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Seasonal Effect on Ocular Sun Exposure and Conjunctival UV Autofluorescence

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Abstract

Purpose—To evaluate feasibility and repeatability of measures for ocular sun exposure and conjunctival ultraviolet autofluorescence (UVAF), and to test for relationships between the outcomes.

Methods—Fifty volunteers were seen for 2 visits 14±2 days apart. Ocular sun exposure was estimated over a two-week time period using questionnaires that quantified time outdoors and ocular protection habits. Conjunctival UVAF was imaged using a Nikon D7000 camera system equipped with appropriate flash and filter system; image analysis was done using ImageJ software. Repeatability estimates were made using Bland-Altman plots with mean differences and 95% limits of agreement calculated. Non-normally distributed data was transformed by either log10 or square root methods. Linear regression was conducted to evaluate relationships between measures.

Results—Mean (±SD) values for ocular sun exposure and conjunctival UVAF were 8.86 (±11.97) hours and 9.15 (±9.47) mm², respectively. Repeatability was found to be acceptable for both ocular sun exposure and conjunctival UVAF. Univariate linear regression showed outdoor occupation to be a predictor of higher ocular sun exposure; outdoor occupation and winter season of collection both predicted higher total UVAF. Furthermore, increased portion of day spent outdoors while working was associated with increased total conjunctival UVAF.

Conclusions—We demonstrate feasibility and repeatability of estimating ocular sun exposure using a previously unreported method and for conjunctival UVAF in a group of subjects residing in Ohio. Seasonal temperature variation may have influenced time outdoors and ultimately calculation of ocular sun exposure. As winter season of collection and outdoor occupation both predicted higher total UVAF, our data suggests that ocular sun exposure is associated with

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APPENDIX

Appendix 1, the questionnaire that shows how subjects were questioned about the proportion of time ocular protection measures were used, is available at [LWW insert link].

Appendix 2, the questionnaire that shows how subjects were asked to estimate the number of hours spent on outdoor activities, is available at [LWW insert link].

conjunctival UVAF and possibly, that UVAF remains for at least several months following sun exposure.

Keywords

ocular sun exposure; conjunctiva; ultraviolet autofluorescence; imaging; repeatability

Ultraviolet (UV) radiation exposure is a significant public health problem. The World Health Organization (WHO) estimates UV exposure is responsible for significant loss of quality of life and approximately 60,000 premature deaths annually.¹ Ambient UV exposure is influenced by distal environmental factors such as cloud cover and latitude, and is further modified by proximal individual factors such as behavior and skin pigmentation. While environmental factors may have equal effects on UV exposure to a population at a specific time and location, significant variance is likely to occur on an individual level. Because methods to evaluate ocular UV exposure are not yet fully developed, many studies report values for ocular UV exposure based on dermatology research which includes factors such as skin pigmentation and sun sensitivity.²⁻⁶ However, the nature of the ocular surface is quite obviously different than that of the skin suggesting that estimations of cutaneous exposure may not be relatable to ocular UV exposure.⁷

While UV exposure is known to be beneficial for systemic vitamin D production, no ocular benefits are known. In fact, the WHO identified nine diseases with enough support in the literature to be associated with UV exposure; among those nine diseases, three are specific to the eye: cortical cataract, pterygium, and squamous cell carcinoma of the cornea and conjunctiva.¹ Assessment of UV exposure in most epidemiologic studies has been through methods subject to recall bias and with disregard for individual activities outside of personal protection habits.⁸ Subsequently, many epidemiologic studies report associations (or lack of association) between UV exposure and disease based on subject recall for place of residence and occupation compared with recorded ambient UV levels for each reported location.⁸ Potential for error is significant using this method as ambient UV levels vary up to 30 percent dependent on factors such as season and shade cover.⁹ Hence, for this reason and those discussed earlier, attempts to assess lifetime UV exposure may have limited relevance for assessing correlations to disease development for individuals. Support for establishment of alternative methods to determine individual levels of ocular sun exposure is apparent.

