Association of Thiol–disulfide Homeostasis with Retinal Layer Thickness in Patients with Diabetes Mellitus

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Abstract

Background: This study aimed to investigate the relationship between early changes in retinal layer thickness and thiol–disulfide homeostasis in patients with type II diabetes mellitus (T2DM).

Materials-Methods: There were 69 patients with T2DM (61 patients without retinopathy, 8 patients with retinopathy) and 21 healthy controls. In patients without retinopathy, 31 of the patients had a disease duration under 10 years, 30 of the patients had a disease duration over 10 years. Retinal layer thickness of the right eye was measured using Spectral Domain Optical Coherence Tomography.

Results: Patients with T2DM and healthy controls had mean ages of 48.40 ± 8.25 years and 45.94 ± 7.32 years, respectively. The ganglion cell layer and retinal pigment epithelium thicknesses were significantly lesser in patients without diabetic retinopathy than those in the control group. In patients without diabetic retinopathy and with a disease duration of under 10 years, there was a negative correlation between the retinal nerve fiber layer thickness (μm) and disulphide/total thiol ratio, between the inner nuclear layer thickness (μm) and disulphide/native thiol ratio as well as disulphide/total thiol ratio ($r = -0.376$, $p = 0.037$; $r = -0.356$, $p = 0.050$; $r = -0.380$, $p = 0.035$, respectively) and positive correlation between the INL thickness (μm) and native thiol/total thiol ratio ($r = 0.359$, $p = 0.047$).

Conclusion: Early changes in retinal layers in patients with DM were associated with thiol–disulfide homeostasis. Administration of therapeutic supplements may aid in the management of low thiol concentrations; this increases the importance of the study findings.

Introduction:

Diabetic retinopathy (DR) is the most common microvascular complication of diabetes mellitus (DM) and the most common cause of blindness [1]. DR is divided into two main categories as non-proliferative DR (NPDR) and proliferative DR (PDR) [2]. Spectral Domain Optical Coherence Tomography. (SD-OCT) is a non-invasive imaging method that provides high-resolution cross-sectional imaging of biological tissues. SD-OCT is widely used in the follow-up of patients with DR because it allows the retinal tissue to be examined in high resolution, is non-invasive, and can be performed in a short duration [3]. DR is the most important cause of blindness between the ages of 20 and 74 years all over the world. Early diagnosis and appropriate treatment of DR findings is the most important strategy in preventing DR-associated blindness [4]. Reactive oxygen species (ROS), hypoxia, and inflammation are believed to play an active role in the pathophysiology of DR [5]. Abnormalities in biochemical and molecular signaling mechanisms trigger ROS formation and cause cytokine and chemokine dysregulation; this results in an increase in oxidative stress, which affects cellular macromolecules, such as protein and nucleic acids [5]. ROS is continuously produced to support normal cellular functions; increase in ROS production or dysfunction in antioxidant defense systems contribute to oxidative stress [6,7]. Activities of enzymes, such as superoxide dismutase, glutathione reductase, thioredoxin and catalase, decrease in the retina in patients with DM [8]. The majority of antioxidants are thiols; they provide defense against ROS (9). Dynamic

thiol–disulfide homeostasis plays a key role in many events, such as signal transduction, apoptosis, and regulation of enzymatic activity [9,10]. Abnormal thiol–disulfide homeostasis is associated with systemic diseases, such as DM and cancer [11,12], as well as eye diseases, such as central serous chorioretinopathy and age-related cataract [13,14]. Studies examining the relationship between thiol–disulfide homeostasis and DR have previously been conducted [15,16]; these studies reported that dynamic thiol–disulfide homeostasis shifts toward the disulfide side in patients with DR [16] and that the serum total thiol levels of patients with NPDR were lower than those in the control group [15]. However, the relationship between early changes in retinal layers and thiol–disulfide homeostasis in patients with DM has not been studied. In 2014, Erel and Neselioğlu developed a new automated spectrophotometric method in which thiol and disulfide could be measured separately [9]. In the present study, we aimed to investigate the relationship between changes in retinal layer thickness measured using SD-OCT and thiol–disulfide homeostasis measured using the new spectrophotometric method in patients with type 2 DM (T2DM).

