



UV damage of the anterior ocular surface – microstructural evidence by in vivo confocal microscopy



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ABSTRACT

Purpose: To evaluate and describe the microstructural changes at the ocular surface in response to habitual ocular sun exposure, correlate them with the UV protection habits and follow their dynamics using in vivo confocal microscopy (ICM).

Methods: For a period of minimum 4 months 200 subjects (400 eyes), aged 28 ± 7.3 years, were recruited with the agreement that they will spend their summer exclusively in the region of the Black Sea coast at 43°N latitude and will be examined before and after the summer. All subjects filled in a questionnaire about habitual UV protection and were examined clinically and by ICM.

Results: Questionnaire results demonstrated that 83.5% (167 participants) of the subjects considered the sun dangerous for their eyes, but 78% (156 subjects) believed that there is danger exclusively during the summer period. Although no clinical changes were detected, microstructural analysis of the cornea demonstrated statistically significant ($p = 0.021$) decrease of the basal epithelial density – from 6167 ± 151 cells/ mm^2 before to 5829 ± 168 cells/ mm^2 after the summer period. Microstructural assessment of the conjunctiva demonstrated characteristic cystic lesions with dark centres and bright borders encountered in only 25 eyes (6%) before, and affecting 118 eyes (29.5%) after the summer. The total area of the cysts after the summer increased fivefold. Spearman analysis proved negative correlation between sun protection habits and number of cysts.

Conclusion: Summer sun exposure for one season leads to clinically undetectable, microstructural changes affecting the cornea, bulbar and palpebral conjunctiva with transient, but possibly cumulative nature.

1. Introduction

UV damage of the skin is common knowledge and has been well documented at histological and microstructural level in scientific literature [1–4]. The knowledge of potential eye damage at microstructural level is somehow lacking, although there are a number of studies evaluating the epidemiological evidence and molecular mechanisms of UV exposure as a causative agent of cataract [5–9]. As the eyelids and the ocular surface are exposed, together with the face, more often than other body areas, it is interesting to understand the nature of the associated with UV exposure microstructural changes at the ocular surface. Such alterations cannot be observed with the naked eye or standard clinical methods such as focal illumination or slit-lamp biomicroscopy. Currently, most of the publications are based on conjunctival autofluorescence and provide solid knowledge that there is significant UV damage of the interpalpebral conjunctiva [8,10,11]. Surface diseases, such as pinguecula and pterygium also have been associated with solar damage, but interestingly pterygium is more often

nasal (prevalence around 7.3%), and in contrast, pinguecula affects almost equally nasal and temporal conjunctiva with prevalence (in either eye or location) up to 69.5% [12–14]. Sasaki et al. demonstrated that the danger of sun exposure for the eyes depends on solar altitude and cannot be compared with the well-recognised diurnal pattern for skin protection. Furthermore, they proved that UV indices are misleading when considering the potential for eye damage [15]. The explanation for this disparity includes the direction of the reflected light and the upright position of the human body.

Currently, the most popular eye protection is sunglasses. However, due to trends in the frame style, most of the time sunglasses are seen more as a fashion accessory and health considerations are not of paramount importance [16]. Nevertheless, the optical industry pays significant attention to the protective properties of sunglasses and corrective lenses and recent advances have shown that the rear surface of the lenses should have an anti-reflective coating to protect from side-reflected light [16]. Currently, there are some contradicting opinions in the contact lens industry about UV protection, mainly due to the fact

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that biofilm changes the properties of contact lenses for multiple usage [17,18]. Although it is a standard to provide UV B protection, UV A protection is something that a limited number of manufacturers are striving towards. The situation is different for intraocular lenses, where even the longer wavelength (blue light) is blocked by some implants. However, this has no impact on the anterior ocular surface, a subject of our current scientific interest.

Considering the lack of knowledge regarding microstructural changes following UV exposure, we created a hypothesis that there should be a detectable change at cellular level imageable by *in vivo* confocal microscopy on the anterior ocular surface and/or adjacent adnexal structures, following intensive, consecutive sun and UV exposure. We had some preliminary observations facilitated by *in vivo* confocal microscopy (data on file), highlighting changes in the conjunctiva and cornea following acute sunburn. Therefore, a prospective study was designed in order to follow randomly selected young, active subjects, regularly exposed to sun (at a sea level 43° latitude) during the summer period. The goal of the study was to evaluate the UV protection habits of those subjects, determine eventual microstructural changes at the ocular surface and follow their dynamics using *in vivo* confocal microscopy.

2. Materials and methods

The study was conducted in the Department of Ophthalmology and Visual Science, Medical University – Varna and its clinical base Centre for Vision (Vision Protect) in Varna, Bulgaria. This study adhered to the tenets of the Declaration of Helsinki and ethical approval was granted by the Ethics Committee (2014).