Conjunctival ultraviolet autofluorescence is supported as a reliable and valid biomarker for time spent outdoors by evidence from studies performed in Australia and the Norfolk Island Eye Study.¹⁰ Clinical support for the utility of conjunctival ultraviolet autofluorescence was demonstrated in subjects with pterygia¹¹ and in those with myopia.¹² As epidemiological evidence supports increased incidence of pterygia¹³ and decreased onset of myopia¹⁴ being associated with time spent outdoors, the possibility of conjunctival ultraviolet autofluorescence as a clinically useful biomarker is noteworthy. In fact, the association for myopia protection was stronger for conjunctival ultraviolet autofluorescence than for time outdoors.¹² Additionally, a dose-response increase in conjunctival ultraviolet autofluorescence was noted with increased time outdoors for those with pterygium.¹¹ Further support is given to conjunctival ultraviolet autofluorescence as a biomarker for

chronic time outdoors in schoolchildren as ultraviolet autofluorescence is more apparent in those who spend more time outdoors and with increasing age.¹⁵ Of interest, although 81% of schoolchildren ages 12 to 15 years showed evidence of conjunctival ultraviolet autofluorescence, clinical evidence of conjunctival tissue changes were not frequently found.¹⁵ Consequently, based on this evidence, it was hypothesized that conjunctival ultraviolet autofluorescence may be useful in detecting areas of preclinical tissue changes thought to be associated with UV radiation exposure, specifically as a sub-acute ocular UV dosimeter.

The present study assessed ocular sun exposure using questionnaires and measurements of conjunctival ultraviolet autofluorescence using a camera system built from currently available components. Specifically, the purpose of this study was to evaluate feasibility and repeatability of measuring ocular sun exposure with novel surveys to reflect individual outdoor behaviors and to evaluate the relationship of ocular sun exposure with conjunctival ultraviolet autofluorescence. We anticipated that subjects would show increased conjunctival ultraviolet autofluorescence with higher amounts of ocular sun exposure.

METHODS

Subjects

Fifty volunteers were seen for 2 visits 14 ± 2 days apart. As this study was designed as a feasibility study, inclusion and exclusion criteria were liberal; all adults 18 years and older were included, with exclusion only for those who self-reported 1) currently pregnant or lactating, or 2) sporadic use of topical ocular medications. Prior to initiation of the study, this research was approved by The Ohio State University Institutional Review Board. Informed consent documents that followed the tenets of the Declaration of Helsinki were obtained from each subject. Following in-person screening for ocular pathology by slit lamp examination, a brief medical history questionnaire and the Ocular Surface Disease Index (OSDI, Allergan)¹⁶ were completed, in addition to ocular sun exposure questionnaires.^{11, 17} For contact lens wearers, current contact lens brand information was collected from each subject to determine UV-absorbing status.

Ocular Sun Exposure Questionnaires

Ocular sun exposure was estimated over the most recent two-week time period by subject responses on two questionnaires. First, a questionnaire of ocular protection habits was administered (see Questionnaire, Appendix 1, available at [LWW insert link] that shows how subjects were questioned about the proportion of time ocular protective measures were used).¹¹ In this questionnaire, subjects were asked to categorize the proportion of time spent wearing ocular protective measures including prescription glasses, protective hats, and sunglasses when outdoors. To minimize recall bias, participants were asked to estimate the proportion of time each protective measure was worn using nominal categories: never, seldom, half time, usually, and always.¹¹ These categories were subsequently used for quantitative analyses with scaled assignments: never = 0, seldom = 0.25, half time = 0.50, usually = 0.75, and always = 1.00. In addition, each protective measure was assigned a number relative to their ocular exposure specific effectiveness¹³: no protection = 1.00, hat

alone = 0.65, clear spectacles alone = 0.23, dark spectacles alone = 0.11, hat with clear spectacles = 0.11, hat with dark spectacles = 0.07. To further clarify this component of ocular exposure calculations, “no protection” provides no ocular exposure protection, thus is given a proportion of 1.00, or 100% ocular exposure. Alternatively, “hat with dark spectacles” provides ocular exposure protection of about 93%, or an ocular exposure proportion of 0.07, or 7%.