Materials And Methods:

The study protocol was approved by Ankara Yıldırım Beyazıt University and complied with the principles of the Declaration of Helsinki. An informed consent form was signed by all the participants. Patients over 18 years of age with T2DM were included in the study. Those with systemic conditions, such as chronic kidney failure, chronic liver failure, pregnancy, hypertension, hypothyroidism, hyperthyroidism, cardiovascular disease, and rheumatological diseases, and a history of malignancy were excluded from the study to eliminate any possible effect on thiol–disulfide homeostasis [17]. In addition, patients who received laser therapy or intravitreal injection; patients who had diabetic macular edema (DME), PDR, myopia, hyperopia or astigmatism ≥ 2 diopters, history of intraocular surgery, or chronic ocular diseases (glaucoma, uveitis, dry eye syndrome, or conjunctivitis); and contact lens wearers were not included in the study. A total of 69 patients with T2DM meeting the inclusion and exclusion criteria and 21 healthy controls were included in this study. Retinal layer thickness in the right eye was measured in the patient and control groups. The patients were grouped according to disease duration (<10 years and ≥ 10 years) to analyze early-stage changes [18,19].

Ocular evaluation

All participants were examined by two retina specialists. Evaluation included biomicroscopic examination to assess the macula and fundus, non-mydratic photographs, visual acuity and OCT. Severity of DRP was classified according to the scale of DRP severity [20,21].

SD-OCT

SD-OCT was performed with a dilated pupil. Whole process was conducted by the same experienced operator and no manual correction was performed on OCT scans. The SD-OCT system automatically

provided Early Treatment Diabetic Retinopathy Study (ETDRS) thickness maps for the following seven layers: retinal pigment epithelium (RPE), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (GCL), retinal nerve fiber layer (RNFL) with the caliper measurement tool embedded in the system. [22]. In this study only the scans with sufficient quality ($Q > 25$) were included in image analysis

Laboratory evaluation

Glucose, albumin, C-reactive protein (CRP), spot urinary albumin/kreatin ratio were measured using commercially available assay kits (Roche Diagnostics, Mannheim, Germany) and an auto analyzer (Cobas 501, Roche Diagnostics, Mannheim, Germany) as well thiol-disulfide tests. HbA1c was quantified by standardized immunoturbidimetry on a Cobas 502 analyzer (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions.

Thiol/disulfide homeostasis

Venous blood samples from the patients and healthy controls were collected into EDTA tubes after 12 hours of fasting. The blood samples were immediately centrifuged for 10 minutes at 1500 rpm, and plasma and serum were separated. Separated serum samples were stored at -80°C and all samples were run at once. During the thiol/disulfide homeostasis tests, first, the reducible **disulfide** bonds were reduced to form free functional thiol groups. **Formaldehyde** was used to remove unused and consumed **sodium borohydride**. Total thiol ($-\text{SH} + -\text{S}-\text{S}-$) and native thiol ($-\text{SH}$) concentrations in the samples were measured using Ellman's and modified Ellman's reagent. The natural thiol content was subtracted from the total thiol content and half of this difference gave the amount of dynamic disulfide bonds ($-\text{S}-\text{S}$). Using these parameters, disulphide/native thiol, disulphide/total thiol, native thiol/ total thiol ratio were calculated.

Statistical evaluation

All statistical analyses were performed with the SPSS 15.0 software package (SPSS, Inc., Chicago, Illinois). Descriptive statistics for the constant variables were expressed as mean $\pm$ SD or median (range), and categorical variables were noted as numerics and percent (%). Student T test was used for comparison between independent groups conforming to normal distribution, and Mann Whitney U test for those, who did not comply with normal distribution. Chi-square was used to compare categorical variables. Analysis of variance (ANOVA) was used to compare normally distributed interval data between patients with DM with and without DR and healthy individuals, and the Kruskal Wallis test was used to compare non-normally distributed interval data. Pearson correlation analysis was used to analyze correlations between normally distributed variables, and Spearman correlation analysis was used to analyze correlations between non-normally distributed variables. To compare interval data between the

groups with a DM duration of <10 years and those with a DM duration of ≥ 10 years, Student's t-test was used for normally distributed data and Mann–Whitney U test was used for non-normally distributed data. The significance level was determined as $p < 0.05$.

