KLINIKINIAI TYRIMAI

Ultrasonic and biochemical evaluation of human diabetic lens

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Key words: *diabetes mellitus, ultrasound, lens, cataract, crystallins.*

Summary. *Objective. To evaluate the ultrasonic attenuation and amount of soluble proteins of human diabetic lens.*

Materials and methods. The examination was performed in the Clinic of Eye Diseases of Kaunas University of Medicine. The study included 4 groups of patients (110 eyes). The first group consisted of healthy subjects (32 eyes), the second group – of patients with initial senile cataract (13 eyes), the third group – patients with type I diabetes mellitus (24 eyes) and the fourth group – patients with type II diabetes mellitus (41 eyes). In vivo examination of human lenses was carried out by Mentor™ A/B ultrasonic imaging system using 7 MHz A-mode probe and the ultrasound attenuation coefficient was calculated. The phacoemulsification technique has been used for cataract extraction. Gel chromatography of the supernatant fraction on the Sepharose CL 6B column was used for fractionation of soluble lens proteins. Protein concentration was determined by the method of Lowry using bovine serum as standard.

Results. The least mean lens thickness of 3.58±0.18 mm was found in the healthy patients' group. There was a significant difference ($p<0.05$) between the thicknesses of the lenses in the healthy group and in the type I diabetic group. The difference between senile cataract and type II diabetic cataract was insignificant ($p>0.05$).

The mean ultrasound attenuation coefficient in the groups of healthy and type I diabetic cataract was nearly the same, as so as in the groups of senile cataract and type II diabetic cataract. The significant difference ($p<0.001$) in the values of attenuation coefficient was found between the groups of type I and type II diabetics.

The amount of soluble proteins was lowest in cataractous lenses of patients with type II diabetes (0.053±0.007 mg / 1 mg tissue) and highest in the lenses of patients with type I diabetes (0.063±0.004 mg / 1 mg tissue), but those differences were statistically insignificant. Distribution of soluble proteins into the different molecular mass fractions in the group of type I diabetic lenses was found to be similar to the type II diabetic lenses and to the patients with senile cataract.

Conclusions. The diabetic changes stronger influence the thickness of lenses of young people; in the elder age the difference between the thickness of senile and diabetic cataract is not so distinct. Ultrasound attenuation coefficient has a tendency to be higher in patients with senile and type II diabetic cataract. Human lens crystallins of patients with type I diabetes and type II diabetes are damaged at the same degree, the amount of soluble proteins decrease with age and biochemical changes of the lens.

Introduction

In 1985 there were about 30 million people with diabetes. In 1995, this number increased to 135 million. It is estimated that in 2025, there will be 330 million people suffering from this disease (1).

According to data by Lithuanian Health Ministry there are about 60 thousand people with diabetes in Lithuania. Diabetes Atlas 2003 published by International Diabetes Federation states that prevalence of impaired glucose tolerance is 17% in Lithuanian society while 10.2% in Europe and 8.2% in the world (2).

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Diabetic eye disease is the leading cause of blindness and visual impairment among Americans aged 20 to 74 years (3). Persons who have diabetes are at an increased risk for impairment due to diabetic retinopathy, glaucoma, and cataracts (4, 5). Overall, these individuals are 25 to 30 times more likely to progress to blindness than individuals without diabetes of similar age and gender (5).

There are some theories regarding the role of diabetes in cataractogenesis. According to S. Duke-Elder (6), cataracts in diabetics are due to hyposmolarity of the plasma and aqueous humor. The lowered osmolarity of the aqueous humor in relation to the lens leads to water influx in the lens, causing swelling and ultimate opacification. J. H. Kinoshita and colleagues (7) suggested that accumulation of excessive sorbitol, a sugar alcohol derived by the action of aldose reductase on glucose in the presence of NADPH, in the lens fibers and epithelium leads to an increase in intracellular tissue osmolarity and its consequent hydration because of water influx from the aqueous humor.

Alternative hypotheses for the genesis of sugar cataract have also been proposed, such as those citing the oxidative stress induced by hyperglycemic conditions (8) and/or protein glycation phenomena (9, 10). These data would seem to show that diabetic cataract etiology and development is a multifactorial event.

