ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

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Is There Any Association Between the Retinal Vein Occlusion and the Thiol-Disulfide Homeostasis Which is an Oxidative Stress Indicator?

Retinal Ven Tıkanıklığı ve Bir Oksidatif Stres Belirteci Olan Tiyol-Disülfit Dengesi Arasında Herhangi Bir İlişki Var mıdır?

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ABSTRACT Objective: In this study, it is aimed to investigate the dynamic thiol-disulfide homeostasis which is an indicator of oxidative stress in retinal vein occlusion patients. **Material and Methods:** Thirty nine retinal vein occlusion patients and 38 age-gender matched control subjects were included in the study. Exclusion criteria for retinal vein occlusion group were: ocular history and symptoms exceeding 3 weeks, any other history of ocular disease except the retinal vein occlusion, any previous ocular surgeries or lasers, any previous treatment or intra-vitreal injection for the retinal vein occlusion, any other systemic disease except the arterial hypertension. The control group included patients without any ocular or systemic diseases. Native thiol, total thiol, disulfide levels were studied in the serum samples of all participants and native/total thiol and disulfide/total thiol ratios were calculated as percentage. **Results:** Total thiol, native thiol, disulfide, native/total thiol and disulfide/total thiol ratio were not significantly different between the retinal vein occlusion and the control groups. In the control group, age was inversely correlated with the total thiol level, but not with the other serum parameters. In the retinal vein occlusion group, there was no correlation among the age and the serum parameters but the body mass index (BMI) was inversely correlated with the total thiol level. **Conclusion:** There was not any significant difference between the retinal vein occlusion patients and the control subjects for the thiol-disulfide homeostasis.

Keywords: Retinal vein occlusion; thiol-disulfide homeostasis; thiol; disulfide

ÖZET Amaç: Bu çalışmada retinal ven tıkanıklığı ile bir oksidatif stres belirteci olan dinamik tiyol-disülfit dengesi arasındaki ilişkinin araştırılması amaçlanmıştır. **Gereç ve Yöntemler:** Otuz dokuz retinal ven tıkanıklığı hastası ve 38 yaş ve cinsiyet eşleştirilmiş kontrol birey olmak üzere toplam 77 katılımcı çalışmaya alınmıştır. Retinal ven tıkanıklığı grubu için çalışmadan dışlanma kriterleri: 3 haftayı aşan göz şikayet ve bulguları, retinal ven tıkanıklığı dışında başka oküler hastalık öyküsünün olması, daha önce oküler cerrahi ve lazer öyküsünün olması, retinal ven tıkanıklığı için daha önce intravitreal enjeksiyon dahil olmak üzere herhangi bir tedavi uygulanmış olması, arteryel hipertansiyon dışında sistemik hastalık öyküsünün olması olarak belirlenmiştir. Kontrol grubu sistemik ve oküler hastalığı olmayan katılımcılardı. Native tiyol, total thiol, disülfit düzeyleri katılımcıların kan serumu örneklerinde çalışılarak belirlenmiştir ve native/total tiyol ve disülfit/total tiyol oranları hesaplanarak yüzde olarak kaydedilmiştir. **Bulgular:** Total tiyol, native tiyol, disülfit, native/total tiyol oranı ve disülfit/total tiyol oranları retinal ven tıkanıklığı ve kontrol grubu arasında karşılaştırıldığında anlamlı fark bulunmamıştır. Kontrol grubunda yaş ve total tiyol seviyeleri arasında negatif korelasyon olduğu, fakat diğer parametreler arasında anlamlı ilişki olmadığı bulunmuştur. Retinal ven tıkanıklığı grubunda yaş ve serum parametreleri arasında anlamlı ilişki bulunmamış, fakat beden kitle indeksi (BKİ) ile total tiyol düzeyleri arasında negatif korelasyon bulunmuştur. **Sonuç:** Retinal ven tıkanıklığı ve kontrol grupları arasında tiyol-disülfit dengesi açısından anlamlı fark bulunmamıştır.