Results:

A total of 69 patients with T2DM (43 females and 26 males) and 21 healthy subjects (13 females and 8 males) participated in the study. The patients with T2DM and healthy controls had mean ages of 48.40 ± 8.25 years and 45.94 ± 7.32 years, respectively. Age and sex distribution were similar in both the groups ($p > 0.05$ for each parameter). A total of 11.6% ($n = 8$) of the patients with T2DM had DR (NPDR) and 88.2% ($n = 61$) did not have DR. Of the patients without DR, 50.8% ($n = 31$) had a disease duration of < 10 years and 49.2% ($n = 30$) had a disease duration of ≥ 10 years. Among the patients with T2DM, fasting blood glucose level was 181.36 ± 77.68 mg/dl; mean Hba1c rate (%) was 8.33 ± 1.95 ; mean native thiol, disulfide, and total thiol levels were 414.03 ± 59.64 $\mu\text{mol/L}$, 17.17 ± 8.98 $\mu\text{mol/L}$, and 448.25 ± 66.15 $\mu\text{mol/L}$, respectively; and disulfide/native thiol rate (%) was 0.042 ± 0.022 . Mean disulfide/total thiol ratio was calculated as 0.038 ± 0.020 , and mean native thiol/total thiol ratio was calculated as 0.924 ± 0.039 . Patients with and without DR demonstrated RNFL, GCL, IPL, INL, OPL, ONL, and RPE thicknesses of 12.79 ± 2.23 μm , 15.27 ± 4.53 μm , 21.21 ± 5.34 μm , 20.56 ± 5.52 μm , 25.27 ± 6.72 μm , 84.76 ± 12.84 μm , and 16.73 ± 1.97 μm , respectively. The age (years); gender distribution; disease duration (years); levels of fasting blood glucose (mg/dl), albumin (g/L), and CRP (mg/L); Hba1c (%) and urinary albumin/creatinine ratio (%); and retinal layer thickness of the patients with and without DR as well as those of the controls are given in Table 1. GCL and RPE thicknesses in the patients without DR were significantly lesser in patients without DR compared with those in the controls ($p = 0.026$, $p = 0.022$, respectively). There was no difference in the GCL and RPE thicknesses between the groups with and without DR ($p = 0.720$, $p = 0.999$, respectively). Additionally, there was no significant difference between other retinal layers (Table 1). No correlation was found between RNFL, GCL, IPL, INL, OPL, and ONL thicknesses (μm) and disease duration in patients without DR ($r = -0.006$, $p = 0.965$; $r = -0.032$, $p = 0.805$; $r = 0.061$, $p = 0.639$; $r = 0.100$, $p = 0.443$; $r = 0.049$, $p = 0.709$; and $r = -0.033$, $p = 0.801$, respectively). There was a weak negative correlation between disease duration and the RPE thickness (μm) ($r = -0.261$, $p = 0.042$). The correlation analysis in which we compared the relationship between thiol–disulfide homeostasis and the retinal layer thickness in patients without DR is shown in Table 2. Accordingly, weak negative correlation was detected between the IPL thickness (μm) and disulphide levels ($\mu\text{mol/L}$) ($r = 0.278$, $p = 0.030$), disulphide/native thiol ratio ($r = -0.303$, $p = 0.018$), and disulphide/total thiol ratio ($r = 0.297$, $p = 0.020$). A weak negative correlation was found between the INL thickness (μm) and disulphide/total thiol ratio ($r = -0.266$, $p = 0.038$). A weak positive correlation was detected between the ONL thickness (μm) and total thiol levels ($\mu\text{mol/L}$) ($r = -0.263$, $p = 0.040$). A very weak negative correlation was detected between the RNFL thickness (μm) and disulphide/native thiol ratio ($r = -0.248$, $p = 0.054$) as well as disulphide/total thiol ratio ($r = -0.230$, $p = 0.075$) and between the INL thickness (μm) and disulphide/native thiol ratio ($r = -0.246$, $p = 0.056$) as well as disulphide levels ($\mu\text{mol/L}$) ($r = 0.239$, $p = 0.063$), however was slightly significant. Thiol–disulfide homeostasis and the retinal layer thickness measurements were compared according to disease duration

