

# Oxidative Stress in Pseudoexfoliation Syndrome

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## Abstract

**Purpose:** The objective of this study was to investigate the total antioxidant status (TAS) and DNA damage markers of serum and aqueous humor in patients with pseudoexfoliation (PEX) syndrome.

**Methods:** Twenty-seven cataract patients with PEX and twenty-seven without PEX syndrome were included in the study and groups were matched for age and gender. Patients had no elevated intraocular pressure (IOP) or glaucoma. Aqueous humor and serum samples were taken at the time of surgery and TAS and 8-hydroxy-2'-deoxyguanosine (8OHdG) levels of all samples were determined by spectrophotometric and enzyme-linked immunosorbent assay (ELISA) methods, respectively.

**Results:** Mean 8OHdG concentration in the PEX aqueous ( $3.34 \pm 1.93$  ng/ml) and serum ( $17.63 \pm 6.78$  ng/ml) samples were significantly higher than that measured in the control aqueous ( $1.98 \pm 0.70$  ng/ml) and serum ( $13.63 \pm 3.54$  ng/ml) samples, respectively ( $P=0.002$ ,  $P=0.010$ ). TAS of serum ( $0.60 \pm 0.15$  vs.  $0.70 \pm 0.14$  mmol/lit,  $P=0.022$ ) and aqueous humor ( $0.33 \pm 0.13$  vs.  $0.34 \pm 0.15$  mmol/lit,  $P=0.003$ ) in PEX patients were lower than that of the control group.

**Conclusion:** The increased 8OHdG levels in the aqueous humor and serum of PEX patients suggests that oxidative DNA damage may play a role in the pathophysiology of PEX.

**Keywords:** 8-Hydroxy-2'-Deoxyguanosine, Aqueous Humor, Total Antioxidant Status, Pseudoexfoliation Syndrome

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## Introduction

Pseudoexfoliation (PEX) syndrome is a clinically significant systemic disorder of extracellular matrix that is frequently associated with severe chronic secondary open angle glaucoma and cataract. Its ocular manifestations affect all of the structures of the anterior segment as well as conjunctiva and orbital tissues. PEX syndrome may affect up to 30% of people older than 60 years of

age in a worldwide distribution. Despite its wide prevalence and clinical importance, the pathogenesis of PEX and the exact composition of the exfoliation material remain unknown.<sup>1-3</sup>

The oxidative stress mechanisms in ocular tissues have been hypothesized to play a major role in the pathogenesis of PEX.<sup>4-6</sup>

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Oxidation of DNA is known to generate adducts of base and sugar groups, single strand and double strand breaks in the backbone and cross links to the other molecules. Among more than 20 known products resulting from DNA oxidation; 8-hydroxy-2'-deoxyguanosine (8OHdG) can be easily quantified and is commonly used as a method to assessment oxidative damage to DNA.<sup>7-9</sup>

Our study was designed to compare 8OHdG levels and total antioxidant status (TAS) in the aqueous humor and serum of patients undergoing cataract surgery with and without PEX and investigate the role of DNA oxidation in the development of PEX.

## Methods

This prospective cross sectional study was performed in Nikookari Eye Hospital in Tabriz, Iran. Ethical approval was obtained from the ethical committee of Tabriz University of Medical Sciences and written informed consent was received from all patients according to the tenets of the Declaration of Helsinki.

Consecutive patients scheduled for phacoemulsification surgery with the diagnosis of PEX and cataract and those with senile cataract only were enrolled in the study.

All patients underwent a complete ophthalmic examination which include slit-lamp examination with pupil dilation, applanation tonometry, fundus examination, and gonioscopy.

PEX syndrome was diagnosed if clinical examination revealed deposition of PEX material on the anterior lens capsule or at the pupillary border, the presence of transillumination defects near the pupil accompanied by normal optic nerve head finding, and intraocular pressure (IOP) less than 21 mmHg.

Patients with history of glaucoma, high IOP, antiglaucomatous drop use, or any other systemic or ocular condition and drug taking that might influence oxidative stress were excluded from the study.

All participants had normal renal and liver function as assessed by plasma urea, creatinine, alanine aminotransferase and aspartate aminotransferase. Serum levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C)

and low-density lipoprotein cholesterol (LDL-C) were determined using commercial reagents with an automated chemical analyzer (Abbott laboratories, Chicago, IL).

Aqueous humor samples were carefully collected at the beginning of cataract surgery through a paracentesis using a 27-gauge needle attached to a tuberculin microsyringe. Serum specimens were also collected from patients just before surgery for measurement of TAS and 8OHdG levels. Aqueous humor and blood samples were immediately frozen and stored at -70°C until analyzed.