The identity of diabetic cataracts is well established in relatively younger diabetics (Fig. 1). It is usually bilateral and consists initially of a characteristic band of subcapsular vacuoles extending to approximately one-third the depth of the superficial layers of anterior and posterior cortex. The vacuoles often are interspersed with white flaky opacities. There may also be a simultaneous appearance of water clefts and suture separations. These early stages of cataract development are ultimately followed by the appearance of more diffuse cloudiness and opacification.

In adult diabetics, cataracts are characterized by cortical and nuclear involvement and it is not possible to distinguish them morphologically from the garden variety of senile cataracts (Fig. 2).

However, in diabetic patients, the senile changes of a cortical, posterior subcapsular and mixed form of opacification develop at an earlier age (11) and faster (12) than in nondiabetic patients. The diabetic eye also suffers episodes of transient refractive change, either hypermetropia or myopia (13). Lens thickness is greater in diabetics than in normal subjects (14). Diabetic eyes with advanced stages of diabetic retinopathy or neuropathy have a larger lens and shallower anterior chamber (15, 16). Thickening of the capsule has also been found in diabetic lenses (17).

When examining the patient it is very important to describe the cataracts quantitatively, but it is difficult using only the slit lamp. Ultrasound examinations are widely used in ophthalmology. Acoustic parameters of biologic tissues are described by velocity and attenuation coefficient. It is known that in soft tissues the attenuation coefficient is approximately proportional to the frequency – high frequency components of echoes are attenuated more than the lower frequency components (18).

Normal lens is acoustically homogenous and clear. Its characteristics change according to the density of cataract that is due to the changes in tissue density and structure (19). The crystallins are the main structure of the human lens and constitute approximately 90% of the total protein content (20). Their structural function is to assist in maintaining the proper refractive

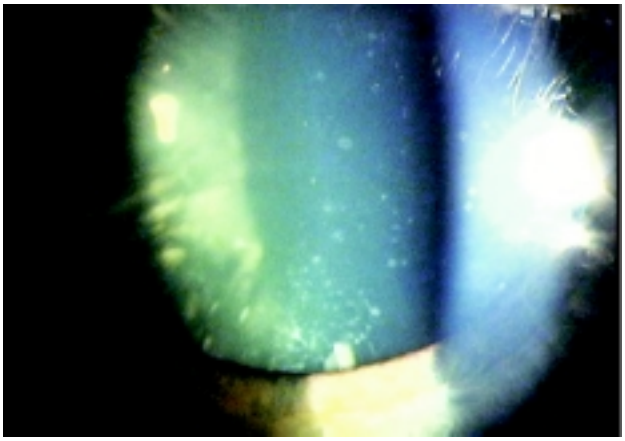

Fig. 1. **Classical "snow-flake" juvenile cataract (type I diabetic cataract)**

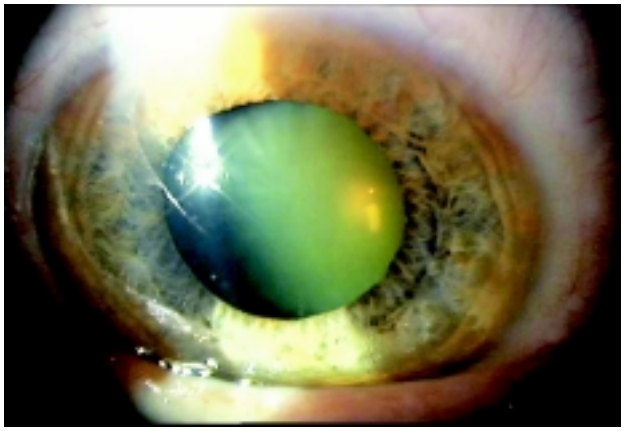

Fig. 2. **Type II diabetic cataract**

index of the lens and its transparency (21). According to molecular weight there are α-crystallins (over 200 kDa), β-crystallins (40–160 kDa) and γ-crystallins (about 20 kDa). Protein aggregation results in the development of high molecular weight aggregates of sufficient size to directly scatter the light and in the creation of protein rich and protein poor phases causing changes in refractive index and increased light scattering (20).

Objective

The purpose of our study was to evaluate the ultrasonic attenuation and amount of soluble proteins of the human diabetic lens.