(< 10 years and ≥ 10 years) in patients without DR ($n = 61$). Disulfide/native thiol and disulfide/total thiol ratio were found to be higher in patients with disease duration ≥ 10 years (0.034 ± 0.017 , 0.045 ± 0.024 , $p = 0.049$ and 0.031 ± 0.015 , 0.040 ± 0.020 , $p = 0.050$, respectively). The RPE thickness was found to be $17.29 \pm 2.44 \mu\text{m}$ in patients with a disease duration of < 10 years and $16.23 \pm 1.75 \mu\text{m}$ in patients with a disease duration of ≥ 10 years. There was no significant difference between other retinal layers and thiol–disulfide parameters (Table 3). On evaluation of the correlation between the retinal layer thickness and thiol–disulfide homeostasis in patients without DR having a disease duration of < 10 years ($n = 31$), a very weak negative correlation was detected between the RNFL thickness (μm) and disulphide/native thiol ratio ($r = -0.350$, $p = 0.054$), and a weak negative correlation was detected between the RNFL thickness (μm) and disulphide/total thiol ratio in these patients ($r = -0.376$, $p = 0.037$). A weak negative correlation was detected between the INL thickness (μm) and disulphide/native thiol ratio ($r = -0.356$, $p = 0.050$) as well as disulphide/total thiol ratio ($r = -0.380$, $p = 0.035$), and a weak positive correlation was detected between the INL thickness (μm) and native thiol/total thiol ratio ($r = 0.359$, $p = 0.047$). No correlation was found between the other retinal layers and thiol–disulfide parameters in patients with a disease duration of < 10 years (Table 4). A weak positive correlation was found between the ONL thickness (μm) and native as well as total thiol levels in patients with a disease duration of ≥ 10 years ($r = 0.391$, $p = 0.033$ and $r = 0.422$, $p = 0.020$, respectively), and a weak negative correlation was found between the native thiol levels and OPL thickness (μm) in patients with a disease duration of > 10 years ($r = -0.366$, $p = 0.047$).

Table 1
Comparison of patient and control groups according to demographic characteristics, biochemical measurements, and retinal layer thickness

	DR-present (n = 8)	DR-absent (n = 61)	Control (n = 21)	p
Age	50.12 ± 6.74	48.18 ± 8.45	45.94 ± 7.32	0.426
Gender	4 (50%)	39 (63.9%)	13(61.9%)	0.744
Female	4 (50%)	22 (36.1%)	8(38.1%)	
Male				
Duration of disease (month)	115.50 ± 78.01	100.78 ± 66.09	-	0.564*
Fasting glucose (mg/dl)	227.75 ± 98.25	175.27 ± 73.40	83.11 ± 8.11	<0.001
Albumin (g/L)	4.36 ± 0.60	4.61 ± 0.30	4.66 ± 0.24	0.087***
CRP (mg/L)	7.96 ± 6.79	4.82 ± 3.19	3.11 ± 1.32	0.601
Urinary albumin/kreatinin ratio (%)	115.33 ± 241.03	12.60 ± 6.60	-	0.101**
Hba1C (%)	9.42 ± 2.44	8.18 ± 1.85	-	0.092*
RNFL (µm)	12.62 ± 1.68	12.81 ± 2.31	13.55 ± 2.09	0.428
GCL (µm)	16.50 ± 5.42	15.11 ± 4.43	18.50 ± 5.54	0.033
IPL (µm)	21.25 ± 4.43	21.21 ± 5.48	23.00 ± 4.24	0.432
INL (µm)	20.50 ± 2.82	20.57 ± 5.80	20.44 ± 5.51	0.996
OPL (µm)	26.75 ± 4.59	25.08 ± 6.96	23.88 ± 4.94	0.568
ONL (µm)	82.62 ± 4.95	85.04 ± 13.54	87.50 ± 10.37	0.622
RPE (µm)	16.50 ± 1.19	16.77 ± 2.06	18.33 ± 2.00	0.012
CRP; c-reactive protein RNFL retinal nerve fiber layer; GCL ganglion cell layer; IPL inner plexiform layer; INL inner nuclear layer; OPL outer plexiform layer; ONL outer nuclear layer; RPE retinal pigment epithelium				

Table 2
Correlation between retinal layer thickness and thiol–disulfide homeostasis in patients without DR (n = 61)

	RNFL	GCL	IPL	INL	OPL	ONL	RPE
Native Thiol (μmol/L)	0.103	0.043	0.068	0.033	-0.108	0.247	-0.016
r	0.428	0.744	0.600	0.799	0.406	0.055	0.903
p							
Disulphide (μmol/L)	-0.207	-0.186	-0.278	-0.239	-0.151	0.177	-0.026
r	0.110	0.151	0.030	0.063	0.246	0.173	0.842
p							
Total thiol (μmol/L)	0.036	0.001	-0.003	-0.035	-0.112	0.263	-0.023
r	0.785	0.996	0.979	0.791	0.390	0.040	0.861
p							
Disulphide/Native thiol	-0.248	-0.218	-0.303	-0.246	-0.100	0.090	0.007
r	0.054	0.092	0.018	0.056	0.445	0.490	0.959
p							
Disulphide/Total thiol	-0.230	-0.197	-0.297	-0.266	-0.148	0.117	-
r	0.075	0.127	0.020	0.038	0.255	0.369	0.009
p							0.942
Native thiol/ Total thiol	0.188	0.164	0.247	0.230	0.150	-0.118	0.026
r	0.147	0.205	0.055	0.074	0.249	0.363	0.843
p							
RNFL retinal nerve fiber layer; GCL ganglion cell layer; IPL inner plexiform layer; INL inner nuclear layer; OPL outer plexiform layer; ONL outer nuclear layer; RPE retinal pigment epithelium							