A second questionnaire was used to estimate the hours each participant spent in outdoor activities (see Appendix 2, available at [LWW insert link], which shows how subjects were asked to estimate the number of hours spent on outdoor activities). The outdoor activity questionnaire asked each participant to estimate the number of hours spent on each of seven categories: driving, gardening, home, walking/light exercise, recreation, water, and leisure.¹⁷ As driving represents a special case of assumed 0% UV transmission due to UV protection inherent in car windshields, the hours reported spent driving effectively did not contribute to the final value obtained.¹⁸

Ocular sun exposure was calculated based on the combined outcomes of the two questionnaires. In general, ocular sun exposure was the sum of percent UV transmission for time spent in each condition reported. A summary formula is presented, followed by a detailed example of the calculation. As driving represents a special case of assumed 0% UV transmission, the formula includes a term indicating time spent driving did not effectively contribute to the final estimate. First, time spent driving was subtracted from the total time reported in outdoor activities to calculate time exposed spent outdoors.

$$t_e = T_o - t_d$$

The summary formula follows as:

$$T_{OSE} = U_p t_p + U_o(t_e - t_p) + U_d t_d$$

T_{OSE} : Time of effective ocular sun exposure (hours)

U_p : Percent UV transmission of protective measures

t_p : Time protective measures used (hours)

U_o : Percent UV transmission outdoors (100%)

t_e : Time exposed spent outdoors (hours)

T_o : Total time spent in outdoor activities (hours)

U_d : Percent UV transmission while driving (0%)

t_d : Time spent driving (hours)

As an example for calculation of ocular sun exposure, consider a participant reporting 40 hours outdoors with 9 of those hours spent driving, for a net total of 31 hours of time exposed. During 31 exposed hours outdoors and wearing sunglasses half time, ocular sun

exposure would be calculated as follows: Thirty-one hours outdoors multiplied by 11% (ocular protection effectiveness for sunglasses) and by 50% (half time wear of sunglasses), then added to thirty-one hours multiplied by 100% (full ocular sun exposure) and by 50% (for half time when sunglasses were not worn). Hours spent driving do not effectively contribute to the estimate as car windshields were assumed to have 0% UV transmission. An equation would appear as below:

$$\text{Ocular sun exposure} = (31 \times 0.11 \times 0.50) + (31 \times 1.00 \times [1 - 0.50]) + (0 \times 9) = 17.21 \text{ hours}$$

Conjunctival Ultraviolet Autofluorescence

Conjunctival ultraviolet autofluorescence was evaluated with the goal of confirming an objective biomarker of UV exposure in our patient population. Conjunctival ultraviolet autofluorescence images were obtained using a camera with appropriate flash and filter system.¹² The camera system was comprised of a Nikon D7000 Digital SLR camera body, 16.2 megapixel (Nikon USA, Melville, NY), fit with a Nikon AF-S VR Micro-NIKKOR 105mm f/2.8G IF-ED lens (Nikon USA, Melville, NY). The camera lens was fit with a Hoya 62mm HMC UV-IR filter (Hoya/Kenko Tokina Co., Ltd., Tokyo, Japan) and a Hoya 62mm polarizer (Hoya/Kenko Tokina Co., Ltd., Tokyo, Japan). In addition, a Nikon R1 Speedlight kit (Nikon USA, Melville, NY) was attached to the system. Flash units on the kit were modified by replacing flash covers with non-UV absorbing glass slides. Additionally, custom holders for necessary flash filters were machined to fit the modified flash units.

Control and ultraviolet autofluorescence images were obtained for temporal and nasal regions of each eye for 34 subjects. Control images were obtained first in normal room lighting to document clinically evident pathology and to aid in identification of subjects and their orientation; the camera was set at 200 ISO and Baader 2" Polarization Filters for Herschel Wedge (Baader Planetarium, Germany) in place over the flash units. Next, for ultraviolet autofluorescence images all room lights were off, the camera was set at 1600 ISO and Baader U-Filter Venus UV ZWL 350nm filters (Baader Planetarium, Germany) were placed over the flash units. A penlight was used to assist in positioning and focusing, yet was turned off prior to image acquisition. Images were saved in RGB JPEG format with 1:1 magnification.

