Ceruloplasmin and Catalase Activites in Pseudoexfoliation Syndrome and Pseudoexfoliation Glaucoma

Psödoeksfoliasyon Sendromu ve Psödoeksfoliasyon Glokomda Seruloplazmin ve Katalaz Aktiviteleri

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ABSTRACT Objective: Pseudoexfoliation syndrome (PEX) is an age-related systemic disorder primarily with ocular manifestations. PEX is considered as the most common identifiable cause of glaucoma (pseudoexfoliation glaucoma, PEG) and loss of vision. Oxidative stress was suggested to be involved in the pathogenesis of PEX and PEG. Catalase is an antioxidant enzyme which catalyzes decomposition of hydrogen peroxide to water and oxygen. Ceruloplasmin was reported to have antioxidant functions, as well as pro-oxidant activity. This study has aimed to determine whether ceruloplasmin and catalase activities have a role in the development of PEX and/or PEG. **Material and Methods:** Ceruloplasmin and catalase activities were determined in serum samples of 32 cases of PEX, 30 cases of PEG and 32 control subjects using spectrophotometric methods. **Results:** Ceruloplasmin activities of PEX patients (32.94±16.36 U/L) and PEG patients (29.98±13.15 U/L) were similar and slightly lower than those of controls (35.22±21.32 U/L). However, the differences were not statistically significant. Catalase activites were found to be 61.96±33.95 U/L and 60.44±50.84 U/L in PEX and PEG patients, respectively. Controls had slightly, but insignificantly, higher catalase activities (64.98±58.14 U/L). **Conclusion:** Neither catalase nor ceruloplasmin activities are significantly related with PEX or PEG in the studied Turkish population. As the mean age of populations keeps increasing, PEX may become more common in the future. Therefore, further studies are needed to decipher the pathophysiology of this important disorder.

Key Words: Exfoliation syndrome; ceruloplasmin; catalase

ÖZET Amaç: Psödoeksfoliasyon sendromu (PES) öncelikle oküler bulguları olan, yaşa bağlı sistemik bir hastalıktır. PES, glokomun (psödoeksfoliasyon glokom, PEG) ve görme kaybının en yaygın tanımlanabilir nedeni olarak kabul edilir. Oksidatif stresin PES ve PEG patogenezinde rolü olduğu öne sürülmüştür. Katalaz, hidrojen peroksitin su ve oksijene ayrışma reaksiyonunu katalize eden antioksidan bir enzimdir. Seruloplazminin antioksidan fonksiyonları yanında, pro-oksidan aktiviteye de sahip olduğu bildirilmiştir. Bu çalışmada seruloplazmin ve katalaz aktivitelerinin PEX ve/veya PEG gelişiminde rolü olup olmadığını belirlemek amaçlanmıştır. **Gereç ve Yöntemler:** Seruloplazmin ve katalaz aktiviteleri spektrofotometrik yöntemlerle 32 PES'li olgu, 30 PEG'li olgu ve 32 kontrol bireyin serum örneklerinde belirlenmiştir. **Bulgular:** PES hastalarının (32,94±16,36 U/L) ve PEG hastalarının (29,98±13,15 U/L) seruloplazmin aktiviteleri birbirine çok benzer seviyede ve kontrollerinkinden (35,22±21,32 U/L) biraz düşüktü. Ancak aradaki fark istatistiki olarak anlamlı değildi. Katalaz aktiviteleri PES ve PEG hastalarında sırasıyla 61,96±33,95 U/L ve 60,44±50,84 U/L olarak bulundu. Kontroller ise, biraz daha yüksek katalaz aktivitesine sahipti (64,98±58,14 U/L). **Sonuç:** Çalışılan Türk popülasyonunda katalaz ve seruloplazmin aktiviteleri PES veya PEG ile istatistiki olarak anlamlı biçimde ilişkili değildir. Popülasyonların ortalama yaşı artmaya devam ettiğinden, PEX gelecekte daha yaygın hale gelebilir. Bu nedenle, bu önemli hastalığın patofizyolojisini deşifre etmek için daha ileri çalışmalar gerekmektedir.