Table 3

Evaluation of retinal layer thickness and thiol–disulfide balance in patients without DR (n = 61) according to disease duration

	Disease duration < 10 years (n = 31)	Disease duration ≥ (10) years ≥ (n = 30)	<i>p</i>
Native Thiol (μmol/L)	425.38 ± 62.63	417.72 ± 54.63	0.613
Total thiol (μmol/L)	455.3473.28	454.45 ± 60.33	0.959
Disulphide (μmol/L)	14.95 ± 8.22	18.55 ± 9.58	0.120
Disulphide/Native thiol	0.034 ± 0.017	0.045 ± 0.024	0.049
Disulphide/Total thiol	0.031 ± 0.015	0.040 ± 0.020	0.050
Native thiol/Total thiol	0.934 ± 0.030	0.919 ± 0.042	0.127
RNFL	12.80 ± 2.44	12.83 ± 2.21	0.964
GCL	15.38 ± 4.52	14.83 ± 4.38	0.630
IPL	21.19 ± 4.70	21.23 ± 6.27	0.428
INL	20.19 ± 5.76	20.96 ± 5.91	0.865
OPL	24.48 ± 5.40	25.70 ± 8.32	0.994
ONL	86.77 ± 10.27	83.26 ± 16.24	0.316
RPE	17.29 ± 2.44	16.23 ± 1.75	0.044
RNFL retinal nerve fiber layer; GCL ganglion cell layer; IPL inner plexiform layer; INL inner nuclear layer; OPL outer plexiform layer; ONL outer nuclear layer; RPE retinal pigment epithelium			

Table 4

The relationship between retinal layer thickness and thiol–disulfide homeostasis in the patients without DR having a disease duration of < 10 years (n = 31)

	RNFL	GCL	IPL	INL	OPL	ONL	RPE
Native Thiol (μmol/L)	0.257	0.301	0.276	0.176	0.006	0.066	-0.038
r	0.163	0.099	0.133	0.343	0.972	0.726	0.839
p							
Disulphide (μmol/L)	-0.240	-0.115	-0.190	-0.298	-0.176	0.190	0.202
r	0.193	0.537	0.306	0.104	0.343	0.306	0.276
p							
Total thiol (μmol/L)	0.165	0.232	0.193	0.084	-0.034	0.099	0.013
r	0.374	0.210	0.298	0.653	0.855	0.597	0.945
p							
Disulphide/Native thiol	-0.350	-0.185	-0.255	-0.356	-0.156	0.197	0.262
r	0.054	0.319	0.166	0.050	0.402	0.289	0.154
p							
Disulphide/Total thiol	-0.376	-0.225	-0.263	-0.380	-0.232	0.160	0.248
r	0.037	0.224	0.153	0.035	0.209	0.391	0.178
p							
Native thiol/ Total thiol	0.301	0.200	0.257	0.359	0.244	-0.241	-0.197
r	0.100	0.281	0.163	0.047	0.185	0.192	0.287
p							
RNFL retinal nerve fiber layer; GCL ganglion cell layer; IPL inner plexiform layer; INL inner nuclear layer; OPL outer plexiform layer; ONL outer nuclear layer; RPE retinal pigment epithelium							

Discussion:

The increase in oxidative stress in DM is believed to be associated with the development of microvascular complications [8,23]. The retina is more sensitive to ROS than other ocular tissues as it requires a large amount of energy for its physiological functions and contains large amounts of polyunsaturated fatty acids. There is accelerated loss of the RNFL thickness independent of DR in patients with T2DM, loss of the RNFL thickness in patients with T1DM, and increase in nerve fiber defects