Materials and methods

The study of human lenses was performed in the Clinic of Eye Diseases of Kaunas University of Medicine. The sample consisted of subjects (total 110 eyes) that were divided into 4 groups. The first group consisted of healthy subjects (32 eyes), the second group – patients with initial senile cataract (13 eyes), the third group – patients with type I diabetes mellitus (24 eyes) and the fourth group – patients with type II diabetes 41 eyes. Patients' age in the first group (healthy) varied between 20 and 35 years, in the second group (initial senile cataract) – between 40 and 65, in the third group (type I diabetics) – between 16 and 35 years, in the fourth group (type II diabetics) – between 42 and 75 years. The first two groups were as control for type I and type II diabetic groups according to their age and sex.

The ultrasonic investigation. In our work we performed examination of human lens *in vivo* by Mentor™ A/B ultrasonic imaging system (Advent, Norwell, MA) using 7 MHz A-mode probe and calculating the ultrasound attenuation coefficient. The contact examination method was used through the open eye after blocking the blink reflex with topical anesthetics. Radio frequency echo signals from lens were digitized by TEKTRONIX 220 oscilloscope at the sampling rate 250 MHz and 8 bit amplitude resolution, bandwidth for analog signal was 100 MHz. Manual trigger of oscilloscope was used according to sound notice from system about the probe correct alignment to eye axis. Signal averaging was not used, but five single signals were acquired (Fig. 3). Digitized echo signals were used for off-line processing.

The assumption that attenuation frequency function is linear $\alpha(f)=\beta\times f$ has been made. The attenuation coefficient β has been calculated from logarithmic spectra difference, taking into account spectra of echo signal from anterior $S_{AN}(f)$ (Fig. 4) and posterior $S_{PN}(f)$ (Fig. 5) nucleus interfaces, distance between interfaces d and frequency range f_2–f_1.

To the frequency function of logarithmic spectrum difference $S_{AN}(f)$–$S_{PN}(f)$ least-squares straight line fit $\alpha_L(f)$ was applied. Last the attenuation coefficient β was calculated as follow:

$$\beta=[\alpha_L(f_2)-\alpha_L(f_1)]/[2d\times(f_2-f_1)].$$

Thickness of lens nucleus was assessed taking into account first zero-crossing moments in echo signals and the sound velocity in cataract lens c=1620 m/s. Echo signals from anterior and posterior interfaces of lens nucleus were selected manually with constant

