Clinical Detection of Precataractous Lens Protein Changes Using Dynamic Light Scattering

Manuel B. Datiles III, MD; Rafat R. Ansari, PhD; Kwang I. Suh, PhD; Susan Vitale, PhD, MHS; George F. Reed, PhD; J. Samuel Zigler Jr, PhD; Frederick L. Ferris III, MD

Objective: To use dynamic light scattering to clinically assess early precataractous lens protein changes.

Methods: We performed a cross-sectional study in 380 eyes of 235 patients aged 7 to 86 years with Age-Related Eye Disease Study clinical nuclear lens opacity grades 0 to 3.8. A dynamic light-scattering device was used to assess α-crystallin, a molecular chaperone protein shown to bind other damaged lens proteins, preventing their aggregation. The outcome measure was the α-crystallin index, a measure of unbound α-crystallin in each lens. The association of the α-crystallin index with increasing nuclear opacity and aging was determined.

Results: There was a significant decrease in the α-crystallin index associated with increasing nuclear lens opacity grades (P < .001). There were significant losses of α-crystallin even in clinically clear lenses associated with aging (P < .001). The standard error of measurement was 3%.

Conclusions: Dynamic light scattering clinically detects α-crystallin protein loss even in clinically clear lenses. α-Crystallin index measurements may be useful in identifying patients at high risk for cataracts and as an outcome variable in clinical lens studies.

Clinical Relevance: The α-crystallin index may be a useful measure of the protective α-crystallin molecular chaperone reserve present in a lens, analogous to creatinine clearance in estimating renal function reserve.

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Clinical Detection of Precataractous Lens Damage in Clinically Clear Lenses and Identification of Patients at High Risk for Cataracts would be useful for many reasons. This ability would help alert a patient in advance of functional change and allow lifestyle adjustments to reduce factors, such as sun exposure, cigarette smoking, alcohol intake, and poor control of diabetes mellitus, which could increase the risk of developing cataracts. It could also help determine patient eligibility for clinical trials of antcataract drugs because studies have suggested that once lens opacities develop, it may be too late to intervene. Exposure to noxious physical agents (such as cigarette smoke, x-radiation, and sunlight) or drug treatments may also result in an increased risk of cataracts: early detection would be important to assess lens damage and avoidance of these agents or treatments. For example, long-term studies of drugs that lower intraocular pressure have suggested a possible increased risk of nuclear opacity.11 Because the development of overt nuclear cataracts can proceed slowly, a highly sensitive and quantitative means of assessing precataractous changes also would be useful as an outcome variable in clinical trials for assessing treatment effects (protective or toxic) on the lens.

Dynamic light scattering (DLS) was developed more than 30 years ago, and it has been used by numerous researchers to study lens protein changes.14-22 Recently, a new DLS device developed by Ansari et al23-29 was tested in animals under a National Aeronautics and Space Administration–National Eye Institute interagency agreement and was shown to detect lens protein changes much earlier than conventional optical methods. These studies were conducted using different animal models of cataract, including cold cataract and radiation-, diabetic-, selenite- and hyperbaric oxygen–induced cataracts.23-29 Following these findings, a clinical device was developed. The DLS probe was mounted on a movable carriage inside a keratoscope (Keratron; Optikon 2000 SpA, Rome, Italy) with a 3-dimensional aiming system to improve repeatability.21,24,30-32 Preliminary clinical studies with this DLS device demonstrated its safety and the repeatability of its results.31 In this article, we describe further clinical studies using this device to assess early lens changes in a large group of healthy and cataractous individuals in a cross-sectional study, and further laboratory studies to clarify the information obtained using DLS.
In this new clinical DLS system, a beam of light is directed to a specific area in the lens, and light scattered by randomly moving particles in that area is collected during a 5-second interval (Figure 1).31 The time-autocorrelation function of the measurements is then processed to estimate a profile of intensities. Because the intensity is directly related to particle size, this provides an estimate of the frequency distribution of particle sizes. This profile is typically bimodal (see the example in Figure 1): the first peak represents scattering from particles in the size range of α-crystallin proteins (see the following paragraph), and the second peak represents scattering from high-molecular-weight particles, which are mainly large aggregates of lens protein, cellular organelles, and membrane components.17,18,20,21,28

SIGNIFICANCE OF α-CRYSTALLINS

Results of recent studies28,33-45 have highlighted an important role played by α-crystallins in the lens. α-Crystallins are members of the small heat shock protein family and have been found to act as molecular chaperones that prevent the unfolding and uncontrolled aggregation of damaged lens proteins. α-Crystallins have been shown to be highly efficient in recognizing, and binding to, proteins in the early stages of unfolding. Because the formation of large protein aggregates in the lens causes light scatter and leads to cataracts, the net effect of the chaperone activity of α-crystallin is the maintenance of lens transparency. As a person ages, there is a loss of α-crystallins, which diminishes the capacity of the lens to prevent uncontrolled protein aggregation due to the irreversible binding of damaged proteins and the fact that most of the lens lacks the capacity to synthesize new proteins. Previous biochemical analyses33,35-38,46-48 of lens extracts have shown that, whereas young, clear lenses have abundant unbound α-crystallin, eyes with nuclear cataracts have little or none. Thus, lenses remain clear as long as there is available α-crystallin binding capacity. When that capacity is overwhelmed, no further chaperone reserve remains to prevent protein aggregation, and lens proteins aggregate in an uncontrolled manner, resulting in light scattering and opacity. Thus, a measure of the α-crystallin remaining in the lens may reflect the level of protective reserve, much as an assessment of creatinine clearance allows us to estimate the renal functional reserve.