Anahtar Kelimeler: Retinal ven tıkanıklığı; disülfit; tiyol; tiyol-disülfit dengesi

Retinal vein occlusion is one of the most frequently encountered retinal vascular diseases in all the ethnic groups and can cause decreased vision which is sometimes permanent.[1] Various factors including hypertension, hyperlipidemia, diabetes mellitus, stiffed retinal arteries compressing on retinal veins, hematological alterations, glaucoma and sleep apnea were blamed in the etiology before.[1,2] The oxidative stress may also play a role in the pathogenesis in this vascular disease.[3,4]

Total plasma thiol is formed by proteins including albumin and smaller peptides and amino acids.[5] Disulfide bonds can be formed by the oxidation reaction of the thiol groups.[6] The oxidative stress can lead to the reversible formation of disulfides and these disulfide bonds can be reversely reduced to the thiol groups indicating a thiol- disulfide homeostasis.[7] The thiol disulfide homeostasis plays crucial roles in various cellular mechanisms like antioxidant protection, detoxification, apoptosis and the enzymatic regulation.[8]

There is an increasing interest in the thiol disulfide homeostasis for various diseases including the cardiovascular disease.[9,10] As a vascular disease, the role of oxidative stress in the retinal vein occlusion pathogenesis was emphasized before, and the thiol-disulfide homeostasis might also be altered.[3,4,11] In this study, we aimed to investigate the dynamic thiol-disulfide homeostasis which is an indicator of oxidative stress in the retinal vein occlusion patients.

MATERIAL AND METHODS

This study was carried out according to the tenets of Declaration of Helsinki. The study was approved by the local ethical committee before patient collection. The informed consent was obtained from the participants before enrollment.

PATIENT ENROLLMENT

Thirty nine retinal vein occlusion patients and 38 age-gender matched control subjects were included in the study. The demographical data was summarized in Table 1. The exclusion criteria for retinal vein occlusion group were: ocular history and symptoms exceeding 3 weeks, any other history of ocular disease except the retinal vein occlusion, any previous ocular surgeries or lasers, any previous treatment or intra-vitreal injection for the retinal vein occlusion, any other systemic disease except the arterial hypertension. The control group included patients without any ocular or systemic diseases except arterial hypertension. There wasn't any significant difference between the groups in the body mass index (Table 1). All the participants underwent full ophthalmological examination including obtaining the visual acuity, intraocular pressure measurement with the air puff tonometer and dilated fundus examination. The retinal vein occlusion patients also underwent imaging with optical coherence tomography (OCT) and fundus fluorescein angiography (FFA). The retinal vein occlusion diagnosis made by the observation of multiple retinal hemorrhages, dilated and tortuous veins, retinal and papillary edema during retinal examination and additional

TABLE 1: The demographic features of retinal vein occlusion (RVO) and control subjects.

	RVO Group n=39	Control Group n=38	P value
Mean age (years)	61.17 (43-85)	63.42 (42-82)	P=0.28
BMI (kg/m^2)	28.7 (19.2-43.7)	27.7 (19.7-39.3)	P=0.34
Gender			
Female	22 (56.4%)	15 (39.5%)	P=0.12
Male	17 (43.6%)	23 (60.5%)	

BMI: Body Mass Index.

hypo-fluorescence due to the capillary non-perfusion and hemorrhages, dilated and tortuous veins, and leakage if macular edema was present on the FFA.

Blood Samples and Thiol-Disulfide Analysis

The venous blood samples (5 mL) from all the patients were taken from the antecubital vein and placed in the separating gel vacuum tubes. The serum was separated by the centrifugation (1500 g, 10 min). The serum samples were stored at -80 °C until the test date.

The thiol-disulfide hemostasis analyzed by the method was described before.[10] The dynamic disulfide bonds in the sample were reduced to the thiol groups by NaBH4 and the remaining NaBH4 remnants were completely removed by the formaldehyde. The total thiol amount was measured using the modified Ellman reagent. The native thiol amount was measured without reduction process by the same method. The native thiol content is subtracted from the total thiol content and divided into 2 to give the disulfide bond content. The native/total thiol ratio and the disulfide/total thiol ratios were also calculated.

STATISTICAL ANALYSIS

The statistical analysis was performed by the SPSS 18.0 software. Mean ages and the body mass index (BMI) between the groups were compared by the student t-test. Normal distribution was examined by Shapiro-Wilks test. Categorical results were compared by Pearson's Chi-squared test (Exact 2-sided). The thiol-disulfide parameters were compared by Mann Whitney-U test. The correlations between the parameters were tested with Spearman test. P values lower than 0.05 considered as statistically significant.

