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Original Article

Assessment of Plasma Thiol-disulfide Balance in Pseudoexfoliation Syndrome and Pseudoexfoliation Glaucoma

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Abstract

Objectives: The thiol-disulfide balance is very important in cellular events such as apoptosis and oxidative stress. This study is a comparison of plasma thiol-disulfide homeostasis in patients with pseudoexfoliation syndrome (PEXS) and pseudoexfoliation glaucoma (PEXG).

Methods: Thirty-one patients with PEXS, 43 patients with PEXG, and 38 healthy controls were included in this prospective study. The plasma level of native thiol and disulfide were measured using a spectrophotometric assay and the native thiol/disulfide ratio was analyzed.

Results: The demographic characteristics of the 3 groups were similar (p>0.05). Statistically significant differences were observed in the plasma disulfide levels (21.6±7.3 μmol/L vs. 17.4±6.8 μmol/L) and the native thiol/disulfide ratio (22.9±9.1 vs. 29.9±14.7) between the PEXG group and the controls (p=0.03, p=0.02, respectively).

Conclusion: Significant differences in the plasma levels of disulfide and the native thiol/disulfide ratio in PEXG patients indicated a breakdown of the thiol-disulfide circuits.

Keywords: Oxidative stress, pseudoexfoliation syndrome, pseudoexfoliation glaucoma, thiol-disulfide balance.

Introduction

Pseudoexfoliation syndrome (PEXS), an age-related disorder, is the most prevalent cause of open-angle glaucoma. It is characterized by the deposition of fibrillar protein in the anterior segment of the eye and other ocular tissues (1,2). Although the exact pathogenesis of PEXS is unknown, oxidative damage is one of the most investigated mechanisms (3-5). Increased levels of the oxidative stress (OS) markers in the serum and aqueous humor, such as nitric oxide, tumor necrosis factor alpha, malondialdehyde, and 8-isoprostaglandin-F2, have been reported in patients with PEXS (6,7).

Pseudoexfoliation glaucoma (PEXG) is the most common form of secondary open-angle glaucoma worldwide, with a faster progression of neurodegeneration than that seen in primary open-angle glaucoma (8). Previous research has indicated that OS causes conformational changes in the extracellular matrix (ECM) of trabecular epithelial cells, leading to reduced aqueous outflow in PEXS and PEXG (9-14).

Thiol oxidoreductases are critical cellular molecules in the defense against reactive oxygen species (ROS) (15). Thiol-containing proteins, such as cysteine, homocysteine, glutathione and albumin, form the plasma thiol pool. Dynamic thiol-disulfide homeostasis plays an important role in detoxification of OS related substances, regulation of intracellular

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signaling pathways, enzymatic reactions, and cell apoptosis. Abnormal thiol-disulfide concentrations have been reported in the pathogenesis of several systemic degenerative diseases (16-18). PEXG has similar clinical findings to other neurodegenerative disorders, such as Alzheimer's disease (19). The aim of this study was to compare the systemic thiol-disulfide redox status in patients with PEXS and patients with PEXG.

Methods

This study was conducted in accordance with Helsinki Declaration and approved by the local ethics committee (#26379996-44; 02/22/2017). Informed consent was obtained from all of the participants.

Thirty-one patients with PEXS, 43 patients with PEXG, and 38 control subjects were enrolled in this prospective study. The study was conducted in the glaucoma section of the Ophthalmology Department of Ankara Atatürk Training and Research Hospital.

Detailed ophthalmological examinations, including an evaluation of best corrected visual acuity, intraocular pressure (IOP) with Goldmann applanation tonometry and slit lamp, gonioscopic, and fundoscopic examination of dilated pupils, as well as visual field defects using a Humphrey visual field analyzer, were performed on all of the subjects. PEXS was diagnosed in patients with an open iridocorneal angle and pseudoexfoliation material deposits on the anterior lens capsule or pupillary margin without glaucomatous optic neuropathy. Patients with an open iridocorneal angle and pseudoexfoliation material deposits on the anterior lens capsule and pupillary margin with an IOP >21 mmHg without any treatment and typical optic nerve head changes and visual field defects of glaucoma were diagnosed with PEXG. In this study, the patients with PEXG were using at least 1 topical antiglaucomatous drug. Age and sex-matched subjects without any ocular disorders were included as a control group.

