Article

A Rayleigh Scatter-Based Ocular Flare Analysis Meter for Flare Photometry of the Anterior Chamber

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Purpose: Existing flare photometers are based on the Tyndall effect, which requires sophisticated laser photometry. The ocular flare analysis meter (OFAM) is a nonlaser photometer that uses quantitative Rayleigh scatter and absorption from visible light to compute a flare value. This study is designed to correlate OFAM measurements with qualitative measurements of flare in vitro and in vivo.

Methods: Following validation of the device on artificial anterior chambers containing known protein concentrations, flare readings were obtained from 90 subjects (46 with and 44 without uveitis) in one eye. Subjects were graded by the Standardization of Uveitis Nomenclature (SUN) working group flare scoring system and received the OFAM flare measurements.

Results: The OFAM showed linear response in vitro to protein concentrations ranging from 0 to 0.5 mg/ml. In clinical use in subjects ranging from SUN flare scores of 0+ to 2+, OFAM showed statistically significant measurement accuracy ($P = 0.0008$ of flare 0 versus flare 2; $P = 0.031$ of flare 0 versus flare 1). Distinction of SUN scores 1 and 2 was borderline significant ($P = 0.057$).

Conclusion: The OFAM photometry correlates with the standard SUN scoring system. This method may provide an objective method to diagnosis and monitor uveitis. Further longitudinal studies are warranted.

Translational Relevance: Currently, ocular flare is assessed qualitatively in most clinical settings. The existing methodology uses only Tyndall effect to measure flare. The OFAM uses an alternate, nonlaser means for measurement of anterior chamber flare by measure of Rayleigh scatter. This pilot clinical study suggests that the OFAM device may be useful in measurement of uveitis activity.

Introduction

Uveitis describes a group of sight-threatening and potentially blinding ocular inflammatory conditions. The activity of uveitis in a patient typically is evaluated in part by grading the relative number of white cells ("cell") and light scatter ("flare") seen in the anterior chamber of the eye using a slit-lamp biomicroscope. The Standardization of Uveitis Nomenclature (SUN) criteria normalized scoring for these measures.¹ Cells are counted in a field size of 1×1 mm slit-beam and scored based on the number of cells observed (0 [<1 cell], 0.5+ [1–5 cells],

1+ [6–15 cells], 2+ [16–25 cells], 3+ [26–50 cells], and 4+ [>50 cells]). The grading for flare is less quantitative, based on 0 (none), 1+ (faint), 2+ (moderate, iris, and lens details clear), 3+ (marked, iris, and lens details hazy), and 4+ (intense, fibrin, or plastic aqueous).

Several studies have demonstrated that quantitative measurement of chronic flare may be a better predictor of outcome than cell in some types of uveitis, particularly juvenile uveitis,^{2–7} suggesting there is clinical use in quantification of flare. Flare photometry is a quantitative and objective method for measuring the amount of flare in an eye. In flare

photometry, the degree of flare is calculated from the Tyndall effect scatter of a laser light beam directed into the eye. Despite apparent utility in assessment of uveitis, laser flare photometry has not achieved widespread clinical use.

We describe a novel Ocular Flare Activity Meter (OFAM), which uses an alternative measure to calculate flare values based on Rayleigh scattering rather than the Tyndall effect. Rayleigh scattering describes the physical scattering of light by small molecules and may be calculated from nonlaser light sources by measuring the angle of scatter and response at several specific wavelengths. This method is fundamentally more sensitive than Tyndall methods in measuring concentration of small molecules in an aqueous medium. The OFAM illuminates the anterior chamber of the eye with 3 light-emitting diodes (LED) in the visible wavelength range and analyzes the scattered light that returns from the eye to calculate a flare value. In the current pilot study, we determined the correlation of OFAM readings with flare measures in vitro and in vivo in subjects with and without uveitis.

