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Free amino acid concentration in aqueous humour of patients with nuclear or cortical cataract

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Aim: Since the free amino acids (a.a.) and derivated a.a. compounds in physiological fluids might reflect physiological or pathological conditions, some researchers have investigated the correlation of a.a. concentrations in physiological fluids with some clinical conditions, including ocular diseases. Here, we aimed to compare free and derivated a.a. in nuclear (generally a physiologic condition) and cortical (generally a pathologic condition) cataract.

Materials and methods: Ten patients with nuclear and 10 with cortical cataract were included in this study. Aqueous humour a.a. and derivated a.a. compounds were determined by HPLC method (HP 1100) with Pickering post-column derivatization.

Results: There was no significant difference between cortical and nuclear cataract groups in terms of aqueous humour total, glyco/ketogenic and standard a.a. contents, and nonessential ($P < 0.01$), glycogenic ($P < 0.001$) and ketogenic ($P < 0.001$) a.a. concentrations were found higher, and derivated ($P < 0.001$) a.a. concentrations were lower in the cortical cataract patients compared to the nuclear cataract patients.

Conclusion: The present study suggests that there may be a difference between the nuclear and cortical cataract groups with respect to the contents of a.a. and derivated a.a. compounds in aqueous humour.

Key words: Cataract, free amino acids, aqueous humour

Nükleer veya kortikal kataraktlı hastaların humor aközündeki serbest amino asit konsantrasyonu tayini

Amaç: Fizyolojik sıvılardaki serbest ve türev amino asit bileşimi fizyolojik ve patolojik durumları yansıtabildiğinden, bazı araştırmacılar göz hastalıklarını da içeren bazı klinik durumlarda fizyolojik sıvılardaki amino asit konsantrasyonlarının korelasyon analizini yapmışlardır. Burada biz, genellikle fizyolojik olarak oluşan nükleer katarakt ve genelde patolojik olarak oluşan kortikal kataraktlılarda serbest ve türev amino asit kompozisyonunu karşılaştırmayı amaçladık.

Yöntem ve gereç: Çalışmaya 10 nükleer kataraktlı ve 10 da kortikal kataraktlı hasta dahil edildi. Humor aköz sıvısı amino asit ve türev amino asit bileşimi kolon sonrası türevlendirmeli Pickering ünitesine sahip (HP 1100) HPLC yöntemiyle tayin edildi.

Bulgular: Aköz sıvının total, glukoketojenik ve standart amino asit içeriği açısından kortikal ve nükleer kataraktlı gruplar arasında önemli bir fark bulunamadı. Nükleer kataraktlılara kıyasla kortikal kataraktlılarda non esansiyel ($P < 0,01$), glukojenik ($P < 0,01$), ketojenik ($P < 0,01$) amino ait konsantrasyonları daha yüksek, türev amino asit konsantrasyonları ise daha düşük bulundu.

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Sonuç: Mevcut çalışma, humor aköz sıvısındaki amino asit ve türev amino asit bileşimleri açısından değerlendirildiğinde nükleer ve kortikal kataraktlı gruplar açısından farklılığın olduğunu göstermektedir.

Anahtar sözcükler: Katarakt, serbest amino asit, humor aköz

Introduction

Aqueous humour (AH) is an intraocular fluid made continuously by the ciliary epithelium and is responsible for the supply of nutrients to and removal of metabolic wastes from the avascular tissues of the eye (1). Ion, amino acid, and protein composition of AH (2,3) have been studied by many researchers. In human AH protein concentrations range from 5 to 30 mg/100 mL (4). It has been shown to increase during ageing (5). Most of the proteins enter the AH can also be released from tissues in the eye (6,7). Ringvold et al. showed that AH from cataract eyes contains high concentrations of proteins with a wide range of sizes (9). Earlier studies by David et al. showed that the process of aging in the normal eye lens is unique among tissues because the structural proteins of the lens undergo little turnover (10). Cataract is the leading cause of blindness, accounting for 50 percent of blindness in the worldwide (11). With the global aging of populations, particularly in industrialized countries, the prevalence of this condition is increasing rapidly. The only therapy currently available is lens extraction.

Three types of cataracts are generally recognized in adults: nuclear, cortical, and subcapsular (12). Nuclear cataract is an exaggeration of physiologic sclerotic change that results in loss of vision. Cortical cataract is the most common form of lens opacity. The degeneration of the lens cortex is presumed to be the result of enzymatic digestion. In the incipient stage of age- dependent cortical cataract, biomicroscopy reveals water clefts, lamellar separation, and cuneiform opacities (13).

Some changes may be the result rather than the cause of cataracts. A decrease in soluble protein accounts for a decrease in total protein. Age-related changes in the lens may be caused by ultraviolet photo-oxidation of tryptophan or other chromophores (12). Since the free a.a. and derivated a.a. compounds in physiological fluids might reflect physiological or pathological conditions, some researchers have investigated the correlation of a.a. concentrations in physiological fluids in some clinical conditions, including ocular diseases (14-17).