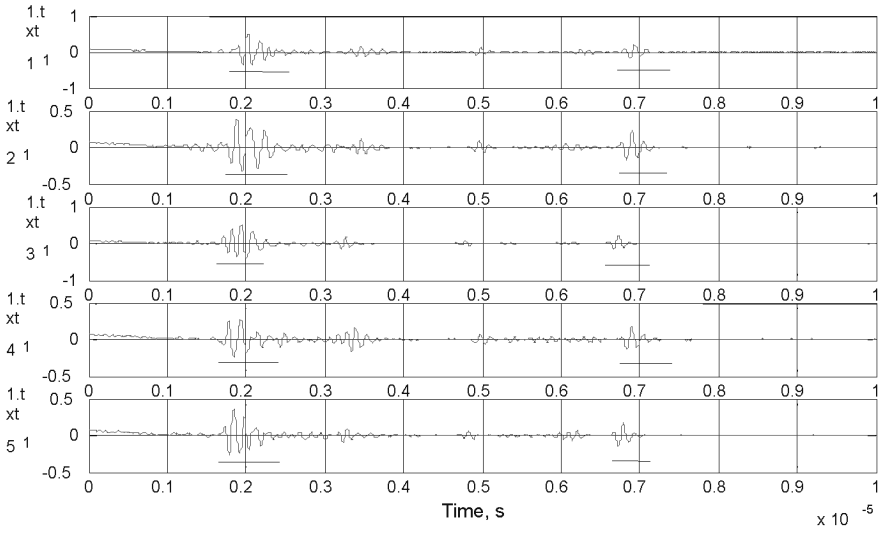

Fig. 3. **Echo signal from diabetic lens**

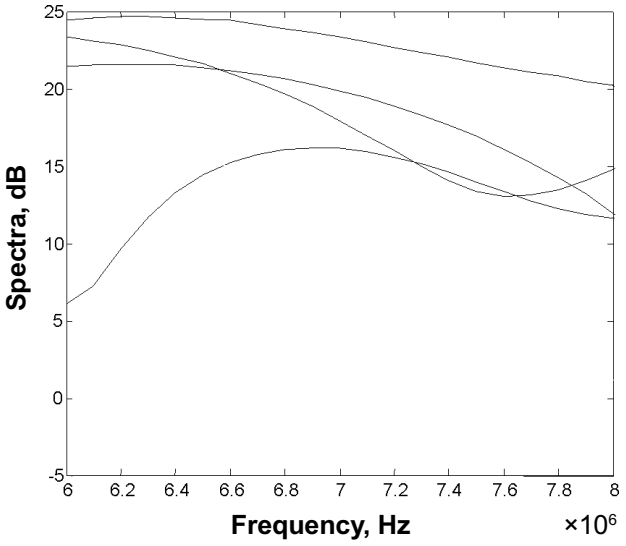

Fig. 4. **Spectra of the anterior interface of the lens ($S_{AN}(f)$)**

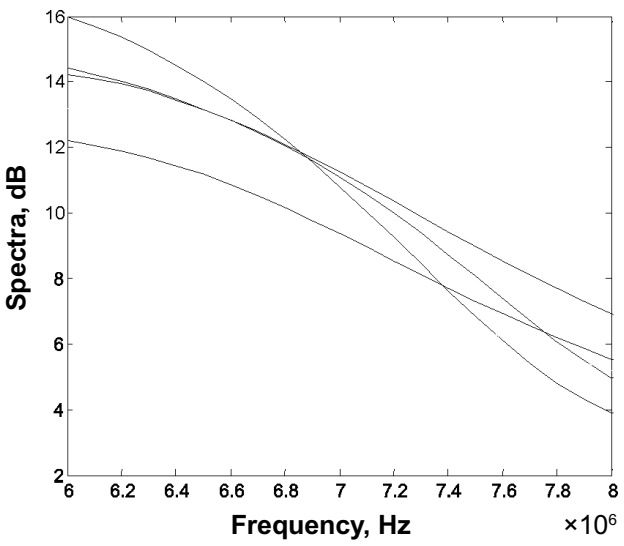

Fig. 5. **Spectra of the posterior interface of the lens ($S_{PN}(f)$)**

time window length of 1.024 μs.

The biochemical investigation. The extraction of cataractous lenses was performed using phacoemulsification technique in all cataractous eyes. The extracted lens masses were soaked in 0.9% NaCl solution and frozen to –20°C until analysis. Lens masses were carefully weighted and homogenized in 5 volumes of buffer A (20 mM sodium phosphate, pH 7.0; 1.0 mM EGTA). Soluble lens proteins were extracted for 10 min at 4°C. Undissolved components were removed by centrifugation at 10,000 x g for 30 min. Gel chromatography of the supernatant fraction on the Sepharose CL 6B column was used for fractionation of soluble lens proteins. Supernatant was diluted with buffer B (50 mM sodium phosphate, pH 7.0; 150 mM NaCl) to give a protein concentration of 10 mg/ml. Aliquot of diluted supernatant (1 mg of protein in 0.1 ml) was applied to a Sepharose CL 6B column (0.7×30 cm) equilibrated with buffer B. Lens proteins were eluted with the same buffer. Protein fractions of 0.5 ml were collected. The column was calibrated with standard proteins: ferritin (440,000 M.W.), aldolase (158,000 M.W.), bovine serum albumin (67,000 M.W.), and chymotrypsinogen (25,000 M.W.). Protein concentration was determined by the method of Lowry using bovine serum as standard.

The statistical analysis of data was performed using the software "SPSS 12.0" for Windows. Data are expressed as a mean ± standard error of the mean (SEM). To compare the data between groups Student's t-test was used. Values of $p<0.05$ were considered significant.

Results

The thickness of lenses and attenuation coefficient β were calculated for all patients using measurement method described above. In the healthy group mean lens thickness was 3.58±0.18 mm, in the group with initial senile cataract – 4.22±0.44 mm, in type I diabetic group – 3.86±0.6 mm, in type II diabetic group – 4.35±0.59 mm (Fig. 6).

There was a significant difference ($p<0.05$) between the thicknesses of the lenses in the healthy group and in the type I diabetic group. The difference between senile cataract and type II diabetic cataract was insignificant ($p>0.05$).

The mean ultrasound attenuation coefficient in the groups of healthy and type I diabetic cataract was near the same, as so as in the groups of senile cataract and type II diabetic cataract (Fig. 7).