Methods

Device Construction

Rayleigh scattering refers to the elastic scattering of light by interaction with particles smaller than the wavelength of the incident light. Equation 1 describes the Rayleigh scattering phenomena where the intensity of light received at a detector/receiver (I) is inversely proportional to the fourth power of the wavelength of light (λ), cosine of the angle between the source and receiver (θ), radius of the particle (r), its molecular weight (M), Avagadro's number (L), its concentration in an aqueous medium (c), and the concentration and wavelength (dispersion) dependent refractive index of the colloidal medium ($n[c, \lambda]$). Equation 2, known as Sellmeier's equation, describes the wavelength dependence of the refractive index of a colloid. Parameters A , B_1 , B_2 , C_1 , and C_2 in the Sellmeier's equation are empirically determined and describe the optical dispersion of a colloid.

$$I[\theta, \lambda, c] = \frac{2\pi}{r^2 \lambda^4} \frac{n^2[c, \lambda]}{L} \left(\frac{dn[c, \lambda]}{dc} \right)^2 Mc(1 + \cos^2 \theta) \quad (1)$$

$$n[c, \lambda] = A + \frac{B_1 \lambda^2}{\lambda^2 - C_1} + \frac{B_2 \lambda^2}{\lambda^2 - C_2} \quad (2)$$

The OFAM device (Fig. 1) illuminates the anterior chamber with an array of LEDs at multiple wavelengths and measures scattered light from the anterior segment of the eye with a highly sensitive avalanche photo-multiplier detector (Hamamatsu PM; Hamamatsu Photonics, Hamamatsu City, Japan). The system uses three wavelengths (405, 465, and 525 nm), and measures Rayleigh scatter from proteins and other macromolecules in aqueous humor (AH) at multiple angles. Scattering information at multiple wavelengths and angles is processed through a set of algorithms that correct for measurement artifacts, including optical absorption in the optical path from cornea and AH, and variation in corneal transparency and iris pigmentation.

θ is fixed by the optical assembly at approximately 70° , and I_0 is the controlled intensity of a given LED of wavelength λ (405, 465, and 525 nm). Each LED source produces incoherent light with a spectral bandwidth of approximately 2 nm. The subject is positioned in a standard slit-lamp chinrest, and values are obtained from the eye in approximately 30 seconds. Pupil alignment software processes an image of the subjects' eye captured with a built-in alignment camera and initially guides the operator to position the OFAM optics such that the LEDs form a 2-mm spot on the cornea at the center of pupil. Once alignment is completed, data collection is initiated. During data collection, the LED sources are intensity modulated by sequentially turning them on and off. As a result, the scattered signal from only one of the three sources reaches the detector at any given instance. Closed loop software processes the pupil position camera feed in real time and only collects the scattered light when the instrument is in correct alignment. This ensures that all scattered light is collected with a spatial accuracy of approximately 0.1 mm. The scattered light collected from the anterior chamber at each LED wavelength is processed offline by the Rayleigh scattering equation by the normalization method given by Equation 3.

$$OFAM\# = H \frac{I[\theta, \lambda_{405}, c] - I[\theta, \lambda_{525}, c]}{I[\theta, \lambda_{465}, c] - I[\theta, \lambda_{525}, c]}, \quad (3)$$

where $I[\theta, \lambda_n, c]$ is scattered signal measured for a given LED of wavelength λ_n given by Equation 1 and meets a signal quality threshold determined empirically for the instrument. H is a normalization factor associated with the selection of the incident power of LEDs.

Figure 1. Photograph of the OFAM device. Three LEDs (405, 465, and 525 nm) are integrated to illuminate the eye. The scattered light is collected by a photomultiplier-based detector. A camera feed is integrated with a software user interface to guide the physician to position the instrument with the patients' eye. The OFAM is mounted on a standard slit-lamp device.

In Vitro Study

Five dilutions of bovine serum albumin (BSA) between 0.1 and 0.5 mg/mL in phosphate buffered saline (PBS) were measured using the OFAM instrument. The range of protein concentration was selected to cover physiological range of normal (0.2 mg/mL) and 2+ flare (~0.4 mg/mL). The samples were placed in a cylindrical cuvette and held in place with a custom-made cuvette holder simulating an artificial anterior chamber. During the measurement, OFAM pupil detection algorithms were turned off.