Early studies have showed that modified crystalline could aggregate and cross-link in human lenses during aging and cataractogenesis (18-19). However, the major challenge has been to identify cataract-specific complexes, and elucidate their modifications as potential mechanisms for cross-linking including identification of participating amino acids in covalent bonding. In patients with cortical cataract, the level of amino acids in aqueous humour increases by destruction of protein molecules and lens capsule (18-19).

In the present study, we aimed to investigate a.a. levels in aqueous humour from patients with cortical and nuclear cataract and to compare the 2 groups of patients in terms of a.a. levels in aqueous humour by high performance liquid chromatography (HPLC) .

Materials and methods

The clinical diagnosis of cataract was based on clinical signs supported by objective findings including the presence of opacity of lens at the slit lamp. Patients did not have any other systemic disease or ocular disorders except cataract. Informed consent was obtained from all participants.

Aqueous humour samples were collected after topical anaesthesia before operation from 10 patients with cortical cataract (4 women and 6 men, aged 55-72 years, mean 64.2 ± 6.0 years) and from ten patients with nuclear cataract (7 women and 3 men, aged 59-76 years, mean 67.1 ± 5.2 years) who were admitted to the Department of Ophthalmology, Medical School, Ataturk University. Aqueous humour samples were obtained with a tuberculin syringe fitted with a 30-gauge needle and stored at –80 °C until a.a. measurements were carried out.

A.a. analysis was performed with a Hewlett-Packard (HP)1100® HPLC with post-column derivatization system (Pickering PCX3100-USA)

containing a high pressure gradient program with a fluorescence detector, a quaternary pump, and an autosampler controlled by computer. A.a. samples were separated on a Pickering high efficiency analytical column, 3 × 150 mm (Cat. No. 0353150) and guard column for 0353150, 2 × 20 mm (Cat. No. 0352020). A.a. samples were derivatizated with orthophtaldehyde (OPA) (Cat. No. 0120). The derivatization time was 122 min. Mobile phases were %Li280, %Li750 and %RG003 (Cats. No. Li280, Li750 and RG003). The injection volume was 5 mL and eluent flow rate was 0.3 mL/min. Native calibration standard (P. Cat. No. 011006P) is the mixture of acidic, neutral, and basic a.a.s and related compounds intended for analysis in lithium buffer systems. Each mL of solution contains 0.250 mmol of each component in 0.27 N lithium citrate buffer, pH 2.36, with 0.01 N phenol as a preservative. To establish relative retention times and response factors, the standards may be diluted to the desired concentration with Pickering Laboratories Cat.No. Li220, Lithium sample diluent. For deproteinization, an aliquot of 50 mL of aqueous humour was thoughly mixed with an equal volume of SERAPREP™ (Cat. No. SP100). The mixture was centrifuged at 9000xg for 5 min. at 4 °C and pH was adjusted to 2.3± 0.2. An aliquot of 80 mL of supernatant was applied to the HPLC instrument (20).

High performance liquid chromatography (HPLC) with post-column derivatization is a technique for rendering analytes more detectable than they would otherwise be in their native forms. Post-column derivatization gives improved sensitivity or better selectivity (reduction of interference) leading to lower detection limits (21). In recent years, the use of HPLC with electrochemical or fluorescence detection has been proved to be a sensitive and easy method (22).

The results are given as mean (CI % Lower limits-Upper limits). Statistical analysis was carried out by Mann- Whitney U test for the comparison of the cortical and nuclear cataract groups. A P value <0.05 was considered statistically significant.

Results

The Table shows the mean aqueous humour levels of free a.a. both in the nuclear and cortical cataract patients. As seen in the Table, the differences between cortical and nuclear cataract subjects with respect to aqueous humour Ile, Lys, Glu, Tyr, His, Phe and Arg levels were not statistically significant. While Asp and Thr levels were higher, those of Ala, Cys, Leu, Ser, Gln, Met, Trp, and Val were lower in nuclear cataract than those of the cortical cataract group. For derivated a.a: o-phosphoserin, ornithine, 3-methylhistidine, a-aminobutyrate values showed no statistically significant difference, and increased levels of o-phosphoethanolamine and anserine, and decreased ethanolamine, taurine and a-aminoadipate levels were detected in the nuclear cataract group. No statistically significant difference was detected between nuclear and cortical cataract groups in terms of total, essential, glycoketogenic, and standard a.a contents. On the other hand, standard/derivated a.a ratio and non-essential, glycogenic and ketogenic a.a contents were higher, and derivated a.a. content was lower in the nuclear cataract group than those of the cortical cataract group.

Discussion

Cataract is a very common condition and is very common among the elderly. More than 90% of individuals over the age of 70 years have some lenticular opacities visible on slit-lamp examination, and these opacities are frequently seen from the age of 40 years onward. There are several forms of age-dependent cataract, some of which may appear simultaneously in the same eye. The three main types of age-dependent cataract are cortical, nuclear, and cupuliform (13). The lens and the inside surface of the cornea are maintained clear and functional by the aqueous humour that is produced by the ciliary body and that fills in the anterior chamber.