Anahtar Kelimeler: Dökülme sendromu; seruloplazmin; katalaz

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Pseudoexfoliation syndrome (PEX) is an age-related systemic disorder which is characterized by accumulation of granular amyloid- like fibrillary extracellular material in anterior structures of the eye. PEX was first described in 1917, and the term pseudoexfoliation was suggested to distinguish it from a similar ailment which sometimes affected glassblowers, called true exfoliation syndrome. The buildup of abnormal fibrillary material can block normal drainage of the aqueous humor and can cause, in turn, a buildup of pressure leading to glaucoma and loss of vision. Although PEX can lead to both open-angle glaucoma and angle-closure glaucoma (pseudoexfoliation glaucoma; PEG), there are persons with PEX without glaucoma.[1] Pseudoexfoliation is included as one of the prognostic factors for the progression of open-angle glaucoma.[2] PEX prevalence changes from 3.4% in a Japanese population to 4.7% in England, 6.3% in Norway, 4% in Germany, 1.1% in Greece, and 5.5% in France.[1] In Turkey, in the Eastern Mediterranean region, frequency of PEX was found to be 7.2% among persons older than 49 years.[3]

Although the exact etiology of this condition remains unknown, involvement of increased oxidative damage and/or impaired cellular protection was suggested in PEX pathogenesis.[4-6] Hydrogen peroxide (H_2O_2) is one of the main reactive oxygen species leading to oxidative stress.[7,8] This molecule is harmful, because in the presence of transition metals such as ferrous iron, it can be converted into a highly reactive oxidant, the hydroxyl radical, which subsequently damages membrane lipids, proteins and DNA.[9] Accumulation of hydrogen peroxide is prevented by catalase, a ubiquitous enzyme which catalyzes the decomposition of hydrogen peroxide to water and oxygen.[10] The reports on the involvement of catalase in PEX are limited and contradictory.[4,5]

Ceruloplasmin is a major protein that circulates in the plasma and contains 95% of serum copper.[11] It is synthesized by hepatocytes and is secreted in plasma with six copper atoms strongly coupled to the molecule. The gene coding for ceruloplasmin is also expressed in extrahepatic tissues such as brain, lungs, spleen, testis and the central nervous system.[12] Ceruloplasmin has several functions including copper transport, iron metabolism, antioxidant defense, and involvement in angiogenesis and coagulation. This protein might have a role in development of exfoliation syndrome; it was detected more frequently in aqueous humor of exfoliation syndrome patients compared to the normal controls.[13] On the contrary, a significant inactivation of ceruloplasmin occurs during oxidative stress, which was suggested to have a role in the development of PEX.[14] However, no information is available in the literature whether the ceruloplasmin concentration is changed in serum in PEX and/or PEG.

Therefore, in the present study, in order to clear our understanding of the complex etiology of PEX/PEG, we aimed to determine catalase and ceruloplasmin activities in these patients and control subjects in Turkish population.

MATERIAL AND METHODS

PATIENT SELECTION

This case-control study included 32 patients with PEX, 30 patients with PEG and 32 control subjects who were seen at the Department of Ophthalmology, Gülhane Military Medical Academy, Turkey. Institutional review board and ethics committee approvals were obtained for the study, and written informed consents were obtained from all subjects. The tenets of the Declaration of Helsinki were followed throughout the study.

The diagnosis of PEX was made on slit-lamp examination following mydriasis, and included the presence of typical pseudoexfoliation material on the anterior lens capsule and/or the pupillary border. PEG patients being followed up by our Glaucoma Department were also recruited into the study. PEG was diagnosed when anterior segment findings of PSX accompanied an IOP >21 mmHg without treatment, typical optic nerve head changes and visual field defects. Consecutive age-matched patients with an IOP ≤21 mmHg, a normal optic disc appearance and no visual field defects were included as controls. Healthy subjects

and all patients received a comprehensive ophthalmological examination including best-corrected Snellen visual acuity testing, slit-lamp examination, Goldmann applanation tonometry, gonioscopic evaluation, dilated fundus examination using a 90-dioptre lens and visual field evaluation using the 30-2 SITA-Standard algorithm (Humphrey Instruments Inc., San Leandro, California, USA).

