibitors at an earlier stage due to the development of complications, causing a positive change in the antioxidant mechanism, since we did not detect a decrease in thiol.

In the female gender group, there was no significant difference in thiol values in patients using SGLT-2 inhibitors, however, disulfide, disulfide/native thiol, disulfide/total thiol values were significantly higher than in the group not using them, and native thiol/total thiol was significantly lower. We can say that there is an effect on TDH due to SGLT-2I use in female gender. However, it is not possible to say that this is oxidative or antioxidant, although the lack of a significant decrease in native thiol may indicate antioxidant side.

In the HbA1c sub-analysis, in the group with HbA1c level ≥ 7%, disulfide, disulfide/native thiol, disulfide/total thiol values were significantly higher in patients using SGLT-2 inhibitors than in patients not using them, while native thiol/total thiol was significantly lower. We found that in patients with HbA1c level 7% and higher in the early stages without developing complications, SGLT-2 inhibitor use probably caused more reparative effects on TDH compared to patients with HbA1c below 7%.

In our study, in patients with diabetes duration of less than 10 years, the disulfide value was found to be significantly higher in the group using SGLT-2 inhibitors than in the group not using SGLT-2 inhibitors. The significant increase in disulfide and the lack of a decrease in native thiol in this study may suggest that the use of SGLT-2 inhibitors in diabetic patients with disease duration of less than 10 years may cause a change in the oxidant-antioxidant mechanism in a way that the result will be in favor of antioxidants.

Disulfide, disulfide/native thiol, and disulfide/total thiol values of patients using SGLT-2I inhibitors were significantly higher in the DPP-4I group than in the group not using SGLT-2I inhibitors. This result may indicate that using SGLT-2I inhibitors and DPP-4I inhibitors together may have an activating effect on the oxidant-antioxidant mechanism.

There are many studies in the literature examining the relationship between oxidative stress and diseases. However, we have not come across a study investigating the effect of SGLT-2I use on this mechanism via TDH. Our study is the first in this regard. However, the fact that the study was cross-sectional, the number of cases was limited, the use of the relevant drug was limited to certain cases due to economic reasons, and the total oxidant level and total antioxidant levels were not measured are the weaknesses of our study. Long-term prospective studies with a larger number of native cases are needed on this subject.

As a result, we found that SGLT2I use caused a change in TDH in cases where the female gender was less than 10 years old, HbA1c was above 7% and complications had not yet developed. We also found an additive effect with DPP4Is. The absence of a significant difference between the groups in thiol levels suggests that this change was in the antioxidant direction. However, since total oxidant and antioxidant activity could not be measured in the study, it is not possible to say anything clear on this subject.

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