The significant difference ($p<0.001$) in the values of attenuation coefficient was found between the groups of type I and type II diabetics. It may be assumed that the alteration in the properties of crystalline, increased scattering and absorption of echo signals are related to the decrease of lens transparency.

Amounts of soluble proteins in human cataractous lenses were determined. Data presented in Table 1 show that the amount of soluble proteins was lowest in cataractous lenses of patients with type II diabetes and highest in the lenses of patients with type I diabetes, but those differences were statistically insignificant.

Gel chromatography on the Sepharose 6B column separated the soluble lens proteins in few fractions

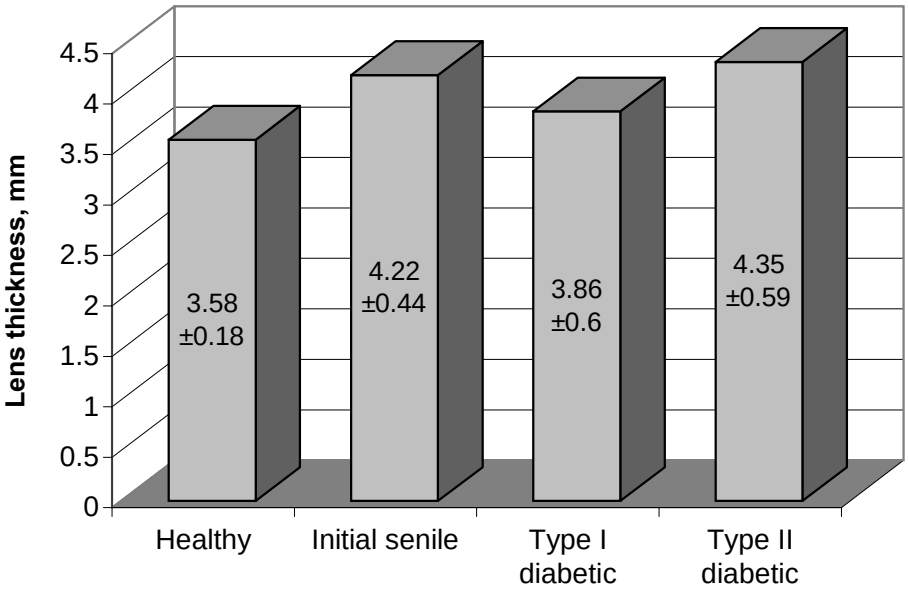

Fig. 6. **The distribution of mean lens thickness in four groups of subjects**

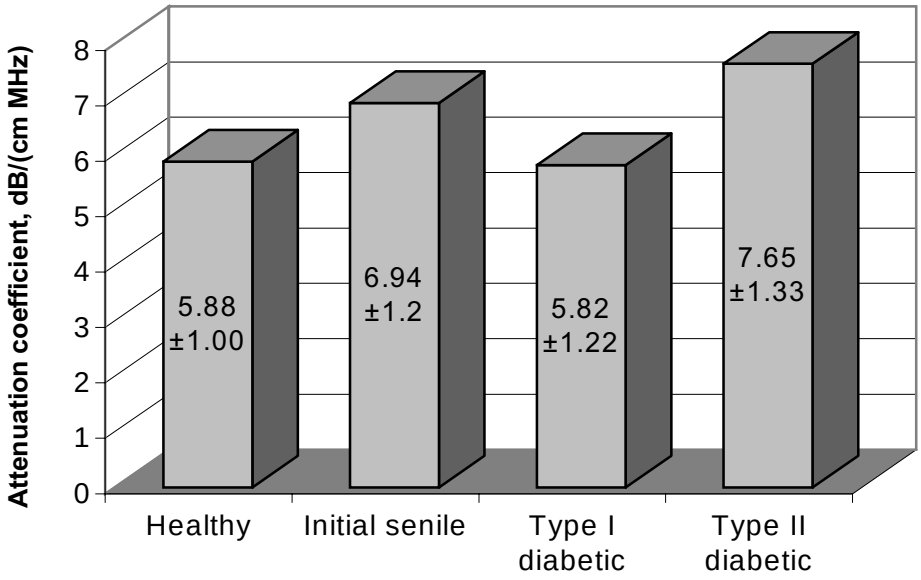

Fig. 7. **The results of mean attenuation coefficient calculations in four groups of subjects**