Clinical Study

This study was designed in compliance with adherence to the Declaration of Helsinki. Institutional review board approval of the University of Washington Human Studies Division was obtained and informed written consent was obtained from all participants. Investigational device exemption (IDE) approval for this nonsignificant risk study was obtained from the University of Washington Human Studies Division institutional review board. This pilot study was registered with clinicaltrials.gov (clinicaltrials.gov ref #NCT01897935).

Inclusion criteria for participation in the study were: subject age greater than 18 years, ability to

understand and willingness to sign a written informed consent document, eligibility for the uveitis arm of the study by the presence of active anterior chamber inflammation in the study (left) eye as determined by the ophthalmologist, and eligibility for the noninflammatory arm of the study by having no history of ocular inflammation in either eye as determined by the ophthalmologist. Exclusion criteria for participation were: inability to give informed consent, no active disease (uveitis arm of the study), and signs of current or previous uveitis (noninflammatory arm).

For each subject, demographic data, including age; sex; ethnicity; underlying diagnosis; presence and doses of immunomodulating drugs; vision; clinical cell on exam; clinical flare on exam; laterality; intraocular pressure; and presence or absence of posterior synechiae, cystoid macular edema, cells in the vitreous, and keratic precipitates, were recorded. Standard ophthalmic examination including SUN slit-lamp grading of flare were performed by one investigator (RVG) before pupillary dilation. To ensure consistency, the same eye (left) was measured by OFAM.

Statistical Analysis

Linearity of OFAM response to in vitro protein concentrations was determined by least squares

Figure 2. OFAM measurement of known protein concentrations in artificial anterior chamber. The normalized response of the scattered light measured by OFAM is shown for all the LEDs. The 95% confidence intervals are shown in blue.

regression fit (Microsoft Excel; Microsoft Corp., Redmond, WA). For the clinical study, statistics were performed using Statistica (StatSoft, Inc., Tulsa, OK), using analysis of variance (ANOVA) to determine overall significance for correspondence of clinical and OFAM flare scoring, and post-hoc Tukey test with Holm inference testing for pairwise significance testing.

Results

To establish repeatability and reproducibility of the device, we tested the ability of OFAM to measure flare caused by BSA in PBS in an artificial anterior chamber. The OFAM response was linearly proportional to concentration (Fig. 2; $R^2 = 0.92$, $P = 0.0091$). Based on these results, the instrument shows a limit of detection <0.1 mg/mL and a dynamic range of >0.5 mg/mL. It should be noted that normal human eyes have a total protein concentration of approximately 0.14 mg/mL and a 2+ flare categorization by the SUN working group¹ is equivalent to a protein concentration of approximately 0.4 mg/mL (2 times the normal protein concentration in the human eye).⁸

We next sought to determine if OFAM readings correlate with SUN flare assessments in a clinical population. A total of 67 subjects with and 54 without uveitis were enrolled in the study. Demographics of enrolled subjects are summarized in Table 1. Satisfactory OFAM readings were obtained in 90 of 121

Table 1. Median Demographic and Exam Findings of Subjects in Clinical Study

	Uveitis	Control
N	46	44
Age, y	42	50
Sex	F:31; M:15	F:21; M:23
Median visual acuity	20/50	20/25
Median cell	1+	0
Median flare	1+	0

subjects. Reasons for inability to obtain OFAM readings included: macular scars precluding fixation, marked keratic precipitates on the endothelium, aphakia, and subject not able to sit for the entire measurement, all of which limited the ability of our prototype to obtain adequate readings. Of those subjects with successful OFAM recordings, 44 had 0, 34 had 1+, and 12 had 2+ flare scored by one examiner (RVG) by SUN criteria.