Patients with other ophthalmic diseases (uveititis, age-related macular degeneration, etc.) and any central nervous system disease that might have interfered with visual field test were excluded from this study. A detailed medical history was also obtained. All subjects were questioned about smoking and medications (non-steroidal anti-inflammatory drug, supplemental vitamin C and/or vitamin E, etc.) and excluded from the study if answered yes. Blood samples were obtained from all the subjects by venipuncture. The blood samples were centrifuged at 2000xg for 15 min, and the sera were separated and stored at -24^{0}C until use.

Demographic features of patients and controls are given in Table 1. There was no statistically significant difference among groups in terms of age and the frequency of males and females.

BIOCHEMICAL ANALYSES

Biochemical analyses were performed without prior knowledge of the diagnosis, or the clinical information.

Measurement of Ceruloplasmin Activity

Enzymatic activity of ceruloplasmin was measured according to Erel's method using a Jenway 6715 UV-VIS spectrophotometer (England).[15] In this method, ferrous ion is oxidized to ferric ion via ceruloplasmin ferroxidase activity. The amount of oxidized iron is directly proportional to the enzymatic activity. The enzymatic activity of ceruloplasmin is inhibited by sodium azide.

Measurement of Catalase Activity

Catalase activity was measured using hydrogen peroxide (H_2O_2) as substrate.[16] The disappearance of H_2O_2 was followed at 240 nm, and enzyme activity was expressed in U/L. Measurements were done on an autoanalyzer (Advia 1800, Siemens, Japan).

STATISTICAL ANALYSES

Normality of the sample distribution of each continuous variable was tested with the Kolmogorov-Smirnov test. Normally distributed continuous variables were expressed as mean ± SD and their differences were evaluated by the independent samples t-test. Distribution of age was skewed, thus it was expressed as median and interquartile range and compared using the Mann-Whitney U test. Categorical variables (gender) were expressed as proportions and compared using the Chi-square test. Statistical Package for Social Sciences version 16.0 (SPSS, Chicago, IL, USA) was used for these statistical analyses. A p value less than 0.05 was evaluated as statistically significant.

RESULTS

Table 2 summarizes the mean ceruloplasmin and catalase enzyme activities of PEX, PEG and control groups. We observed that ceruloplasmin was slightly lower in PEX (32.94 ± 16.36 U/L) as compared to controls (35.22 ± 21.32 U/L), however the difference was not statistically significant

TABLE 1: Demographic features of patients with pseudoexfoliation syndrome (PEX) and pseudoexfoliation glaucoma (PEG) and control subjects.

Parameter	PEX (n=32)	PEG (n=30)	Control (n=32)
Age	70 (9)[a]	72 (13.3)[b]	70.5 (16)
Gender (male)	16 (50%)[c]	16 (53.3%)[d]	17 (53.1%)

Data represented as median (interquartile range) for age, frequencies for gender. Comparisons were with respect to control and were carried out by Mann-Whitney U test for age and chi-square test for gender. [a]p=0.454, [b]p=0.475, [c]p=0.802, [d]p=1.

TABLE 2: Ceruloplasmin and catalase activities of pseudoexfoliation syndrome (PEX) patients, pseudoexfoliation glaucoma (PEG) patients and controls.

	PEX (n=32)	PEG (n=30)	Control (n=32)
Ceruloplasmin (U/L)	32.94±16.36[a]	29.98±13.15[b]	35.22±21.32
Catalase (U/L)	61.96±33.95[c]	60.44±50.84[d]	64.98±58.14

Data are mean ± SD. Comparisons were with respect to control and were carried out by independent samples t-test [a]p=0.636, [b]p=0.252 [c]p=0.806, [d]p=0.745.

(p=0.636). Likewise, ceruloplasmin activity was insignificantly lower in PEG (29.98 ± 13.15 U/L) as compared to controls.

Catalase activities of PEX and PEG patients were very similar (61.96±33.95 U/L and 60.44±50.84 U/L, respectively). Although catalase activities of both patient groups were slightly lower compared to controls (64.98±58.14 U/L), the difference was not significant.

