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Research article

Non-invasive tryptophan fluorescence measurements as a novel method of grading cataract

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Development of non-invasive treatments for cataract calls for a sensitive diagnostic assay. We conducted a study to test whether the ratio of folded tryptophan to non-tryptophan fluorescence emission (F-factor) may be used for grading cataracts in human lenses. The F-factor was measured on aspirated lens material from eyes undergoing femtosecond laser assisted cataract surgery (FLACS) and was compared to a preoperative optical grading of cataract using Scheimpflug imaging. The preoperative optical grading allocated the cataracts to 1 of 4 categories according to the density of the cataract. All cataracts were age-related. Lens material from 16 eyes of 14 patients was included in the study. Cataracts were preoperatively graded in categories 1, 2 and 3. No lenses were category 4. For nuclear cataracts mean values of F-factor were 52.9 (SD 12.2), 61.7 (SD 5.3) and 75.7 (SD 8.9) for categories 1, 2 and 3 respectively. Linear regression on F-factor as a function of preoperative grading category showed increasing values of F-factor with increasing preoperative grading category, $R^2 = 0.515$. Our experiment showed that preoperative optical grading of cataracts by Scheimpflug imaging may correlate to measures of tryptophan and non-tryptophan fluorescence in human lenses. Based on our results we find that measuring the ratio between tryptophan- and non-tryptophan fluorescence may be a future tool for grading cataracts, but further research is needed.

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1. Introduction

Cataract remains the leading cause of blindness worldwide (Resnikoff et al., 2004). In most westernized countries, cataract extraction followed by intraocular lens implantation is the second most frequently performed elective surgical procedure, only outnumbered by intravitreal anti-VEGF injections (Avery et al., 2014) and vast resources are spent on the monitoring and treatment of cataracts. Earlier surgical intervention and the growing population of elderly citizens put considerable strain on the health care systems worldwide and the challenges will be even more pronounced in the years to come due to the demographic changes (Kessel, 2011; Tuulonen et al., 2009). Thus, it is essential to prioritize health care resources and an objective grading of cataract may assist in deciding when it is the optimal time for surgery.

New non-invasive methods for treatment of cataract are under development. These methods include femtosecond photolysis (Kessel et al., 2010), pharmacological chaperones (Makley et al., 2014), Lanosterol (Zhao et al., 2015) and the antioxidant N-acetylcysteine amide (Carey et al., 2011). These methods target the biochemical changes to lens proteins that ultimately result in cataract formation rather than replacing the crystalline lens. The biochemical changes to lens proteins are evident prior to formation of visible cataracts and hence non-invasive methods for treatment of cataract may be applicable before a visible cataract is formed or in early stages of visible cataracts (Ponce et al., 2006). To test the efficacy of and determine the indication for non-invasive methods for treatment of cataract, it is important to be able to precisely detect early changes in (pre)cataractous lenses and to monitor the grade and extent of the cataract after treatment. This calls for a sensitive diagnostic assay.

Abbreviations: FLACS, Femtosecond laser assisted cataract surgery; PBS, phosphate buffered saline; PMT, photomultiplier tube; PSC, posterior subcapsular cataract; DLS, dynamic light scattering; NFK, N-formylkynurenine; OH-Trp, hydroxytryptophan; ArgP, argpyrimidine.

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1.1. Tryptophan fluorescence

Tryptophan is an amino acid present in human lens proteins and it is intrinsically fluorescent. It emits fluorescence upon excitation in the ultraviolet or near-ultraviolet spectrum. The red edge of the excitation spectrum for tryptophan includes the 310–320 nm spectral range which can be transmitted through the cornea and aqueous humour (Ambach et al., 1994; Gakamsky et al., 2011). The spectral range of tryptophan emissions shows a red shift when proteins containing multiple tryptophan sidechains unfold (Gakamsky et al., 2011), a property that may be used for detecting conformational changes of the lens proteins (Lakowicz, 2006). Such conformational changes are important in the development of cataracts (Bloemendal et al., 2004). Non-tryptophan residues contribute to lens autofluorescence with an emission peak around 435 nm. These residues are thought to be products of tryptophan following exposure to UV-light and advanced glycation end products. Early literature describes that cataract is formed following exposure to sunlight which causes tryptophan to photo-oxidize(Pirie, 1971). The role of tryptophan and its' derivates on cataract formation was first studied several decades ago (Borkman and Lerman, 1978; Van Heyningen, 1971). The ratio of tryptophan to non-tryptophan fluorescence, termed the F-factor, was found to be very sensitive to structural changes induced by various durations of UV lighting in pigs' lenses (Gakamsky et al., 2011). Measuring the ratio of folded tryptophan to non-tryptophan fluorescence emission (F-factor) may be an important non-invasive diagnostic tool for further research towards developing non-invasive treatments for cataract. The purpose of this study was to test whether measuring the F-factor may be used for grading cataracts in human lenses by comparing measures of tryptophan fluorescence to an optical grading of cataract using Scheimpflug imaging.