We have measured the a.a. concentrations in aqueous humour of cortical and nuclear cataract patients. In the nuclear cataract group, 2 standard and 2 derivated a.a. concentrations were higher, and 5 standard and 3 derivated a.a. concentrations were lower compared to the cortical cataract group.

Hypothetically, a.a. may reflect the abnormal metabolism in the anterior eye segment. Wuu et al. (23) and Watling (24) measured serum and aqueous humour a.a. contents in cataract patients and reported

Table. Standard and derivated amino acid levels of aqueous humour from patients with cortical and nuclear cataract.

	Cortical cataract X (CI 95%) (μmol/L) (n = 10)	Nuclear cataract X (CI 95%) (μmol/L) (n = 10)	P <
Asp	1.4 (1.3-1.5)	2.3 (1.8-2.8)	0.01
Ala	343 (331.6-354.4)	234 (203.2-264.8)	0.001
Cys	2.3 (2.1-2.5)	1.7 (1.4-1.2)	0.001
Ile	136 (127.4-144.6)	184 (146.1-221.9)	ns
Leu	125 (112.8-137.2)	103 (82.1-123.0)	0.05
Lys	174 (165.4-182.6)	148 (132.7-151.3)	ns
Thr	99 (88.3-109.7)	142 (132.7-151.3)	0.001
Ser	155 (151.4-158.6)	140 (127.8-152.2)	0.05
Glu	25 (23.3-26.6)	23 (21.2-24.8)	ns
Gln	714 (647.4-780.5)	533 (452.9-613.1)	0.01
Gly	14 (10.7-17.3)	18 (15.9-20.6)	ns
Met	250 (224.3-275.8)	194 (176.1-211.9)	0.01
Tyr	89 (81.9-96.2)	91 (72.4-109.6)	ns
His	320 (308.6-331.4)	336 (288.8-383.2)	ns
Trp	30 (27.4-32.7)	23 (18.5-27.5)	0.05
Val	249 (221.8-276.2)	173 (152.3-193.7)	0.001
Phe	92 (81.3-102.7)	94 (66.1-121.9)	ns
Arg	169 (148.3-189.7)	183 (170.1-195.9)	ns
o-phosphoserine	396 (376.7-415.3)	427 (391.10-462.0)	ns
Ethanolamine	17 (16.1-17.9)	7.1 (6.0-8.2)	0.001
o-phosphoethanolamine	11.3 (9.7-12.9)	35 (30.1-39.9)	0.001
Ornithine	77 (74.6-79.4)	83 (76.4-89.7)	ns
3-methylhistidine	21 (18.6-23.4)	24 (20.4-27.6)	ns
Anserine	12 (11.3-12.7)	14 (13.0-15.0)	0.01
α-aminobutyric acid	16 (13.6-18.4)	16 (13.7-18.3)	ns
Taurine	49 (44.9-53.2)	39 (32.7-45.3)	0.05
α-aminoadipic acid	3.6 (3.4-3.8)	2.4 (2.1-2.7)	0.01
Ammonia	33 (31.5-34.5)	32 (30.0-3.9)	ns
total amino acid	*3623 (3355.5-3890.5)*	*3676 (3101.6-4250.4)*	*ns*
essential	*1645 (1506.2-1783.8)*	*1597 (1197.1-1996.9)*	*ns*
nonessential	*1341 (1247.3-1434.7)*	*1041 (895.1-1186.9)*	*0.01*
glycogenic a.a	*1255 (1168.5-1341.6)*	*952 (824.7-1079.3)*	*0.001*
glycoketogenic a.a	*1433 (1308.5-1557.5)*	*1435 (1060.2-1809.8)*	*ns*
ketogenic a.a	*299 (278.3-319.7)*	*251 (207.4-294.6)*	*0.05*
standard a.a	*2986 (2753.5-3218.5)*	*2638 (2091.5-3184.5)*	*ns*
derivated a.a	*636 (600.1-671.0)*	*1038 (1010.1-1065.9)*	*0.001*

that Trp was not detectable in aqueous humour of senile cataract patients examined. Additionally, Schonhender et al. proposed that low tryptophan was cataractogenic. For this reason, a.a. concentrations in aqueous humour have been studied in many ocular disorders (14,15,16,25,26). We could not find any study investigating aqueous humour content both in cortical and nuclear cataracts. In the present study, we compared aqueous humour a.a. content in cortical and nuclear cataract and found that only derivated a.a. was lower and that glycogenic, ketogenic, and nonessential a.a. significantly occurred in higher concentrations in the cortical cataract group when compared to the nuclear cataract group. Although we

determined Trp in aqueous humour in one study performed in a Chinese population; this a.a. could not be detectable with liquid chromatography. This observation may be attributed to the resolution of the equipment used.

Since we could not measure a.a. concentrations in aqueous humour in normal healthy subjects, we were unable to compare the cataract patients and normal subjects in terms of aqueous humour a.a. contents. Further studies are needed to make such comparisons.

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