Table 1. **Amount of soluble proteins in human lens**

Patient group	Soluble proteins* (mg / 1 mg tissue)
Initial senile cataract (n=13)	0.056±0.003
Type I diabetic cataract (n=24)	0.063±0.004
Type II diabetic cataract (n=41)	0.053±0.007

*The quantities of soluble proteins were determined in supernatant fractions after centrifugation of eye nucleus extracts at 10,000 x g.

with molecular mass 600, 270, 170, 50, 30, and 20 kDa. Since crystallins are major proteins of the lens, obtained protein fractions are most likely separate groups of crystallins. Data on the relative amount of protein in different molecular mass fractions, obtained by the gel chromatography of soluble human lens nucleus proteins, are summarized in Table 2.

Distribution of soluble proteins between different molecular mass fractions in the group of patients with type I diabetes was found to be similar to the patients with type II diabetes and to the patients with senile cataract.

***Table 2*. Distribution of soluble proteins of human lens among the fractions of different molecular mass**

Patient group	Relative amount of proteins (%)*					
	I fraction	II fraction	III fraction	IV fraction	V fraction	VI fraction
Initial senile cataract (n=13)	8.0±1.1	5.7±0.4	7.4±0.4	17.5±0.6	28.0±1.0	33.6±1.0
Type I diabetic cataract (n=24)	7.9±1.0	6.2±0.5	8.5±0.6	15.6±0.6	26.7±1.0	35.2±2.4
Type II diabetic cataract (n=41)	6.7±1.5	5.2±0.6	7.5±0.6	16.8±1.0	27.1±2.5	34.2±3.3

*100% – quantity of total soluble proteins of lens nucleus (see Table 1).

I fraction was eluted from Sepharose CL 6B column at the range of 400–700 kDa, II fraction – 200–400 kDa, III fraction – 100–200 kDa, IV fraction – 50–100 kDa, V fraction – 20–50 kDa, VI – fraction 10–20 kDa.

Discussion

Improvements in the medical management of diabetes mellitus during the last 50 years have increased the life expectancy of a large number of diabetic patients. This increase has resulted in a significant rise in diabetic complications, including diabetic cataract and diabetic retinopathy. These complications are leading causes of visual dysfunction and blindness in patients with diabetes mellitus. About 10–15% of patients undergoing cataract surgery are diabetics, and this number is increasing (22).

N. Brown and J. Hungerford (1982) estimated the difference of lens thickness between diabetics and normal subjects. According to our results the diabetic changes influence the thickness of lenses of young people stronger; in the elder age the difference between the thickness of senile and diabetic cataract is not so distinct.

Ultrasound attenuation by a biological medium is largely influenced by the presence of high-molecular-weight compounds and increased protein aggregation in cataract lenses contributes to the hardening of the lens and increased ultrasound attenuation (19). Y. Sugata and co-authors (1992) examined normal and cataract lenses and suggested the possibility of diagnosing cataract by measuring the attenuation characteristics of the lens. In our work we analyzed diabetic lenses and compared them with senile cataract and normal lenses. Our results show that ultrasound attenuation has a tendency to be higher in senile and type II diabetic cataract, but there is no possibility of separating them by ultrasound.

Many studies of human cataractous lenses demonstrate that cataractogenesis is a multifactorial process and in most cases oxidative stress initiates a series of processes leading to cataract formation. H. Rink and co-authors (1995) found the increased amount of water insoluble proteins in nuclear cataract lenses (23). Redistribution of soluble crystallins from lower molecular weight form to higher was observed in nuclear and cortical regions (24). Protein aggregation results in development of high molecular weight aggregates causes changes in refractive index and increases light scattering. Earlier we revealed the decrease of soluble proteins and redistribution of the portion of soluble lens proteins into the higher molecular weight fraction in the case of advanced senile cataract in comparison with the initial senile cataract (25). The present data show, that human lens crystallins of patients with type I diabetes and type II diabetes are damaged at the same degree. Their content and redistribution among different molecular weight fractions were the same as in the case of senile cataract. We did not find the statistically significant difference in the amount of soluble proteins in cataractous lenses between the patients with type I and type II diabetes. Moreover, no differences were observed in the distribution of soluble lens proteins between different molecular mass fractions for those two groups of patients. We think that insignificant differences were determined because of lens phacoemulsification. Lens masses taken do not reveal all the protein content of lens nucleus.