Patients with ophthalmic diseases, such as age-related macular degeneration, retinal dystrophy, uveitis, grade 3 or 4 cataract severity according to the Lens Opacities Classification System III, were excluded from the study. Patients with chronic systemic diseases, such as diabetes mellitus, cardiovascular, renal and liver diseases, were also excluded since these conditions may affect OS status. In addition, patients who were current smokers and taking antioxidant supplements, such as vitamin C or vitamin E, were excluded.

Biochemical Analysis

Blood samples were collected after a 12-hour overnight fast. Venous blood samples were centrifuged in tubes including ethylenediaminetetraacetic acid at 1500 rpm for 10 minutes and the plasma was extracted for analysis.

Native thiol, total thiol, and disulfide levels were measured as novel OS parameters. Dynamic disulfide bonds (-S-S-) in the serum sample were reduced to native thiol groups (-SH) using sodium borohydride. Total thiol was measured with modified Ellman reagent. The native thiol value was subtracted from the total thiol, and half of the difference produced the disulfide bond quantification. Measurements were performed with a spectrophotometer (UV-1800; Shimadzu Corp., Kyoto, Japan) and an automated analyzer (Cobas c 501 Roche Diagnostics, Basel, Switzerland), as described in the study published by Erel and Neselioglu (20). After measuring the native thiol and disulfide concentrations, the native thiol/disulfide ratio (-SH/-S-S-) was calculated.

Statistical Analysis

Statistical analysis was performed by using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA). Normal distribution of the continuous variables was assessed using the Kolmogorov-Smirnov test. One-way analysis of variance and the Tukey test for post hoc analysis were used to compare the mean data of the 3 study groups. The p value accepted for statistical significance was <0.05.

Results

The demographic and clinical data of the patients and control subjects are shown in Table 1. There was no statistically significant difference between the groups in terms of age or gender (p=0.12, p=0.23, respectively). The mean plasma native thiol, disulfide and native thiol/disulfide ratio of the groups are shown in Table 2. The mean plasma native thiol

Table 1. Comparison of demographic data

	PEXS (n=31)	PEXG (n=43)	Control (n=38)	p*
Age (years)	70.13±6.32 (62-88)	69.09±8.72 (50-84)	68.42±8.03 (52-81)	0.12
Gender (female/male)	14/17	20/23	19/19	0.23
Visual acuity	0.59±0.08 (0.50-0.8)**	0.58±0.77 (0.4-0.7)**	0.89±0.10 (0.7-1.0)	p=0.02
Baseline IOP	17.83±1.24 (15-20)	16.09±1.63 (12-19)	16.28±1.45 (14-19)	p=0.79

IOP: Intraocular pressure; PEXG: Pseudoexfoliation glaucoma; PEXS: Pseudoexfoliation syndrome; * The p value was calculated using one-way analysis of variance; ** p <0.001, comparison of PEXS, PEXG groups with control group, post hoc Tukey test.

Table 2. Comparison of thiol-disulfide balance

	PEXS (n=31)	PEXG (n=43)	Controls (n=38)	p*
Native thiol (μmol/L)	437.9±47.7	437.6±48.7	437.2±41.7	0.99
Disulfide (μmol/L)	21.2±8.4***	21.6±7.3**	17.4±6.8	0.01
Native thiol/disulfide	24.2±1.3*****	22.9±9.1****	29.9±14.7	0.01

PEXG: Pseudoexfoliation glaucoma; PEXS: Pseudoexfoliation syndrome; * The p value was calculated using one-way analysis of variance; ** p=0.03, comparison of PEXG group with control group, post hoc Tukey test; *** p= 0.09, comparison of PEXS group with control group, post hoc Tukey test; **** p=0.02, comparison of PEXG group with control group, post hoc Tukey test; ***** p=0.11, comparison of PEXS group with control group, post hoc Tukey test.

value was 437.9±47.7 μmol/L in the PEXS group, 437.6±48.7 μmol/L in the PEXG group, and 437.2±41.7 μmol/L in the control group. The results were statistically similar between groups (p=0.99).