RESULTS

Thirty nine retinal vein occlusion patients and 38 age-gender matched control subjects 77 patients in total were included in the study. There was not any significant difference with respect to the mean age and the gender distribution (Table 1). The total thiol, native thiol, disulfide, native/total thiol and disulfide/total thiol ratio were compared between the retinal vein occlusion patients and the control subjects and there were no significant differences between the groups (Table 2). Even we included hypertension patients in the control group, significantly higher percentage of hypertension were included in the retinal vein occlusion group (26% vs 56%, p=0.011).

In the control group, the age was inversely correlated with the total thiol levels ($p<0.001$, $r= -0.539$), but not with the other serum parameters (Table 3, Figure 1). In the retinal vein occlusion group, there was no correlation with the age and the serum parameters but the BMI was inversely correlated with the total thiol levels (P=0.39, r= -0.332) (Table 3, Figure 2).

DISCUSSION

The role of oxidative stress in pathogenesis of various ocular disease including the cataracts, age related macular degeneration, central serous

TABLE 2: The total thiol, native thiol, disulfide, native/total thiol ratio and disulfide/total thiol ratio were not significantly different between the retinal vein occlusion and the control groups.

	RVO Group Median (Min-Max)	Control Group Median (Min-Max)	P Value
Total Thiol (µmol/L)	330 (195.5-510.7)	331.96 (218.5-552.4)	P= 0.69
Native Thiol (µmol/L)	117 (22-338)	129 (3 - 306)	P=0.48
Disulfide (µmol/L)	90.8 (45.1-189)	79.1 (42.3 - 242.7)	P=0.66
Native/Total Thiol (%)	29.5 (9.8-67.4)	48.25 (1.1- 67.4)	P=0.48
Disulfide/Total Thiol(%)	35.2 (16.3-45.1)	25.9 (16.2-49.5)	P=0.46

(RVO: Retinal vein occlusion).

TABLE 3: The correlations among the age and the BMI (body mass index) and the serum parameters.

		Total thiol	Native thiol	Disulfide	Native/total thiol	Disulfide/Total Thiol
Control Group n=38						
AGE	r	**-0,539***	-0,145	-0,307	-0,114	0,109
	P value	**<0,001**	0,386	0,061	0,497	0,513
BMI	r	-0,066	-0,108	0,008	-0,095	0,094
	P value	0,692	0,520	0,964	0,571	0,573
RVO Group n=39						
AGE	r	-0,216	-0,206	-0,020	-0,127	0,129
	P value	0,187	0,209	0,902	0,440	0,436
BMI	r	**-0,332***	-0,201	-0,103	-0,122	0,121
	P value	**0,039**	0,221	0,531	0,461	0,462

(r: correlation coefficient; *: statistically significant parameters correlations).

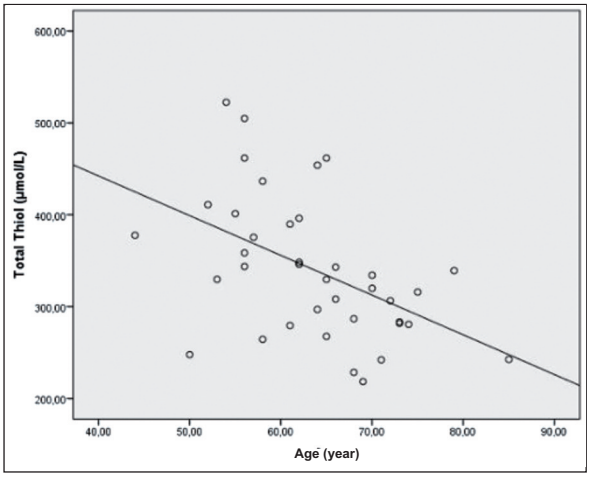

FIGURE 1: The inverse correlation was found between the age and the total plasma thiols in the control group (p<0.001, r=0.539).

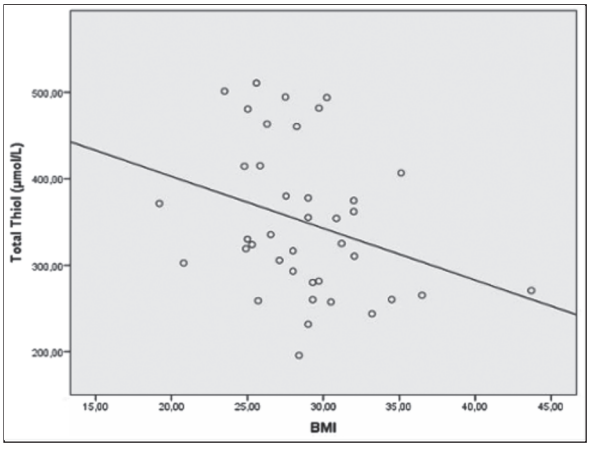

FIGURE 2: BMI was inversely correlated with the total thiol levels in retinal vein occlusion group (P=0.39, r= -0.332).
BMI: Body mass index.