DISCUSSION

Pseudoexfoliation syndrome (PEX) is considered to be the most common identifiable cause of glaucoma and loss of vision. PEX is rarely seen before age 50, but its incidence increases steadily with age.[1] As the body ages, the balance between oxidants and antioxidant defense is disrupted towards the oxidants. There is evidence suggesting that oxidative stress might have a role in the pathogenesis of PEX. Koliakos et al. reported that ascorbic acid concentration was reduced in the aqueous humor of patients with PEX.[4] Another study by Yılmaz et al. confirmed the results of Koliakos et al., and found significantly lower serum vitamin C concentrations and significantly higher malondialdehyde levels in PEX compared to the control group.[4,5] A significant shift of the prooxidant balance in favor of oxidants was detected in the PEX and PEG groups compared with controls.[5]

Catalase serves as the first line of defense against hydroperoxides. We found no significant difference in serum catalase activities among PEX patients, PEG patients and controls. Previous studies reported inconsistent results. Koliakos et al. found that catalase activity in the aqueous humor from PEX (10.1±4.5 U/ml) and PEG (12.2±6 U/ml) patients was significantly lower than that measured in the normal aqueous humor (14.6±1.9 U/ml).[17] Similarly, a significantly lower serum catalase activity was found in PEX (103±21.4 U/ml) and PEG (116±38 U/ml) patients compared to the controls (189.6±84.3 U/ml).[17] The difference between the study of Koliakos et al. and ours might be due to the differences in life style, dietary habits and genetic background of the subjects in the two studies.[17] Hence, in another Turkish study, Yılmaz et al. reported nonsignificantly lower catalase activities in PEX patients compared to controls, similar to our study.[5] In addition to these studies on PEX, catalase activity was also measured in other disorders of the eye. For example, significantly lower erythrocyte catalase activity was reported in age-related macular degeneration and cataract patients.[18]

Ceruloplasmin functions as a copper transporter that is able to couple and transport 90–95% of serum copper.[11] It is an essential ferroxidase, an enzyme which catalyzes the oxidation of ferrous iron to ferric iron. Even though it was reported to have antioxidant functions, ceruloplasmin was also found to exhibit pro-oxidant activity and catalyse the oxidation of low-density lipoprotein at acidic pH.[19,20] That's probably why high levels of ceruloplasmin were found to associate with atherosclerosis and myocardial infarction.[21] Ceruloplasmin was found to be related to neurodegenerative process in humans, as well.[12]

Complete absence of ceruloplasmin, called aceruloplasminemia, has been observed in some cases. This condition is characterized by accumulation of iron in the pancreas, liver and brain, causing diabetes and progressive neurodegeneration.[22] The balance between the compartmentalization and storage of iron and the mobilization and transport of iron is disrupted. Ceruloplasmin knockout mice exhibited morphologic and molecular features of age-related macular degeneration.[23] Furthermore, ceruloplasmin was detected in seven of ten patients with exfoliation syndrome, but in only one of the eight controls.[13] In our study, we did not find a significant difference between patients and controls in terms of ceruloplasmin activity. All of the subjects expressed some degree of ceruloplasmin activity and there was no individual with aceruloplasminemia.

It is crucial to note that a significant inactivation of ceruloplasmin occurs during oxidative stress, this phenomenon being associated with the uncoupling of copper atoms from the ceruloplasmin molecule.[14] This might explain the slightly lower ceruloplasmin activities in PEX/PEG group compared to controls observed in this study. We could not collect aqueous humor samples in this

study; and this might be considered as a limitation of this work.

CONCLUSION

To the best of our knowledge, ceruloplasmin activities were not measured before in serum of PEX and PEG patients. However, this parameter came out to be insignificant in PEX and PEG. Likewise, catalase activity was not significantly related with these two pathologies. As populations get older and older, PEX may become more prevalent in the future. Therefore, further studies, preferably prospective ones with higher number of patients, are required to find the molecular clues of the pathophysiology of this important disorder.

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