Areas of conjunctival ultraviolet autofluorescence were analyzed using ImageJ (National Institutes of Health) software. Image analysis was conducted with minimal light in a darkened, windowless room using a single computer monitor for improved contrast detection, accuracy of image tracings, and subsequent measurements. First, a millimeter (mm) ruler image taken at 1:1 magnification was used to calibrate the measurement scale; the straight line selection tool in ImageJ was used to measure the distance in pixels for a known ruler distance of 3mm. Details measured were entered into ImageJ under the "Analyze" then "Set Scale" menus as follows: distance in pixels: 632; known distance: 3; pixel aspect ratio: 1.0; unit of length: mm; "Global box" was checked. Therefore, the scale was set at 210.67 pixels/mm. Next, to enhance edges of complex low contrast ultraviolet autofluorescence areas, all ultraviolet autofluorescence images were converted to grayscale in 8-bit format using the "Image", "Type", and "8-bit" menu selections. In comparison with

8-bit format, conversion to 16-bit format was found to increase brightness of both the areas of ultraviolet autofluorescence and of the non-fluorescent background, thus slightly diminishing detection of ultraviolet autofluorescence areas. Conjunctival ultraviolet autofluorescence area was estimated by outline tracing with the freehand area selection tool to allow for maximum tracing flexibility (Figure 1). Measurements of the areas in square millimeters were calculated by ImageJ using the “Analyze” then “Measure” selections. Each area for the nasal and temporal regions was measured twice and the average obtained. Subsequently, total ultraviolet autofluorescence was calculated by adding the average areas for each region of both eyes, or the sum total of four measured areas per subject.

Statistical Analysis

Data was analyzed using IBM SPSS Statistics 21 (IBM Corporation, Armonk, New York, USA) software. Means, standard deviations, percentages, and frequencies were used to summarize data collected. In addition, repeatability based on test-retest variability for ocular sun exposure and conjunctival ultraviolet autofluorescence was evaluated using Bland-Altman plots. Full data sets for ocular sun exposure and conjunctival ultraviolet autofluorescence were identified as non-normally distributed; hence each was transformed to best approximate a normal distribution (ocular sun exposure: $\log_{10}$ transformation; conjunctival ultraviolet autofluorescence : square root transformation) for comparison with raw data. Univariate linear regression was conducted to evaluate relationships between measures. Statistical significance was accepted at probability level less than 5%.

RESULTS

Subject Characteristics

The study included 50 subjects, 58% female, with a mean age of 38 ± 13 (range 21-64) years from The Ohio State University, College of Optometry. Ocular sun exposure was calculated for all 50 subjects. Nine subjects reported outdoor occupations, 41 reported indoor occupations. Conjunctival ultraviolet autofluorescence images were acquired from 34 subjects, including all subjects with outdoor occupations. Four subjects were classified as having mild dry eye by the OSDI. Corrective lenses were worn by 30 subjects: 20 spectacles, 10 contact lenses. Of the 10 subjects who wore contact lenses, three reported wearing known UV absorbing lenses, three reported wearing known non-UV absorbing lenses, and the remaining four were not aware of the specific brand of contact lenses worn. All subjects completed the study and there were no adverse events.

Descriptive Statistics

Descriptive statistics were generated for measures of ocular sun exposure and conjunctival ultraviolet autofluorescence (Table 1).

Bland-Altman scatter plots were generated for ocular sun exposure (Figure 2) and conjunctival ultraviolet autofluorescence (Figure 3) to obtain estimates of repeatability based on test-retest variability for each measure, with mean differences (bias) and 95% limits of agreement calculated (Table 1). Additionally, seasonal effects for ocular sun exposure were evaluated using Bland-Altman plots comparing spring and winter season of collection

(Figures 4 and 5). A range of agreement was defined as mean bias $\pm$ 1.96 standard deviations.

Linear Regression Analyses

Univariate linear regression was conducted to identify covariates between variables (Table 2). Sensitivity testing was done for specific concerns. First, sensitivity was tested for the single outlier for ocular sun exposure (Figure 2) by elimination; no significantly different results were found. Second, the four subjects determined to have mild dry eye based on their responses to the OSDI were eliminated from the data set; again, no significantly different results were found. Linear regression was also performed for subject reported portion of time spent on activities by visit.