There was a statistically significant difference between the groups in the mean plasma disulfide level (p=0.03). The plasma disulfide level was significantly higher in the PEXG group (21.6±7.3 μmol/L) compared with the control group (17.4±6.8 μmol/L) (p=0.03).

In addition, the plasma native thiol/disulfide ratio was significantly lower in the PEXG group (22.9±9.1) compared with the control group (29.9±14.7) (p=0.02).

Discussion

PEXS is a known risk factor for several ocular complications, including cataract, zonular instability and PEXG. Although the exact etiopathogenesis is unknown, several factors, such as age, genetic variations, high IOP, and OS burden, have been reported in the progression from PEXS to PEXG (7).

Following the establishment of a relationship between aging and OS, the role of OS has been investigated in the sera and aqueous humors of patients with PEXS and PEXG, like many other age-related eye diseases (3-6,21,22). OS-induced retinal ganglion cell death was found to be consistent with high IOP and visual field defects in glaucoma (2,23-26). Further, several studies have reported a correlation between lower systemic antioxidant capacity and insufficient local ocular oxidative defense capacity (27,28).

Recently, the thiol-disulfide balance was introduced as a novel biochemical parameter to assess systemic OS. Normally, native thiol, disulfide, and total thiol molecules are balanced. The thiol molecule, containing sulfhydryl functional group (SH), forms disulfide bonds (-S-S) with ROS under OS (29). The resulting disulfide bonds can be reduced again to the thiol molecule with the help of the anti-oxidant system. Disulfide bonds have an important function in the stabilization of protein structure, signal transduction, and thiol and redox enzyme protection (30). Therefore, a highly dynamic thiol disulfide balance plays an important role in cell defenses against OS and the regulation of total plasma enzymatic activity (29).

In this study, we analyzed the native thiol/disulfide homeostasis in patients with PEXS and PEXG using a novel, easy, and practical automated method developed by Erel and Neselioglu (20). We found a significant difference in the plasma disulfide level and the native thiol/disulfide ratio between the PEXG group and the control group. The results of this study corroborate previous observations of Tetikoglu et al. (31). In the current study, we included patients with PEXS (n=31) and PEXG (n=43), while Tetikoglu et al. divided PEXS (n=35) into 2 subgroups of PEXS without glaucoma (n=19) and with glaucoma (n=16). Similar to the present study, they found no significant difference in the plasma total or native thiol or disulfide levels, or the native thiol/disulfide ratio between the PEXS and PEXG subgroups (31). Other studies have also evaluated systemic redox status in glaucoma. Demirdogen et al. (32) used the Ellman method for serum thiol analysis and found similar thiol levels in PEXS and PEXG patients. Tanito et al. (33) reported lower plasma total thiol levels in PEXS and PEXG than controls using a modified Ellman method.

In contrast to literature findings, we observed a similar plasma native thiol level between groups, in spite of the disulfide and native thiol/disulfide levels. Significantly higher levels of disulfides in PEXG indicate that the thiol disulfide balance can be shifted to the disulfide bond formation. Neseloglu et al. (34) also reported significantly decreased native total thiol and disulfide levels in ulcerative colitis groups, contrary to much of the literature. They linked these results to inflammation causing a decrease in thiol-containing protein synthesis. The authors suggested that thiol-containing molecules in plasma may be reduced in inflammation, leading to a weakened thiol disulfide system.

There are several limitations to the present study. PEXS and PEXG are complex neurodegenerative disorders, however, our results were based on the difference in several plasma oxidative markers. Another limitation is the small number of patients.