Mean normalized OFAM readings for each group were 0.10 ± 0.24 for 0 flare, 0.85 ± 0.22 for 1+ flare, and 1.8 ± 0.54 for 2+ flare (overall $P = 0.001$ by ANOVA; Fig. 3). ANOVA with post hoc analysis (Tukey method with Holm inference for multiple comparisons) demonstrated significance for the difference between 1+ and 0 flare ($P = 0.031$) and between 2+ and 0 flare ($P = 0.0008$). The difference in OFAM measurement between 1+ and 2+ flare reached borderline significance ($P = 0.057$).

Discussion

Ocular flare is a critical measure in the assessment of patients with uveitis. For certain forms of uveitis, flare has been shown to be a strong predictor of outcomes, particularly in chronic uveitis in children,^{3–6} HLA-B27-associated uveitis,⁹ and postcataract surgery inflammation,^{10,11} among others.³ Flare measurements also have been used to monitor disease course and response to therapy.^{11–13} Qualitative scoring of flare has been used for many years¹⁴ and was clinically defined by the SUN working group.¹ While physical exam grading of flare is rapid and straightforward, validation of scoring between expert observers has revealed only moderate concordance level ($\kappa = 0.5–0.64$).¹⁵ Because of the qualitative nature of this scoring system and lack of clear cut-off between levels it is unlikely to achieve higher levels of concordance.

Laser flare photometry has been used for the past 25 years for more precise, quantitative monitoring of

Figure 3. OFAM readings for subjects with various levels of clinical flare. Box and whisker plots for normalized OFAM readings. Box represents limits of second and third quartile; bold horizontal band is median and small cross is mean. Whiskers represent 95% percentile. Width of box proportional to number of subjects. P values from post hoc ANOVA analysis.

flare.^{16–18} Currently, the sole FDA-approved bench-top ocular flare meter is the KOWA FM series. The KOWA Flare Meter consists of a laser (630 nm) and photodetector. The laser scans the anterior chamber of the eye at a grazing angle. The change in signal received at the photodetector is used as a direct measure of scatter (Tyndall effect) by the cells and proteins (flare) present in the anterior chamber. While this technique has been well-validated in a number of studies as being reproducible and tracking closely with clinically observed inflammation,⁵ the instrument has had limited clinical use.¹⁶

Rayleigh scattering is an alternative method for assaying for the presence of proteins and other small molecules in a liquid. It has the advantage of using a noncoherent (i.e., nonlaser) light source and is rapid in practice. The Rayleigh and Tyndall methods rigorously describe the phenomenon of classical scattering of light by particles suspended in an aqueous/gaseous medium. However, Tyndall equations are suitable primarily for measuring concentration of colloidal particles that are larger (~400–900 nm) when using a light source in the visible spectrum. In contrast, the Rayleigh scattering method provides a more complete description of

the underlying phenomenon of scattering by particles, including those that are significantly smaller than the wavelength of light. In visible wavelengths, this translates to particle sizes in the range of few nm to approximately 100 nm. For reference, a large spherical 500 kD protein is expected to have a diameter of approximately 10 nm.¹⁹ As a result, a system based on Rayleigh scattering, such as the OFAM, may be fundamentally more sensitive to small/trace protein concentrations in the AH as the Tyndall effect would be expected to detect primarily protein aggregates, which may occur only at higher concentrations. For instance, in studies using two-dimensional gel electrophoresis of aqueous humor, approximately one-third of the identified proteins are below 50 kD in size,^{20,21} which would not produce a significant Tyndall effect under visible light. Similarly, the major upregulated protein found in aqueous humor of patients with juvenile arthritis-associated uveitis is transthyretin,²² which has a molecular weight of 13 kD and an expected diameter of 3 nm. Based on Equation 1, it would be predicted that the Rayleigh-based OFAM system would have approximately 6 times stronger signals when compared to laser flare- or slit-lamp-based Tyndall

observational methods for equivalent concentrations of protein. Additionally, the OFAM device is able to sample a larger proportion of the aqueous than current laser flare photometers. The KOWA system, for example, probes an approximately 0.075 mm^3 volume, compared with an observed slit-lamp volume of 1 mm^3 . The OFAM system uses a 2-mm diameter LED beam and has a 6-mm^3 analytic volume, which may further increase sensitivity for detection of inflammation.