CLINICAL STUDY

We conducted a prospective cross-sectional study of control subjects with clear lenses and patients with varying ages and grades of nuclear lens opacity using the DLS device at the National Eye Institute. This study was approved by the National Eye Institute institutional review board. All the tenets of the Declaration of Helsinki on human subjects were followed, and all the participants gave informed consent. Individuals who had tear film disorders, corneal opacities or disorders, uveitis, or glaucoma were excluded. Those who were thought to be at risk for or who had a history of allergic or adverse reaction to one of the dilating or anesthetic agents used were also excluded. No one was excluded based on sex or race.
RESULTS

LABORATORY STUDY

To determine whether the first peak in the DLS distribution could be attributed to scatter from unbound α-crystallin, we chose a fetal calf lens model to ensure that the crystallins would be in their nascent state without aging effects. Figure 2 shows the results of DLS analysis of the soluble proteins from fetal calf lenses and the purified α-crystallin and the mixture of β- and γ-crystallin proteins. The profile of the complete mixture of soluble proteins showed 2 peaks (similar to the human distributions), with the relative proportion of scattering from the first peak being particularly high. The α-crystallin proteins scattered light only in the first DLS peak; the mixture of β- and γ-crystallin proteins also scattered light in the first peak, as would be expected for these relatively small molecules. These in vitro measurements were made at much higher light intensities than are used clinically because the β- and γ-crystallin fraction did not yield a detectable peak at the intensities used clinically (limited to <100 μW for patient safety). For comparison, the DLS profile for a 28-year-old volunteer with a clear lens nucleus (nuclear opacity grade N=0) is shown in Figure 2D.

In a further experiment, we modeled the effects of aging on the crystallins by exposing solutions of total lens soluble proteins to elevated temperatures (50°C-60°C), inducing denaturation of β- and γ-crystallins. We found that this stress caused the first peak to diminish, with a concomitant increase in the second scattering peak, indicating an increase in protein aggregates. This was demonstrated in Figure 3 by comparison of a representative sample before heating and after 60 minutes at 55°C. Note the shift in both scattering peaks to a higher molecular size and the decrease in area for the first peak and the increase for the second.

CLINICAL STUDY

We examined 235 patients (380 eyes) aged 7 to 86 years. Age-Related Eye Disease Study clinical nuclear lens opacity grades ranged from 0 (clear) to 3.8 (opaque). The Table provides the distribution of age, nuclear lens opacity grade, and ACI values for study participants. The ACI values ranged from 0% to 41.4%. Figure 4 shows a significant decrease in the ACI with increasing nuclear lens opacity grade. For each 1-U increase in nuclear grade, the ACI decreased by 8.9 U (P < .001). Results were similar for photographic grades (data not shown).

In a multiple regression model, ACI values significantly decreased with increasing nuclear grade even after adjusting for age. For each 1-U increase in nuclear opacity grade, the ACI decreased by 4.0 U (P < .001). The mean value of the ACI in clear lenses from control subjects younger than 22 was 31%, whereas the mean value of the ACI in eyes with nuclear grades of 2 or greater (significant
nuclear opacity) was 2%. Two-thirds (67%) of persons who were 70 years or older with a clinical nuclear grade of 2 or more had an ACI of 0%. This finding also suggests that ACI measurements may be most useful and meaningful when used for lenses with nuclear grades of less than 2.

In participants with clinically clear nuclear regions (nuclear grade 0-1), there was a significant decrease in the ACI associated with an increase in age (P<.001) (Figure 5). The mean amount of unbound α-crystallin is highest (ACI=31%) in those younger than 22 and declines at each successive decade, approaching 0% for persons older than 75 years. Thus, even in clinically clear lenses, there are demonstrable losses of α-crystallin associated with aging.

Figure 6 shows the distribution of DLS measurements in a patient with grade 3 cataracts. In this lens, there is complete disappearance of the first peak, representing unbound α-crystallin (ACI=0%), and a single large particle size peak, representing high-molecular-weight particles (mainly large lens protein aggregates, cellular organelles, and membrane components). For comparison, the DLS profile is shown for the clear lens of a 28-year-old individual (nuclear opacity grade N=0). The 2 peaks in the profile indicate higher particle sizes as is the case for all species tested. This is in part due to the increased age of the tissue but also reflects interactions among the protein species in the very protein-dense milieu of the intact lens and the fact that the second peak also includes scattering from cellular constituents, such as membranes and organelles that are removed in the soluble protein preparations.