In conclusion, we observed a significant difference in the plasma disulfide level and the native thiol/disulfide ratio between the PEXG patients and the controls. Collectively, this

novel assay demonstrated that patients with PEXG had a lower systemic antioxidant capacity. Further understanding of the thiol-disulfide circuits is still needed to develop new treatment options and the understanding of the complex etiopathogenesis of age-related ocular diseases.

Disclosures

Ethics Committee Approval: This study was conducted in accordance with Helsinki Declaration and approved by the local ethics committee (#26379996-44; 02/22/2017).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Involved in design and conduct of the study (TT, DY, NY); preparation and review of the study (TT, DY, NY); data collection (TT, DY, MAY, AS, OE); and statistical analysis (DY, NY).

References

1. Schlötzer-Schrehardt UM, Koca MR, Naumann GO, Volkholz H. Pseudoexfoliation syndrome. Ocular manifestation of a systemic disorder? Arch Ophthalmol 1992;110:1752–6.
2. Naumann GO, Schlötzer-Schrehardt U, Küchle M. Pseudoexfoliation syndrome for the comprehensive ophthalmologist. Intraocular and systemic manifestations. Ophthalmology 1998;105:951–68.
3. Gartaganis SP, Georgakopoulos CD, Patsoukis NE, Gotsis SS, Gartaganis VS, Georgiou CD. Glutathione and lipid peroxide changes in pseudoexfoliation syndrome. Curr Eye Res 2005;30:647–51.
4. Erdurmuş M, Yağcı R, Atış Ö, Karadağ R, Akbaş A, Hepşen IF. Antioxidant status and oxidative stress in primary open angle glaucoma and pseudoexfoliative glaucoma. Curr Eye Res 2011;36:713–8.
5. Yağci R, Gürel A, Ersöz I, Keskin UC, Hepşen IF, Duman S, et al. Oxidative stress and protein oxidation in pseudoexfoliation syndrome. Curr Eye Res 2006;31:1029–32.
6. Yilmaz A, Adigüzel U, Tamer L, Yildirim O, Oz O, Vatansever H, et al. Serum oxidant/antioxidant balance in exfoliation syndrome. Clin Exp Ophthalmol 2005;33:63–6.
7. Schlötzer-Schrehardt U, Naumann GO. Ocular and systemic pseudoexfoliation syndrome. Am J Ophthalmol 2006;141:921–37.
8. Netland PA, Ye H, Streeten BW, Hernandez MR. Elastosis of the lamina cribrosa in pseudoexfoliation syndrome with glaucoma. Ophthalmology 1995;102:878–86.
9. Alvarado JA, Murphy CG. Outflow obstruction in pigmentary and primary open angle glaucome. Arch Ophthalmol 1992;110:1769–78.
10. Lütjen-Drecoll E, Shimizu T, Rohrbach M, Rohen JW. Quantitative analysis of 'plaque material' in the inner- and outer wall of Schlemm's canal in normal- and glaucomatous eyes. Exp Eye Res 1986;42:443–55.
11. Izzotti A, Bagnis A, Sacca SC. The role of oxidative stress in glaucoma. Mutat Res 2000;612:104–5.
12. Tezel G. Oxidative stress in glaucomatous neurodegeneration: mechanisms and consequences. Prog Retin Eye Res 2006;25:490–513.
13. Koliakos GG, Befani CD, Mikropoulos D, Ziakas NG, Konstas AG. Prooxidant-antioxidant balance, peroxide and catalase activity in the aqueous humour and serum of patients with exfoliation syndrome or exfoliative glaucoma. Graefes Arch Clin Exp Ophthalmol 2008;246:1477–83.
14. Schlötzer-Schrehardt U, Lommatzsch J, Küchle M, Konstas AG, Naumann GO. Matrix metalloproteinases and their inhibitors in aqueous humor of patients with pseudoexfoliation syndrome/glaucoma and primary open-angle glaucoma. Invest Ophthalmol Vis Sci 2003;44:1117–25.
15. Biswas S, Chida AS, Rahman I. Redox modifications of protein-thiols: emerging roles in cell signaling. Biochem Pharmacol 2006;71:551–64.
16. Go YM, Jones DP. Cysteine/cystine redox signaling in cardiovascular disease. Free Radic Biol Med 2011;50:495–509.
17. Smeyne M Smeyne RJ. Glutathione metabolism and Parkinson's disease. Free Radic Biol Med 2013;62:13–25.
18. McBean GJ, Aslan M, Griffiths HR, Torrão RC. Thiol redox homeostasis in neurodegenerative disease. Redox Biol 2015;5:186–94.
19. Reniewska B, Mulak M, Misiuk-Hojło M, Kostuś E. Coexistence of Alzheimer's disease with pseudoexfoliation syndrome PEX]. Klin Oczna 2004;106:107–9.
20. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014;47:326–32.
21. Zoric L, Mitric D, Milenkovic S, Jovanovic P, Trajkovic G, et al. Pseudoexfolition syndrome and its antioxidative protection deficiency as risk factors for age-related cataract. Eur J Ophtalmol 2006;16:268–73.
22. Koliakos GG, Konstas AGP, Schlötzer Schrehardt U, Hollo G, Katsimbris IE, Georgiadis N, et al. 8-Isoprostaglandin F 2A and ascorbic acid concentration in aqueous humour of patients with exfoliation syndrome. Br J Ophthalmol 2003;87:353–6.
23. Yokoyama Y, Maruyama K, Yamamoto K, Omodaka K, Yasuda M, Himor N et al. The role of calpain in an in vivo model of oxidative stres-induced retinal ganglion cell damage. Biochem Biophys Res Commun 2014;451:510–5.
24. Munemasa Y, Ahn JH, Kwong JM, Caprioli J, Piri N. Redox proteins thioredoxin 1 and thioredoxin 2 support retinal ganglion cell survival in experimental glaucoma. Gene Ther 2009;16:17–25.
25. Izzotti A, Saccà SC, Cartiglia C, De Flora S. Oxidative deoxyribonucleic acid damage in the eyes of glaucoma patients. Am J Med 2003;114:638–46.
26. Beyazyıldız E, Cankaya AB, Beyazyıldız O, Ergan E, Celik HT, Yılmazbaş P, et al. Disturbed oxidant/antioxidant balance in