We showed in the current preliminary studies that the OFAM device functions accurately in vitro to measure protein concentrations linearly in artificial aqueous humor over a greater than 5-fold range corresponding to physiologic concentrations. We further showed in a pilot clinical study that OFAM readings correlated with clinical flare readings in subjects with and without active uveitis. These studies are similar to early studies on laser flare photometry, which also demonstrated linear response to in vitro BSA concentrations⁵ and general correlation with observed qualitative flare levels.^{5,18,23–25} As in previous studies with laser flare photometry (see Fig. 3 of the study of Ladas et al.⁵) we saw substantial range of flare values for subjects within one clinical grade, and saw substantial overlap in grading of individuals with particular flare values.

The current study has several limitations. First, OFAM was used to characterize a single point in time in the clinical study. As initial severity of uveitis is not difficult to assess, the true use of OFAM would be in longitudinal analysis of patients, which awaits future study. Additionally, flare was scored by only a single investigator. It is known that interobserver reliability is only moderate for flare between observers.¹⁵ Thus, the observed correlation between OFAM and clinical grading may understate the agreement on cases with clear-cut agreement between multiple observers. In the current study, no patients had 3+ or 4+ flare; future studies will need to assess this uncommon but important population of uveitis patients. Finally, the observed correlations were based on analysis of the population. Individuals within this population showed variability, and there was some overlap between outliers with no uveitis but higher OFAM scores and vice versa. Analysis of the origin of outlier signals will be important for future use of this device.

While the OFAM showed high correlation with clinical flare scoring generally, we observed substantial variation in readings even among patients with

minimal flare. This led to some patients with minimal clinical flare having higher scores than individuals with 2+ flare. As clinically observed flare is a manifestation of the Tyndall effect, these disparities may reflect the greater sensitivity of a Rayleigh-based method to the presence of small molecules in the aqueous. Thus, subjects with high concentrations of small metabolites or peptides, which would not produce visible flare or significant Tyndall laser flare readings, could have elevated readings with devices using Rayleigh scatter. Further study is required to determine the genesis of these signals and their clinical significance.

The OFAM was not able to generate reliable readings from all subjects. Opacities in the cornea, such as dense keratic precipitates, pose a challenge for any flare reading device. The device works optimally with nondilated pupils; patients with postoperative mydriasis from surgical aphakia currently pose difficulty for the pupil tracking component of the device, as do patients with marked synechiae resulting in chronic effective mydriasis. We have not yet evaluated the effect of changing pupil size on serial measurements. Inability to fixate similarly poses a challenge to the device. The requirement of the patient to fixate for 30 to 60 seconds may be problematic in children or patients with attention deficit. The current study has identified these as areas for improvement in the prototype device. Further potential challenges for this device may be common to those faced by laser flare photometry, and include pupil size, changes with aging and cataract, diurnal changes in aqueous protein, changes in constituents of aqueous, and use of medications (reviewed by Ladas et al.⁵).

Availability of a Rayleigh-based flare meter may allow more detailed analysis of uveitis and its response to therapy. In particular, it will be of interest to see if changes in measured Rayleigh scatter flare associated with subclinical qualitative flare scores are predictive of disease course. Subtle increases in laser flare readings ($\sim 20\%$) have been associated with increased risk of full disease recurrence in a cohort of patients with pars planitis, Behcet disease, and sarcoidosis.²⁶ In a similar study, subclinical increases in of Tyndall laser flare readings have been associated with HLA-B27 disease recurrence.⁹ Refinement of the current prototype will allow formal testing including direct comparison with laser flare photometry and longitudinal testing in patients under therapy.

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