We hope that the results of our work will be useful for further developments of the cataractous lens examination.

Conclusions

1. The diabetic changes influence the thickness of lenses of young people stronger; in the elder age the difference between the thickness of senile and diabetic cataract is not so distinct.

2. Ultrasound attenuation coefficient has a tendency to be higher in senile and type II diabetic cataract.

3. Human lens crystallins of patients with type I diabetes and type II diabetes are damaged at the same degree; the amount of soluble proteins decreases with age and biochemical changes of the lens.

Cukriniu diabetu sergančio žmogaus akies lęšiuko ultragarsiniai ir biocheminiai tyrimai

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Raktažodžiai: cukrinis diabetas, ultragarsas, lęšiukas, katarakta, kristalinai.

Santrauka. *Darbo tikslas.* Įvertinti lęšiuko ultragarso slopinimo koeficientą ir apskaičiuoti tirpių baltymų kiekį cukrinio diabeto pažeistame lęšiuke.

Tyrimo medžiaga ir metodai. Tyrimas atliktas Kauno medicinos universiteto Akių ligų klinikoje. Ištirtos keturios grupės pacientų (110 akių). Pirmąją grupę sudarė 32 sveikų asmenų akys, antrąją – 13 akių su pradine senatvine katarakta, trečiąją – 24 pirmojo tipo cukriniu diabetu (CD) sergančių tiriamųjų akys; ketvirtąją – 41 antrojo tipo cukriniu diabetu sergančių tiriamųjų akis. Ultragarsiniai lęšiuko tyrimai *in vivo* atlikti ultragarsine A/B vizualizavimo sistema „Mentor™“ (Advent, Norwell, MA) naudojant 7 MHz dažnio A tipo keitiklį. Radiodažniniai aido iš lęšiuko signalai skaitmenizuoti „Tektronix 220“ osciloskopu, atlikta skaitmenizuotų signalų analizė ir apskaičiuotas ultragarso slopinimo koeficientas β. Atlikus kataraktos operaciją fako-emulsifikacijos būdu, pašalintos lęšiuko dalys buvo patalpintos į fiziologinį 0,9 proc. NaCl tirpalą, vėliau užšaldytos (iki tyrimo). Tirpių baltymų frakcijos nustatytos gelio chromatografijos būdu. Baltymų koncentracija nustatyta Lowry metodu.

Rezultatai. Mažiausias vidutinis lęšiuko storis (3,58±0,18 mm) rastas sveikų tiriamųjų grupėje. Statistiškai reikšmingas ($p<0,05$) lęšiukų storio skirtumas rastas tarp sveikų ir pirmojo tipo CD sergančių tiriamųjų. Gauti senatvinės ir antrojo tipo diabetu sergančių tiriamųjų lęšiukų storio duomenys statistiškai reikšmingai nesiskyrė ($p>0,05$).

Gautos ultragarso slopinimo koeficiento reikšmės reikšmingai nesiskyrė sveikų tiriamųjų ir sergančiųjų pirmojo tipo CD bei sergančiųjų senatvine katarakta ir antrojo tipo CD. Statistiškai reikšmingas ($p<0,001$) ultragarso slopinimo koeficiento reikšmių skirtumas rastas tarp pirmojo ir antrojo tipo CD sergančių tiriamųjų. Mažiausias tirpių baltymų kiekis buvo rastas antrojo tipo CD sergančių tiriamųjų grupėje (0,053±0,007 mg / 1 mg audinio), didžiausias – pirmojo tipo CD sergančių tiriamųjų grupėje (0.063±0.004 mg / 1 mg audinio), bet šie skirtumai statistiškai nereikšmingi. Tirpių baltymų pasiskirstymas į frakcijas pagal molekulinę masę buvo panašus visų grupių tiriamųjų.

Išvados. CD sukelti pokyčiai daugiau įtakos turi jauno amžiaus žmonių lęšiuko storiui, vyresniems žmonėms šie pakitimai ne tokie ryškūs. Ultragarso slopinimo koeficientas turi tendenciją didėti vyresnio amžiaus ir sergant antrojo tipo CD. Lęšiuko kristalinų pažeidimo laipsnis yra panašus sergant ir pirmojo, ir antrojo tipo CD, tirpių baltymų kiekis mažėja su amžiumi ir vykstant biocheminiams lęšiuko pokyčiams.

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