aqueous humour of patients with exfoliation syndrome. Jpn J Ophthalmol 2014;58:353–8.
27. Engin KN, Yemisci B, Yigit U, Agachan A, Coskun C. Variability of serum oxidative stress biomarkers relative to biochemical data and clinical parameters of glaucoma patients. Mol Vis 2010;16:1260–71.
28. Sorkhabi R, Ghorbanihaghjo A, Javadzadeh A, Rashtchizadeh N, Moharrery M. Oxidative DNA damage and total antioxidant status in glaucoma patients. Mol Vis 2011;17:41–6.
29. Turell L, Radi R, Alvarez B. The thiol pool in human plasma; central contribution of albumin to redox processes. Free Radic Biol Med 2013;65:244–53.
30. Messens J, Collet JF. Thiol-disulfide exchange in signaling: disulfide bonds as a switch. Antioxid Redox Signal 2013;18:1594–6.
31. Tetikoğlu M, Aktas S, Sağdik HM, Özcura F, Uçar F, Koçak H, et al. Thiol Disulfide Homeostasis in Pseudoexfoliation Syndrome. Curr Eye Res 2017;42:876–9.
32. Demirdögen BC, Ceylan OM, Işikoğlu S, Mumcuoğlu T, Erel O. Evaluation of oxidative stress and paraoxonase phenotypes in pseudoexfoliation syndrome and pseudoexfoliation glaucoma. Clin Lab 2014;60:79–86.
33. Tanito M, Kaidzu S, Takai Y, Ohira A. Status of systemic oxidative stresses in patients with primary open-angle glaucoma and pseudoexfoliation syndrome. PLoS One 2012;7:e49680.
34. Neselioglu S, Keske PB, Senat AA, Yurekli OT, Erdogan S, Alisik M, et al. The relationship between severity of ulcerative colitis and thiol-disulphide homeostasis. Bratisl Lek Listy 2018;119:498–502.