Ocular Sun Exposure—Due to the exploratory nature of the present study, univariate linear regression for ocular sun exposure was conducted to identify possible covariates (data not shown). For the raw data, outdoor occupation was found to be a predictor of higher ocular sun exposure ($p=0.003$), with an increase of nearly 13 hours of ocular sun exposure expected for those with outdoor occupations when compared with those having indoor occupations. No significant covariate relationships were identified for any of these possible predictors of ocular sun exposure using the transformed data.

Conjunctival Ultraviolet Autofluorescence—Univariate predictors for total conjunctival ultraviolet autofluorescence were occupation and season of collection (Table 2). When using either raw or transformed data, outdoor occupation and winter season of collection both predicted higher total ultraviolet autofluorescence (Table 2). Raw data estimates on average those with outdoor occupations have 8.46 mm² greater total conjunctival ultraviolet autofluorescence than those with indoor occupations; winter season of collection predicts an average increase of 7.56 mm² total conjunctival ultraviolet autofluorescence when compared with spring season of collection. Furthermore, an increased portion of day spent outdoors while working was found to have a positive relationship with increased total conjunctival ultraviolet autofluorescence for both visits (Table 3).

DISCUSSION

Ocular Sun Exposure

While environmental factors may have similar effects on UV exposure to a population at a specific time and location, significant variance is likely to occur on an individual level.¹ Ocular UV exposure is heavily influenced by the angle of incident light, often affected by the solar zenith angle. Ocular tissues are protected by the upper brow bone, eyebrows, and lashes from some solar zenith angles, particularly when the sun is directly overhead.^{19, 20} Although there is some seasonal variation,²¹ evidence supports that the time of day most significant to ocular tissues occurs when the sun is lower in the sky and is thus bimodal, occurring approximately three to four hours before and after the period of most intense overhead ambient UV.^{22, 23} One limitation of the present study is that we did not assess the

time of day sun exposure occurred; future studies will be designed to further investigate the potential significance of this variable to conjunctival ultraviolet autofluorescence.

Simplification of outdoor activity recall by Kwok and colleagues was shown to account for approximately 94% of time spent outdoors,¹⁷ and was thus chosen for use in our study. Additionally, Threlfall and colleagues demonstrated that accounting for individual variations in ocular protective measures, as done in the current study with questionnaires, could significantly improve ocular UV exposure estimates when compared to calculations derived from ambient estimates of UV exposure alone.¹³ The present study combines the two questionnaires in a new way to estimate ocular sun exposure: use of ambient UV is eliminated in calculations by replacing it with Kwok and colleagues' activity questionnaire,¹⁷ while using Threlfall and colleagues' ocular protective measure estimates of percent UV transmission.¹³

Descriptive statistics are reported here for the first time using the present study method of estimating ocular sun exposure.^{14,15,22} During spring data collection all subjects worked indoors, resulting in few ocular sun exposure hours, ranging from approximately 30 minutes to 10 hours with a mean of 4.38 (± 3.02) hours. When compared with the full data set, including 9 subjects reporting outdoor occupations, mean ocular sun exposure approximately doubled to 8.86 (± 11.97) hours. Although the full data set shows a significant increase in ocular sun exposure compared with the initial spring study group, the resulting mean ocular sun exposure was not strikingly greater. This is most likely a reflection of the subjects reporting outdoor occupation enrolling in the present study during a cold, harsh winter season. Regardless of this seasonal confounder, self-reported increased portion of day spent outdoors while working predicted higher levels of ocular sun exposure (Table 3). A larger sample size, recruiting those with markedly increased amounts of ocular sun exposure, would help confirm the usefulness of the study surveys in assessment of ocular sun exposure. Additionally, comparison with alternative methods, such as personal UV dosimeters, to gather data regarding UV exposure during time outdoors would further establish usefulness of methods used in this study.²⁴