The concentration of 8OHdG in serum and aqueous humor was determined using an enzyme-linked immunosorbent assay; (ELISA, Serum 8OHdG check; Japan Institute for control of aging, Shizuko, Japan, Lot 050 KOG 200/SE). The TAS of samples was measured by spectrophotometric assay with Randox Total Antioxidant Status kit (Lot:115813). In this method, incubation of 2,2'-azino-di (3-ethylbenzthiazoline sulfonate) (ABTS) with a peroxidase (metmyoglobin) results in production of the radical cation ABTS<sup>+</sup>. This product is blue-green in color and can be detected at 600 nm. Antioxidants in the added sample inhibit this color production in proportion to their concentration.

All statistical analyses were carried out using SPSS for windows, version 13 (SPSS Inc, Chicago, IL, USA). All data were presented in mean±SD. The differences in measured parameters between two groups were analyzed by independent samples t-test. The differences were considered significant when the probability was less than 0.05.

## Results

A total of 54 aqueous and 54 serum samples from 27 PEX and 27 non PEX, control patients were analyzed. The two study groups were matched for age and gender. The 8OHdG levels and TAS in the serum and aqueous humor of PEX patients and controls are shown in table 1.

The mean concentration of 8OHdG in the aqueous humor and serum of PEX group was found to be significantly higher than that of controls.

TAS of serum and aqueous humor in PEX patients were lower than control group. There were no significant correlation between 8OHdG or TAS levels in serum and aqueous

humor of patients with PEX; however there was a statistically significant correlation

between serum 8OHdG and serum TAS levels in these patients (Table 2).

**Table 1.** Demographics and levels of 8-hydroxy-2'-deoxyguanosine and total antioxidant status in serum and aqueous humor of patients in both study group

Variable	PEX group	Control group	P
<b>Age (years)</b>	63.89±7.37	65.22±9.79	0.095
<b>Sex</b>			
Male %	53.57	51.8	0.800
Female %	46.43	48.2	0.790
<b>Markers</b>			
Serum TAS (mmol/L)	0.60±0.15	0.70±0.14	0.022
Aqueous TAS (mmol/L)	0.33±0.13	0.34±0.15	0.003
Serum 8OHdG (ng/ml)	17.63±6.78	13.63±3.54	0.010
Aqueous 8OHdG (ng/ml)	3.34±1.93	1.98±0.70	0.002
<b>Lipid profile</b>			
TG (mg/dl)	165.11±71.21	173.44±10.34	0.41
TC (mg/dl)	210.22 ±37.95	208.89±53.17	0.81
HDL-C (mg/dl)	49.59±6.69	49.04±10.37	0.80
LDL-C (mg/dl)	130.44 ±36.80	126.74±45.10	0.20

8OHdG: 8-hydroxy-2'-deoxyguanosine

TAS: Total antioxidant status

TG: Triglyceride

TC: Total cholesterol

HDL-C: High density lipoprptein cholesterol

LDL-C: Low density lipoprptein cholesterol

**Table 2.** Correlations between aqueous humor and serum 8-hydroxy-2'-deoxyguanosine and total antioxidant status levels in study groups

Correlations	P	R <sub>s</sub>
<b>Pseudoexfoliation</b>		
Aqueous humor 8OHdG vs. serum 8OHdG	0.22	0.06
Aqueous humor TAS vs. serum TAS	0.10	0.11
Aqueous humor 8OHdG vs. aqueous humor TAS	0.14	0.08
Serum 8OHdG vs. serum TAS	0.00	0.57
<b>Controls</b>		
Aqueous humor 8OHdG vs. serum 8OHdG	0.07	0.13
Aqueous humor TAS vs. serum TAS	0.00	0.59
Aqueous humor 8OHdG vs. aqueous humor TAS	0.00	0.39
Serum 8OHdG vs. serum TAS	0.12	0.09

Linear regression test

8OHdG: 8-hydroxy-2'-deoxyguanosine

TAS: Total antioxidant status

## Discussion

The hallmark of PEX is the pathologic production and progressive accumulation of abnormal fibrillar extracellular material in anterior segment tissues. Despite its wide prevalence and clinical importance, the pathogenesis of PEX and the exact composition of this material remains unknown.<sup>3</sup> PEX represents a complex, multifactorial and late onset disease involving both genetic and nongenetic factors in its etiopathogenesis. Secretion of PEX material by ocular cells is closely related to aqueous humor circulation and thus, is influenced by substances contained in the aqueous humor. Hence investigation of qualitative and quantitative variations of aqueous humor composition in patients with PEX may identify important pathogenetic factors involved in this disorder.<sup>10</sup>

Sever oxidative stress progressively leads to cell dysfunction and ultimately cell death. Oxidative stress is defined as an imbalance between prooxidants and/or free radicals on one hand and antioxidantizing systems on the other.<sup>11</sup> In the ocular tissues, the role of tissue damage induced by oxygen radicals and lipid peroxidation has been demonstrated in many experimental studies.<sup>12-17</sup> Cataract is a multifactorial disease process that has also been suggested to be associated with oxidative stress.<sup>18-21</sup>

There is increasing evidence that cellular stress conditions, such as oxidative stress and ischemia/hypoxia, constitute major mechanisms involved in the pathobiology of the PEX syndrome.<sup>2</sup> There are controversial findings about serum and aqueous humor antioxidant activity and their correlations with oxidative stress markers in PEX patients.