in the retina with the progression of DR [24–27]. In the present study, the RNFL thickness was found to be lower in patients with and without DR compared with controls, but the difference was not statistically significant. In addition, there was no difference in the RNFL thickness between the patients with and without DR. In the present study, a very weak negative correlation was found between the RNFL thickness and disulphide/native thiol ratio as well as disulphide/total thiol ratio. However, we found that the level of correlation between the RNFL thickness and disulphide/total thiol ratio became stronger (from very weak to weak) and statistically significant in patients without DR having a disease duration of < 10 years. We found no such association in these patients with a disease duration of ≥ 10 years. Accordingly, this suggests that the relationship between the increased oxidative stress and RNFL thickness is stronger in the early stages of DM; however, the occurrence of vascular problems in the late stages of DM leads to a weakening in the correlation between these variables. It is believed that neurodegenerative damage in the retina occurs in the early stage of the disease in patients with DM and vascular problems occur later as the disease progresses [28,29]. Decreased RNFL thickness has been attributed to astrocyte and ganglion cell loss, direct toxicity due to hyperglycemia, and Müller cell dysfunction [29,30]. The RNFL thickness loss is higher in patients with a T2DM duration of ≥ 10 years [18,31]. In the present study, the retinal layers (excluding RPE) in patients without DR did not show any difference in thickness with respect to disease duration (< 10 years and ≥ 10 years). This may be due to the fact that the study comprised independent patient groups. The European Consortium for the Early Treatment of Diabetic Retinopathy reported a lower GCL-IPL thickness in patients with T2DM compared with that in the normal population [32]. Progressive loss of the GCL/IPL is an independent risk factor for progression in early-stage DR [32]. In a study conducted in Turkey, the GCL and IPL were found to be thinner in patients with DM compared with those in the control group, but no difference was found between the GCL and IPL thicknesses in patients with and without DR [33]. In the present study, we did not find any difference between the patients with and without DR in terms of the GCL thickness. However, the GCL thickness was lesser in patients without DR compared with that in the controls. Mohammed et al. reported that the GCL demonstrated the difference in its thickness in patients with DR versus that in controls [34]. In the present study, we found that the IPL thickness was similar in patients with and without DR but lower than that in the control group; however, the difference was not statistically significant. We found no correlation between the GCL thickness and thiol–disulfide parameter measurements. Correlation analysis revealed a weak negative correlation between the IPL thickness and disulphide levels, disulphide/native thiol, and disulphide/total thiol ratio. However, no similar correlation was observed in patients with a disease duration of < 10 years.

Mohammed et al., in their study, suggested that the change in the INL thickness is the best indicator that distinguishes patients with DR from controls; it has an even higher discriminative power than the early loss of RNFL thickness [34]. Changes in the INL were found to be more pronounced in animal studies [35]. In the present study, the INL thickness was not different between the patients with and without DR and controls, and we found a weak negative correlation between the INL thickness and disulphide/total thiol ratio. This was a remarkable finding between the INL thickness and thiol–disulfide homeostasis. In our correlation analysis, we also found a weak correlation between the INL thickness and ratio of

disulphide/total thiol, disulphide/native thiol, and native thiol/total thiol in patients with a disease duration of < 10 years, and we found that the level of correlation became stronger. The absence of this correlation in patients with a disease duration of ≥ 10 years suggests that oxidative stress-related neurodegenerative damage in the RNFL is also present in the INL. The hypersensitivity of Müller glial cells in the INL to hyperglycemia causes an increase in the number of cell nuclei and hypertrophy, leading to the apoptotic process [29,36,37]. Mohammed S et al., in their study, argued that the difference between patients with and without DR was best observed in terms of the OPL thickness [34]. In another study, a decrease in the RNFL and OPL thicknesses and an increase in the ONL thickness were observed in the patients without DR compared with those in controls [38]. Vujosevic S et al. did not detect a significant difference in the ONL/ELM thicknesses between patients without DR and controls. In the present study, we found that the OPL and ONL thicknesses were not different in patients without DR compared with those in controls; however, the RPE thickness was different between these two patient groups. To the best of our knowledge, such a finding has not been previously reported in literature. As seen in many of the studies cited here, differences in results were observed in terms of retinal layer thicknesses in patients with DM based on the selected patient group. Variations in genes related to oxidative stress among patients with DM make some patients more predisposed to develop DR than others [39]. Thiol–disulfide homeostasis is considered as a biomarker that may be effective in the early diagnosis and treatment of DR [39]. Inhibition of proinflammatory molecules has also been shown to reduce DM-related vascular and neurodegenerative changes in animal models [29,37]. Elucidating this situation becomes even more important as therapeutic supplementation may aid in the management of low circulating thiol concentrations [40].

In conclusion, early changes in retinal layer thickness in patients with DM were found to be associated with thiol–disulfide homeostasis. The results obtained in the present study should be confirmed by performing similar studies on a larger sample and the possible underlying mechanisms should be elucidated.