Bland-Altman Assessment of Repeatability—Test-retest repeatability of ocular sun exposure over two weeks was good with mean difference (bias) close to zero and a single outlier (Figure 2). Further inspection of the outlier revealed the subject reported a large number of outdoor hours at each visit, yet use of sunglasses was reported 75% of the time for visit one, and 25% of the time for visit two. This outlier highlights the challenges associated with accurately assessing ocular sun exposure. Visual inspection of the scatter plot suggests significant skewness of the data toward lower levels of ocular sun exposure and slightly increased variability with increased ocular sun exposure. The variability may be explained by disparities in weather or schedules between the two visits, affecting subject motivation or ability to spend time outdoors. However, no systematic variation is apparent within the data. The plot shows that 95% of the time ocular sun exposure falls within approximately ± 15 hours over two weeks of time; those with greater than 15 hours may be influenced by other individual behavior factors (Figure 2). Although clinically acceptable variation for ocular sun exposure remains to be established, the reported test-retest variability appears acceptable for use of this measure in future studies.

Further comparison of ocular sun exposure in winter and spring shows distinct seasonal disparity. Bland-Altman scatter plot shows spring ocular sun exposure data (Figure 4) to be fairly evenly distributed with a narrow spread. In contrast, the scatter plot for winter ocular sun exposure (Figure 5) shows definite skewness toward lower values and much wider 95% limits of agreement than present for spring. These findings demonstrate seasonal differences for questionnaire-based ocular sun exposure as a reliable outcome variable, contradictory to the findings of Sherwin and colleagues.¹⁰

Linear Regression Analysis—Although exploratory in nature, as expected, outdoor occupation is supported as a predictor of higher ocular sun exposure. No significant covariate relationships were identified for other tested possible predictors of ocular sun exposure, supporting for the potential usefulness of this measure. Ocular sun exposure for activities reported by visit for wear of prescription eyeglasses, sunglasses, and a protective hat when outside were used to calculate estimated hours of ocular sun exposure; thus, these are not considered possible predictors. However, as expected, increasing the proportion of the day spent outdoors while working was associated with higher levels of ocular sun exposure, while increasing the proportion of the day spent outdoors while not at work was not found to be significant (data not shown).

Conjunctival UV Autofluorescence

Several potential sources of conjunctival ultraviolet autofluorescence have been considered including altered collagen, the presence of NADH, altered cellular mitochondrial activity, and lipofuscin.^{15, 25-27} One plausible source of conjunctival ultraviolet autofluorescence is from collagen cross-linking, demonstrated to autofluoresce following UVA exposure.²⁸ The majority of UVB and approximately 35% of UVA is absorbed by ocular surface tissues.²⁹ While potential downstream influences of UVB-induced DNA damage and mutations should not be minimized, UVA is established as a cause of collagen cross-linking,^{28, 30, 31} with altered collagen structure often permanently changing the functionality of affected tissue.³² In fact, the excitation wavelengths for conjunctival ultraviolet autofluorescence in the present study were in the UVA range (320 to 380nm), previously found to be associated with collagen cross-linking autofluorescence in human skin.³³

Median total ultraviolet autofluorescence in the present study was relatively low (6.31 mm²) when compared with previous findings reported by Sherwin, et al (28.2mm²).³⁴ However, a recent report by Wolffson and colleagues of conjunctival ultraviolet autofluorescence in European eye care providers reports comparable estimates to the present study, although differences in study design prevent conclusive comparison.²⁷ Several factors may have influenced our more than four-fold reduction found in total ultraviolet autofluorescence compared with Sherwin and colleagues. Most notably, although time spent outdoors is not reported in hours by Sherwin¹⁰ it is likely total hours spent outdoors is substantially higher for the population of Norfolk Island when compared with our study subjects living in Ohio; this is expected due to the sub-tropical climate in Norfolk Island, while Ohio has a seasonally variable climate.