Koliakos et al suggested that free radical-induced damage plays a role in the pathophysiology of PEX. The aqueous level of 8-isoprostaglandin F<sub>2α</sub> (8-IPGF<sub>2α</sub>) a marker of oxidative stress was found to be five times higher in PEX than control samples and its increased concentration correlated well with the reduction in ascorbic acid concentration in the same sample.<sup>6</sup> In contrast Ucakhan et al detected increase in superoxide dismutase (SOD) activity in the lens capsule of patients with PEX and cataract and suggested that oxidative mechanisms play a role in the pathogenesis of cataract in PEX. This means

that SOD activity may be increased as a compensatory mechanism to eliminate this oxidative stress.<sup>11</sup>

Yilmaz et al indicated that antioxidant, serum vitamin C concentrations were much lower and malonildialdehyde concentrations were much higher in PEX subjects reflecting free radical damage to lipid peroxides.<sup>22</sup> Yagci and coworkers showed increased protein oxidation in both aqueous humor and serum of patients with PEX.<sup>23,24</sup> Gartaganis and coworkers suggested a role for oxidative stress in the pathogenesis and progression of PEX syndrome.<sup>25</sup>

Thus we investigated the aqueous humor and serum levels of 8OHdG and TAS in patients with PEX and compared these levels with controls. As mentioned above Ucakhan et al in their study suggested that increased antioxidative activity in PEX patients is a compensatory response to increased oxidative stress due to cataract formation. So we compared PEX patients with senile cataract patients for balancing confusing effect of cataract formation in evaluating oxidative DNA damage marker in PEX patients. In this study we found statistically significant higher aqueous humor 8OHdG levels in patients with PEX compared with controls. So in agreement with previous studies we also suggest that oxidative stress is one of major mechanisms involved in the pathobiology of PEX syndrome. Although aqueous and serum levels of 8OHdG were higher in PEX patients, there were no significant correlation between serum and aqueous levels of 8OHdG among patients with PEX. In addition we showed that both aqueous humor and serum TAS were decreased in patients with PEX. In our study the lack of correlation between serum and aqueous levels of 8OHdG and TAS in PEX patients may indicate a localized oxidative burden on DNA in addition to what occurs during senile cataract formation due to generalized aging process; may contribute to occurrence and progression of PEX syndrome.

To the best of our knowledge this is the first study to compare serum and aqueous levels of 8OHdG and TAS and their correlations in patients with PEX. Although we found a significant correlation among serum levels of

8OHdG and TAS in PEX patients, but there was no statistically significant correlation between aqueous levels of these markers in them. The lack of significant correlation among 8OHdG and TAS levels in aqueous humor of our PEX patients may be due to sharing other age related systemic or ocular oxidative pathologies in these patients.

Although we matched two study groups according to age and senile cataract and excluded patients with known systemic or ocular disease; unknown oxidative process may responsible for lack of correlation between these markers and this is one of limitations of our study.

The level of 8OHdG in aqueous humor of PEX patients is 2.9 times higher than control group whereas this ratio in serum levels decreases to 1.37. This may be due to a faulty antioxidative defense system and increased oxidative stress in the anterior chamber of PEX eyes more than a systemic insult and it indicates that anterior segment structures are exposed against free radicals, suggesting that localized oxidative stress may contribute to the formation and development of PEX.

Nevertheless the true pathogenetic origin of this finding is difficult to evaluate, given the fact that 8OHdG increase and TAS decrease may be an outcome of a potential sequence of

events during process. The potential involvement of reactive oxygen fragments in the pathogenesis of PEX suggests that free radical scavengers and antioxidants might have therapeutic uses. Antioxidant therapy may offer potential therapeutic venues for controlling PEX related ocular morbidity.

### **Conclusion**

In conclusion we have documented significantly increased aqueous humor and serum 8OHdG concentrations in patients with PEX compared with that of control patients without PEX. The finding suggests a pathophysiologic role for DNA oxidation and oxidative stress in the development of PEX; however more controlled studies with larger sample size are needed to advance our understanding more on mechanism of PEX syndrome and help to find more effective treatments against this syndrome.

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