2. Methods

Fluorescence measurements were performed on samples from human lenses obtained via emulsification of age-related cataracts. The cataracts were graded prior to the surgical procedure by Scheimpflug imaging and gross biomicroscopy. The study was conducted at the Department of Ophthalmology, Rigshospitalet-Glostrup, Denmark from August through October 2016. The study was approved by the National Committee on Health Research Ethics in Denmark, protocol number H-16022860, and reported to the Danish Data Protection Agency, journal number RH-2016-186, I-Suite number 04745.

Eligible patients were identified among patients scheduled for femtosecond laser assisted cataract surgery (FLACS) since the laser procedure included a preoperative custom grading of the cataract by Scheimpflug imaging. Patients were contacted via mail prior to the surgery. At the day of surgery patients were informed orally about the study and asked for consent to participation. The surgeon decided whether the procedure could be performed as a FLACS procedure or not. We included consenting patients above 18 years of age with age-related cataract scheduled for FLACS at the cataract unit at Rigshospitalet-Glostrup. Patients were excluded if FLACS could not be performed, if the cataract was not age-related (e.g. traumatic, congenital, cataract induced by previous eye diseases or surgery) or a cataract that was too hard to be removed by classical coaxial phacoemulsification technique.

2.1. Collection of lens material

The custom grading allocated the cataractous lenses to 1 of 4 categories according to the density of the cataract (Fig. 1). In addition, the cataract was graded as nuclear, cortical or posterior subcapsular by the surgeon (AM) at the initiation of the surgical procedure.

The FLACS procedure consists of several steps. Once the patient is docked to the laser, the first step performed by the Lensar© device is to analyze the cataract density using Scheimpflug imaging. The cataract is customly graded and a predefined nucleus fragmentation pattern is associated to each case. Anterior capsulorhexis is then performed by the femtosecond laser, followed by custom-graded nucleus fragmentation. The fragmented nucleus is consecutively aspirated using a Kelmann 45° miniflared tip using minimal ultrasound energy. An intraocular lens was implanted in all cases. Lens material removed during the nucleus aspiration procedure was collected in the manufacturer case set along with a phosphate buffered saline (PBS).

2.2. Sample preparation

Following surgery, the extracted lens material and suspension fluid (PBS) was collected and immediately cooled on ice to avoid further degradation of proteins. Excess fluid was removed by pouring the mix of PBS and lens material into tubes followed by centrifugation at 2000 rpm for 15 min. The supernatant was then removed, leaving about 1 ml and the pellet. The pellet was mixed with the remaining supernatant, transferred to Eppendorf tubes and centrifugated at 1000 rpm for 10 min. The supernatant was removed using pipettes and the pellet was resuspended in 1.2 ml phosphate buffered saline solution. The samples were then stirred using a Vortex mixer and homogenized on ice for 10 s. Samples were kept on ice whenever they were not handled. Samples were transferred to a test cuvette and were left in room temperature for 6 min to reach a temperature equilibrium before analyzing tryptophan fluorescence using the CataCure Diagnostic 1 Equipment supplied by Edinburgh Biosciences.

2.3. Fluorescence measures

The CataCure Diagnostic 1 tool was used for measuring fluorescence intensity, I, at 340 nm and 430 nm after excitation at 310 nm using a light emitting diode (LED) source. In addition to the LED source the CataCure Diagnostics 1 tool consisted of a lens holder, a photomultiplier tube (PMT) and two filters. The LED source was placed in a 90-degree angle to the lens sample (Fig. 2). The $I_{340\ nm}$ can be ascribed to the folded lens proteins containing multiple tryptophan side chains and $I_{430\ nm}$ is non-tryptophan emission (Gakamsky et al., 2011). The fluorescence ratio, F, was calculated as the ratio of $I_{340\ nm}/I_{430\ nm}$. A single measurement was taken from each sample. Bandwidth of the filters used for fluorescence detection was 10 nm. The CataCure Diagnostic 1 was enclosed in a black casing and placed in a dark room during all measurements. Only source of ambient light in the room was the screen of the computer attached to CataCure Diagnostic 1, which was placed on a shelf below the instrument to avoid any direct light entering the casing. The voltage of the LED drive was kept constant during all measurements. This was done by fitting a marker to indicate the right position of the knob. The knob was not turned during the experiment but before each test we made sure that the knob was in the right position. The power supply of the PMT, controlling the sensitivity of the PMT, was kept constant at 0.5 V.

3. Results

The study included lens material from 16 eyes of 14 patients. Mean age was 71.7 years (SD 8.8), 13 were female and 3 were male. Of the 16 lenses 3 were graded in category 1, 7 were graded in category 2, and 6 were graded in category 3 according to the

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