Bland-Altman Plot Assessment of Repeatability—Conjunctival ultraviolet autofluorescence demonstrated good intra-observer test-retest repeatability, with a mean difference of nearly zero (Figure 3). Although initially the point spread may appear somewhat wide, the limits of agreement are relatively narrow. There is no evidence of systematic bias of conjunctival ultraviolet autofluorescence in our data and the test-retest variability found is acceptable. Clinically acceptable variability is yet to be determined for conjunctival ultraviolet autofluorescence. However, our findings are in agreement with Sherwin and colleagues¹⁰ who have demonstrated reliability and validity for this measure in a genetically isolated group dwelling in a region with a fairly homogenous climate.¹⁰ In fact, intra-observer repeatability was notably better in the present study compared with the previous report by Sherwin and colleagues (mean difference 0.36 mm²; 95% limits of agreement -5.54 to 6.25 mm²).³⁵

Linear Regression Analysis—Outdoor occupation and winter season of collection predicted higher levels of conjunctival ultraviolet autofluorescence. Increased UV exposure during winter of 2014 in Ohio was not anticipated given that ambient UV levels are known to be lower in winter,^{36, 37} there was little snow fall, and temperatures were near 0°F for several weeks. Limited UV exposure during winter sample collection of the present study is supported by evidence described above showing that even those having outdoor occupations had relatively few hours of ocular sun exposure. It is more likely conjunctival tissue retains UV autofluorescent properties for a minimum of several months resulting from chronic UV exposure, such as may occur with those who have outdoor occupations. Based on findings in this study, it is possible that conjunctival ultraviolet autofluorescence may be a reflection of prior UV exposure sustained for at least several months. Thus, the persistence of conjunctival ultraviolet autofluorescence is perhaps longer than postulated by Sherwin and colleagues, who suggested ultraviolet autofluorescence may serve as an indicator of acute or sub-acute UV exposure over weeks to months.^{12, 34}

As expected, univariate linear regression of portion of time spent on activities reported by visit demonstrated an increased portion of the day spent outdoors while working was associated with increased area of conjunctival ultraviolet autofluorescence (Table 3). This finding is consistent with Sherwin and colleagues' report of increased median total ultraviolet autofluorescence with increasing proportion of day spent outdoors.¹⁰

The present study demonstrates feasibility and repeatability of a previously unreported method for estimating ocular sun exposure by questionnaires, and for conjunctival ultraviolet autofluorescence using a camera system built from currently available components in a group of subjects residing in Ohio. Furthermore, while a direct relationship was not identified for ocular sun exposure and conjunctival ultraviolet autofluorescence, this is likely due to the confounding variable of winter season of data collection from subjects with higher levels of ultraviolet autofluorescence. Given that outdoor occupation, winter season of collection, and larger portion of day spent outdoors while working predicted higher ultraviolet autofluorescence, our data suggests that ocular sun exposure is associated with conjunctival ultraviolet autofluorescence and that ultraviolet autofluorescence may remain for at least several months following sun exposure. Alternatively, it may ultimately be demonstrated that a proportional estimate of time spent outdoors yields adequate

information to classify subjects, with little additional benefit from obtaining a more precise continuous variable, such as proposed here, with a calculated estimate of ocular sun exposure. Findings from this study establish a baseline in normal subjects for future studies comparing these outcome measures in normal groups with ocular surface disease groups.

Study limitations are recognized; designed as a feasibility study to demonstrate repeatability of our outcome measures, “all comers” were included with few restrictions. Inclusion of those with dry eye and contact lens wearers may have confounded the findings. Future studies with more restrictive inclusion and exclusion criteria will help to clarify and delineate the significance of our findings to ocular surface disease. It is our hope the outcome measures reported here will stimulate additional studies to provide further insight into the importance of accurately estimating ocular sun exposure, as well as its potential role in the onset of ocular surface disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Representative tracing of conjunctival ultraviolet autofluorescence (UVAF) area on 8-bit grayscale image. Tracing done using freehand area selection tool in ImageJ software (National Institutes of Health).

Figure 2.
Bland-Altman plot illustrating test-retest variability of raw data ocular sun exposure (n=50).
Solid line shows bias (mean differences); dotted lines show 95% limits of agreement.

Figure 3.
Bland-Altman plot illustrating variability of raw data for total conjunctival UVAF (n=34).
Solid line shows bias (mean differences); dotted lines show 95% limits of agreement.