Figure 7 shows that in the group of older persons aged 60 to 70, a significant decrease in the ACI is associated with increasing nuclear lens opacity grade. For each 1-U increase in nuclear opacity grade, the ACI decreased by 4.8 U (P<.001). This decrease in the ACI remained significant even after adjusting for age within the 60- to 70-year interval; for each 1-U increase in nuclear opacity grade, the ACI decreased by 3.9 U (P<.001). Thus, the ACI may be useful for identifying older persons who still have clear lenses who are at higher risk for cataract development.

Figure 2. Results of dynamic light scattering (DLS) analysis of the soluble proteins from fetal calf lenses. A, The bimodal DLS distribution for the total soluble protein isolated from a fetal calf lens. B, The DLS profile for α-crystallin isolated from the same soluble lens protein preparation. Note that there is only a single scattering peak, which coincides with the first peak in A. C, The DLS profile for the purified β- and γ-crystallins is also a single peak at the leading edge of the first peak in A. This is to be expected because these proteins are much smaller than α-crystallin. D, For comparison, the DLS profile is shown for the clear lens of a 28-year-old individual (nuclear opacity grade N=0). The 2 peaks in the profile indicate higher particle sizes as is the case for all species tested. This is in part due to the increased age of the tissue but also reflects interactions among the protein species in the very protein-dense milieu of the intact lens and the fact that the second peak also includes scattering from cellular constituents, such as membranes and organelles that are removed in the soluble protein preparations.

Figure 3. The effect of heating on the dynamic light scattering profile of fetal calf soluble protein. A, The initial profile obtained before the sample was heated to 55°C. B, The profile of the same sample after 60 minutes at 55°C. Note the shift in scattering intensity from the first to the second peak and the increased particle size seen in both peaks after heating.
For measurement of DLS system error, because there were 2 or 3 independent ACI measurements per eye, a variance components model\(^5\) was fit to the data to estimate the within-eye variance, the square root of which is the standard error of measurement. This estimate was 3%, regardless of the size of the mean ACI.

Figure 8 shows baseline and 11-month follow-up ACI measurements for a 42-year-old patient who had rapid development of presenile nuclear cataracts, providing an example of the potential of this system for use in longitudinal clinical studies of nuclear cataracts.

**COMMENT**

These data show that the clinical DLS device can detect early precataractous protein changes in the living lens nucleus even as the lens remains clinically clear on slit-lamp examination (grades 0-1). Support for the claim that DLS detects \(\alpha\)-crystallin is provided by the biochemical studies, which demonstrate that the low-molecular-weight component (the first peak in the DLS measurement profile, ie, the ACI) represents unbound \(\alpha\)-crystallin (Figure 2).\(^{19,21,26-29}\) Based on these clinical and laboratory results, we propose the ACI as a clinical measure of unbound \(\alpha\)-crystallin available as molecular chaperones in the lens nucleus (\(\alpha\)-crystallin reserve) and that it will be useful as a screening tool and as an outcome variable.

In this study, we found a strong association between clinical DLS measurements, represented by the ACI, and nuclear lens opacity grading and aging. The association between ACI and nuclear lens grade remains even after adjusting for age. However, these data provide evidence that ACI changes may precede the appearance of clinically observable cataracts because the ACI was 0% for clini-
Loss of α-crystallin has recently been found to be associated with presbyopia. The ACI may therefore also be useful in studying, in middle-aged patients, presbyopia and other bothersome visual symptoms that are undetectable by slitlamp and other optical examinations at the molecular level.

The DLS measurements also may be helpful in a variety of clinical situations. For example, in patients with glaucoma, those who have had cornea transplantation, or those who have had retinal surgery and display an ACI of 0% in their lenses, a combined cataract procedure may be preferred. When deciding between laser in situ keratomileusis and lens exchange surgery, if the patient’s ACI is low or close to 0%, lens exchange may be preferred because cataract surgery may be needed in the near future anyway. For our National Aeronautics and Space Administration collaborators, ACI measurements may help assess the effects of cosmic radiation in outer space, may aid in developing methods to prevent lens damage from radiation, and may help select individuals at lowest risk for long missions.

In summary, this study demonstrates the usefulness of DLS measurements in assessing early precataractous lens changes. The ACI may be useful in future studies as a screening tool to detect individuals at high risk for cataracts and as a reproducible, sensitive outcome variable in clinical trials or epidemiologic studies. We present data supporting the applicability of the ACI as a measure of α-crystallin chaperone reserve in the lens nucleus, reflecting the risk of cataracts in an individual.

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Correspondence: Manuel B. Datiles III, MD, National Eye Institute, National Institutes of Health, 10 Center Dr, Bldg 10, Room 10N226, Bethesda, MD 20892-1860 (DatilesM@NEI.NIH.GOV).

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