chorioretinopathy, pterygium, dry eye and the keratoconus were reported before.[3,12-18] Altınisik and et al. showed increased oxidative stress index (OSI) and lower total antioxidant status (TAS) in the humor aqueous samples of the retinal vein occlusion patients and they blamed hypoxia, ischemia and reperfusion damage by the reactive oxygen radicals.[3] Becatti et al. found higher reactive oxygen species production and membrane lipid peroxidation in the erythrocytes of the retinal vein occlusion patients due to the oxidative stress.[4] Considering these previous results we hypothesized that there might be an increased systemic oxidative status affecting the thiol-disulfide hemostasis in the retinal vein occlusion patients. In this study, even the median of disulfide/total thiol ratio which would indicate the increased oxidative stress in retinal vein occlusion patients was higher, it was not statistically significant (Table 2).

According to the results, we concluded that the systemic oxidative stress status wasn't significantly different between the groups to alter the serum thiol-disulfide homeostasis significantly. Thus, the oxidative stress may not be a significant factor in the retinal vein occlusion pathogenesis. However, as the local increased oxidative status shown in previous study, it might be a result of the ischemia following the retinal vein occlusion.[3] In this study, the thiol-disulfide homeostasis was stud-

ied in the serum samples and we even assumed that there was a significant difference in the local ocular levels between the groups, it could be masked by the dilution in the whole blood serum as the amount of blood circulation in the eye is too less than the whole body circulation. There are limited number of studies evaluating local ocular samples for the oxidative stress in retinal vein occlusion and future studies investigating the thiol parameters from the local ocular samples might give better understanding.

An inverse correlation between the increasing age and the total plasma thiols were shown before.[19] In our study, inverse correlation was found between the age and the total plasma thiols ($p<0.001$, $r=0.539$) in the control group, but not in the retinal vein occlusion patients ($p=0.18$) (Table 3). The native and disulfide ratios were not significantly correlated, this may show that there wasn't remarkable shift to the oxidized disulfide groups.

In the retinal vein occlusion group, the BMI was inversely correlated with the total thiol levels ($p=0.39$, $r=-0.332$) but no correlation was found in the control group ($p=0.69$) (Table 3). Decreased plasma thiol level was reported in the obese children before, but according to our best knowledge no previous study reported the relationship between the thiol levels and the body mass index in adults before.[20] It is unclear why the BMI correlation was different between the groups therefore it needs further investigation.

There are limitations of this study. Firstly, even we included hypertension patients in the control group, significantly higher percentage of hypertension were included in the retinal vein occlusion group. Additionally, we studied serum samples in the central vein occlusion patients and we did not study the local ocular samples which may not directly affect the systemic levels.

CONLUSION

In conclusion, there was no significant difference between the retinal vein occlusion patients and the control subjects with respect to the thiol-disulfide homeostasis.

Ethics Committee Approval

The study was approved by the local ethical committee before patient collection.

Informed Consent

The informed consent was obtained from the participants before enrollment.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Osman Ahmet Polat, Ali Kurt, Raşit Kılıç;* ***Design:*** *Osman Ahmet Polat, Ali Kurt, Raşit Kılıç, Rukiye Nar;* ***Control/Supervision:*** *Osman Ahmet Polat, Ali Kurt, Raşit Kılıç, Rukiye Nar, Özkan Kocamış;* ***Data Collection and Processing:*** *Osman Ahmet Polat, Ali Kurt, Raşit Kılıç, Rukiye Nar;* ***Analysis and Interpretation:*** *Osman Ahmet Polat, Ali Kurt, Raşit Kılıç, Rukiye Nar, Özkan Kocamış;* ***Literature Review:*** *Osman Ahmet Polat, Ali Kurt, Raşit Kılıç, Özkan Kocamış;* ***Writing the Article:*** *Osman Ahmet Polat, Ali Kurt, Raşit Kılıç;* ***Critical Review:*** *Osman Ahmet Polat, Ali Kurt, Raşit Kılıç, Özkan Kocamış.*

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