Figure 4.

Bland-Altman plot illustrating test-retest variability of ocular sun exposure during spring season of collection (n=20). Solid line shows bias (mean differences); dotted lines show 95% limits of agreement.

Figure 5.

Bland-Altman plot illustrating repeatability for ocular sun exposure during winter season of collection (n=15). Solid line shows bias (mean differences); dotted lines show 95% limits of agreement.

Table 1

Descriptive statistics for measures of ocular sun exposure and total conjunctival ultraviolet autofluorescence (UVAF).

Measure	n	Min	Max	Mean (±SD)	Median	Bias	95% Limits of Agreement	
Raw data								
Ocular sun exposure (hours)	50	0.55	60.24	8.86 (±11.97)	5.57	+0.57	−14.52	+15.66
Total UVAF (mm ²)	34	0	30.64	9.15 (±9.47)	6.31	+0.19	−1.75	+2.12
Transformed data								
Ocular sun exposure (log ₁₀ hours)	50	−0.26	1.78	0.69 (±0.47)	0.75	−0.15	−1.28	+0.98
Total UVAF (sqrt mnf)	34	0	5.54	2.43 (±1.82)	2.51	+0.01	−0.46	+0.48

Table 2

Univariate linear regression of raw and transformed data for outcome variable total conjunctival ultraviolet autofluorescence (UVAF). As male gender and outdoor occupation are each assigned the higher value, a positive relationship with total conjunctival UVAF would demonstrate increased UVAF for male gender and outdoor occupation. As contact lens wear is assigned the higher value, an inverse relationship with total conjunctival UVAF would indicate that increased UVAF occurred with no contact lens wear. As spring season collected is assigned the higher value, the inverse relationship with total conjunctival UVAF demonstrates increased UVAF during winter season collected. Two-tailed test of significance used to determine p values.

Total conjunctival UVAF			
	Regression Coefficient	p value	95% CI
Raw data			
Ocular sun exposure	0.03	0.821	−0.22 to 0.28
Total conjunctival UVAF			
Age	0.03	0.855	−0.26 to 0.31
Gender (Female=1, Male=2)	5.36	0.099	−1.07 to 11.80
Occupation (Indoor=1, Outdoor=2)	8.46	0.019	1.49 to 15.44
Contact lens wear (Yes=1, No=0)		0.440	−16.23 to 7.23
Season collected (Winter=1, Spring=2)	−7.56	0.018	−13.75 to −1.36
Transformed data			
Ocular sun exposure	0.03	0.969	−1.37 to 1.43
Total conjunctival UVAF			
Age	0.007	0.801	−0.05 to 0.06
Gender (Female=1, Male=2)	0.95	0.133	−0.30 to 2.20
Occupation (Indoor=1, Outdoor=2)	1.51	0.031	0.15 to 2.87
Contact lens wear (Yes=1, No=0)	−1.25	0.265	−3.48 to 0.99
Season collected (Winter=1, Spring=2)	−1.36	0.028	−2.57 to −0.16

Table 3

Linear regression results for self-reported portion of time spent on activities by visit for total conjunctival ultraviolet autofluorescence (UVAF). Two-tailed test of significance used to determine p values.

Total UVAF			
Activities	Regression Coefficient	p value	95% CI
Visit 1			
Wear of prescription eyeglasses when outside	−0.03	0.876	−0.41 to 0.34
Wear of protective hat when outside	0.37	0.202	−0.21 to 0.94
Wear of sunglasses when outside	0.23	0.348	−0.26 to 0.70
Portion of day spent outdoors while working	0.72	0.034	0.06 to 1.38
Portion of day spent outdoors while not at work	−0.07	0.876	−0.95 to 0.82
Visit 2			
Wear of prescription eyeglasses when outside	−0.12	0.522	−0.48 to 0.25
Wear of protective hat when outside	0.22	0.445	−0.36 to 0.81
Wear of sunglasses when outside	0.26	0.291	−0.23 to 0.75
Portion of day spent outdoors while working	0.87	0.020	0.16 to 1.59
Portion of day spent outdoors while not at work	−0.57	0.310	−1.68 to 0.55