Limitations

There are certain limitations of this study. First, we did not use another oxidative stress marker to compare with thiol–disulfide parameters. As thiol–disulfide homeostasis is impaired in patients with DM, we did not separately compare the control group and the patients with DM in terms of thiol–disulfide parameters. Second, the study was designed as a pilot study in a small group of patients.

Declarations

Funding

No funding was received for conducting this study.

Compliance with ethical standards

Conflict of interest

The authors declare no relevant conflicts of interest.

Ethical approval

The study protocol was approved by Ankara Yıldırım Beyazıt University and complied with the principles of the Declaration of Helsinki

Consent for publication

Patients signed informed consent regarding publishing their data.

References

1. Stewart JM, Coassin M, Schwartz DM. Diabetic Retinopathy.(2021) In: Feingold KR, Anawalt B, Boyce A, et al., eds. Endotext. South Dartmouth (MA): MDText.com, Inc.2021; January 3.
2. Wilkinson CP, Ferris FL 3rd, Klein RE, et al (2003) Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. *Ophthalmology*. 110(9):1677-1682.
3. Davoudi S (2016) Optical coherence tomography characteristics of macular edema and hard exudates and their association with lipid serum levels in type 2 diabetes. *Retina (Philadelphia, Pa.)*. 2016;36(9):1622.
4. CDC 2018. Centers for Disease Control and Prevention [Internet]. (2018) [Accessed May, 30, 2018].Access address: <https://www.cdc.gov/visionhealth/basics/ced/index.html>.
5. Das A, McGuire PG, Rangasamy S (2015) Diabetic Macular Edema: Pathophysiology and Novel Therapeutic Targets. *Ophthalmology*. 122(7): 1375-1394.
6. Calderon GD, Juarez OH, Hernandez GE, et al (2017) Oxidative stress and diabetic retinopathy: development and treatment. *Eye (Lond)*. 31(8):1122-1130.
7. Dröge W (2002) Free radicals in the physiological control of cell function. *Physiol Rev*. 82(1):47-95.
8. Kowluru RA, Chan PS (2007) Oxidative stress and diabetic retinopathy. *Exp Diabetes Res*. 2007:43603.
9. Erel, O., Neselioglu, S. (2014) A novel and automated assay for thiol/disulphide homeostasis. *Clin. Biochem*. 47 (18), 326–332.
10. Saibal Biswas, Asiya Seema Chida, Irfan Rahman (2006) Redox modifications of protein–thiols: Emerging roles in cell signaling, *Biochemical Pharmacology*. Volume 71, Issue 5, Pages 551-564.

11. Matteucci E, Giampietro O (2010) Thiol signalling network with an eye to diabetes. *Molecules*. 15(12):8890–903.
12. Prabhu A, Sarcar B, Kahali S, et al (2014) Cysteine catabolism:a novel metabolic pathway contributing to glioblastoma growth. *Cancer Res*. 74(3):787–96.
13. Turkoglu EB, Dikci S, Çelik E, et al (2016) Thiol/Disulfide Homeostasis in Patients with Central Serous Chorioretinopathy. *Curr Eye Res*. 41(11):1489-1491.
14. Sagdik HM, Ucar F, Tetikoglu M, et al (2018) Investigation of dynamic thiol-disulphide homoeostasis in age-related cataract patients with a novel and automated assay. *Int Ophthalmol*. 38(2):655-661.
15. Kirboga K, Ozec AV, Kosker M, et al (2014) The Association between Diabetic Retinopathy and Levels of Ischemia-Modified Albumin, Total Thiol, Total Antioxidant Capacity, and Total Oxidative Stress in Serum and Aqueous Humor. *J Ophthalmol*. 2014:820853.
16. Gulpamuk B, Koç M, Karatepe MS, et al (2017) Novel Assay Assessment of Oxidative Stress Biomarkers in Patients with Keratoconus: Thiol-Disulfide Homeostasis. *Curr Eye Res*. 42(9):1215-1219.
17. Erel Ö, Erdoğan S (2020) Thiol-disulfide homeostasis: an integrated approach with biochemical and clinical aspects. *Turk J Med Sci*. 3;50(SI-2):1728-1738
18. Lee, MW., Lim, HB., Kim, MS. et al (2021) Effects of prolonged type 2 diabetes on changes in peripapillary retinal nerve fiber layer thickness in diabetic eyes without clinical diabetic retinopathy. *Sci Rep*. 11, 6813.
19. Lee JS, Oum BS, Choi HY, et al (2006) Differences in corneal thickness and corneal endothelium related to duration in diabetes. *Eye (Lond)*. 20(3):315-318.
20. Wilkinson CP, Ferris 3rd FL, Klein RE, et al (2003) Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. *Ophthalmology* 2003;110:1677–82
21. Chalam KV, Bressler SB, Edwards AR, et al (2012) Retinal thickness in people with diabetes and minimal or no diabetic retinopathy: Heidelberg Spectralis optical coherence tomography. *Invest Ophthalmol Vis Sci*. 53: 8154–8161.
22. Photocoagulation for diabetic macular edema. Early Treatment Diabetic Retinopathy Study report number 1. Early Treatment Diabetic Retinopathy Study research group. *Arch Ophthalmol*.1985; 103 (12):1796–1806
23. Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. *Nature*. 414(6865):813-820.
24. Chihara, E., Matsuoka, T., Ogura, Y., et al (1993) Retinal nerve fiber layer defect as an early manifestation of diabetic retinopathy. *Ophthalmology*. 100, 1147–1151.
25. Lopes de Faria JM, Russ H, Costa VP (2002) Retinal nerve fibre layer loss in patients with type 1 diabetes mellitus without retinopathy. *Br J Ophthalmol*. 86(7):725-728.
26. Takahashi, H. et al (2006) Diabetes-associated retinal nerve fiber damage evaluated with scanning laser polarimetry. *Am. J. Ophthalmol*. 142, 88–94.

27. Lim, H. B., Shin, Y. I., Lee, M. W., Park, G. S. & Kim, J. Y (2019) Longitudinal changes in the peripapillary retinal nerve fiber layer thickness of patients with type 2 diabetes. *JAMA Ophthalmol.* 137, 1125–1132.
28. Jiang J, Liu Y, Chen Y, et al (2018) Analysis of Changes in Retinal Thickness in Type 2 Diabetes without Diabetic Retinopathy. *J Diabetes Res.* 2018:3082893.
29. Vujosevic S, Midena E (2013) Retinal layers changes in human preclinical and early clinical diabetic retinopathy support early retinal neuronal and Müller cells alterations. *J Diabetes Res.* 2013:905058.
30. A. Bringmann and P. Wiedemann (2011) "Muller glial cells in retinal disease," *Ophthalmologica.* Vol. 227, no. 1, pp. 1–19.
31. Wan ZQ, Gao Y, Cui M, Zhang YJ (2021) Association between risk factors and retinal nerve fiber layer loss in early stages of diabetic retinopathy. *Int J Ophthalmol.* 2021;14(2):255-262.
32. K. Kim, E. S. Kim, and S. Y. Yu (2018) "Longitudinal relationship between retinal diabetic neurodegeneration and progression of diabetic retinopathy in patients with type 2 diabetes," *American Journal of Ophthalmology.* Vol. 196, pp. 165–172.
33. Celik Buyuktepe T, Demirel S, Batioğlu F, Özmert E (2021) The correlation of inflammation and microvascular changes with diabetic retinal neurodegeneration. *Curr Eye Res.* 10.1080/02713683.2021.1908567.
34. Mohammed S, Li T, Chen XD, et al (2020) Density-based classification in diabetic retinopathy through thickness of retinal layers from optical coherence tomography. *Sci Rep.* 2020;10(1):15937.
35. Zeng, X. -x, NG, Y. -K. & Ling, E. -a (2000) Neuronal and microglial response in the retina of streptozotocin-induced diabetic rats. *Vis. Neurosci.* **17**, 463–471.
36. Curtis TM, Hamilton R, Yong P-h-H et al. (2011) Muller glial dysfunction during diabetic retinopathy in rats is linked to accumulation of advanced glycation end –products and advanced lipoxidation end-products. *Diabetologia* 54:690-8.
37. Rübsam A, Parikh S, Fort P (2018) Role of Inflammation in Diabetic Retinopathy. *Int J Mol Sci.* 19(4):942.
38. Wanek J, Blair NP, Chau FY, Lim JI, Leiderman YI, Shahidi M (2016) Alterations in Retinal Layer Thickness and Reflectance at Different Stages of Diabetic Retinopathy by En Face Optical Coherence Tomography. *Invest Ophthalmol Vis Sci.* 57(9):OCT341-OCT347
39. Al-Shabraway M, Smith S (2010) Prediction of diabetic retinopathy: role of oxidative stress and relevance of apoptotic biomarkers. *EPMA J.* 1(1):56-72.
40. Panigrahy SK, Bhatt R, Kumar A (2017) Reactive oxygen species: sources, consequences and targeted therapy in type 2 diabetes. *J Drug Target.* 2017;25(2):93-101.