All subjects were prospectively recruited using social media (Facebook, Twitter) with the assistance of the local students' association at the Medical University – Varna. The inclusion criteria were Caucasian ethnicity, having lived permanently in the Varna region (latitude 43° N at sea level) for the last 3 years, general ocular health, no need for optical correction, no contact lens usage, and no history of systemic disease and no pathology detected by biomicroscopy (including pinguecula or conjunctival cysts). For a period of 4 months, 225 subjects (450 eyes) were recruited with the agreement that they will spend their summer exclusively in the Varna region (beach resort at the Black Sea coast at 43° latitude) and will be examined before and after the summer season. After detailed explanation of the benefits and potential risks all participants signed written informed consent forms. The subjects filled in a questionnaire about their eye protection habits, underwent comprehensive eye examination and upon confirmation that they fit the criteria, were included in the study. All assigned subjects were invited to have *in vivo* confocal microscopy between 1–30 April, when the outside activities and sun exposure were still limited because of the cold weather. Subsequently, they were invited for a control examination between 1–30 October, after the summer period. All subjects were warned to report immediately if they have any sunburnt skin areas associated with redness and pain, because those subjects were excluded from the current study and followed separately. Subjects were obliged to report any eye problems immediately, including but not limited to sore, red or itchy eyes. Subjects diagnosed clinically with eye problems, such as blepharitis, conjunctivitis, or any other more serious eye condition were also excluded from the study. Fifty of the subjects (100 eyes) were able to repeat the examination procedure in one year's time (between 1–30 April of the following year) and served as a control group to evaluate consecutive exposure.

Laser scanning *in vivo* confocal microscopy was performed with HRT II Rostock corneal module (Heidelberg Engineering GmbH, Dossenheim, Germany) and took up to 60 min per subject as 5 corneal, 4 conjunctival and 4 lid areas were examined for both eyes. After explanation of the examination procedure and instillation of anaesthetic drops (Alcain, Alcon Inc.), the confocal examination was commenced as follows: central cornea; nasal, temporal, superior and inferior

conjunctiva; and superior lid after eversion (from the conjunctival side). Corneal areas were central and 12, 3, 6 and 9 o'clock, 2 mm from the limbus towards the centre. The four conjunctival areas were corresponding but 2 mm from the limbus in the opposite direction. The upper and lower lids were everted and the medial and lateral areas of each eyelid were examined. The z-dimension always started from the surface towards the deeper structures, for example from epithelium to the endothelium for the cornea. The anaesthetic drops were repeated before each examination. The right eye was done first. The 40× lens was used for all examinations of this study. The size of the images was 400 by 400 microns, with lateral resolution of 2 microns and slice thickness of 5 µm. The images were stored within the proprietary database. Subsequently one experienced examiner selected three images per region (CNG), and two independent experienced analysts (MNR and DIG) counted the cells and measured the structures using analysis 3.1 (Soft Imaging System, Münster, Germany) software and prepared data for statistical analysis.

After careful analysis of the acquired data the outcome parameters were - density of the corneal epithelium, number, size and total area of the cystic lesions in the bulbar and tarsal conjunctiva. Statistical analysis was facilitated by Statistical Package for Social Sciences (SPSS software, version 19.0). Descriptive statistics was used for basic feature extraction. Data were presented as numbers (percentages), mean (\pm SD). *t*-test was selected to test statistical significance, the level of the latter was set at $P < 0.05$. Association between sun protection habits and microstructural damage were evaluated by Spearman correlation analysis.

3. Results

The study included 200 subjects (400 eyes), aged 28 ± 7.3 years, with a slight female predominance of 57%. The 50 re-examined subjects (100 eyes) were at a mean age of 27 ± 6.5 years and 30 (60%) were females. The remaining, initially recruited 25 subjects were excluded from the study because they did not fulfil the inclusion criteria (mainly did not spend the summer on the beach (17 subjects), had excessive sunburn (5 subjects), or other reasons. From the specially developed questionnaire only the information related to the current analysis will be presented. Not surprisingly 83.5% (167 participants) of the subjects considered the sun dangerous for their eyes, but 78% (156 subjects) believed that there is danger exclusively during the summer period. Although 65% (130 subjects) would use sunglasses as eye protection, 112 (89% of the users) would choose the trendiest model, as opposed to the one offering complete protection. The rest (35%) would never consider protection even on the beach. However, none of the subjects included in the current study had any serious (associated with pain) skin burn on the face or body during the period of the study. The re-examination group demonstrated higher values of sun protection for their eyes (96% would use sunglasses and 20% would prefer the best protection regardless of design and fashion trends), which can explain their sustained interest in the study. Demographic and questionnaire data are presented in Table 1.

Microstructural analysis of the cornea demonstrated a slightly decreased level of basal epithelial density – from 6167 ± 151 cells/mm² before the summer period to 5829 ± 168 cells/mm² after the summer period, which also was statistically significant ($p = 0.021$). In the control subjects' group ($n = 100$ eyes) the epithelial density returned to 6097 ± 147 cells/mm² within a year and was not statistically different to the baseline $p = 0.33$. The number of keratocytes (from superficial, mid- and posterior stroma) remained numerically and statistically unchanged during the follow-up period. This was also the case for the endothelial densities.

Analysis of the conjunctiva demonstrated characteristic cystic lesions with dark centres and bright borders (Fig. 1), encountered in only 25 eyes (6%) before the summer season. After the summer season their presence increased, affecting 118 eyes (29.5%). From the eyes "affected

Table 1

Demographic and questionnaire data for the 200 subjects participating in the study and 50 random subjects re-examined as a control group in one year's time.

Groups	Main group (N = 200 subjects, n = 400 eyes)	Control group (N = 50 random subjects from the study group, n = 100 eyes)
Age	28 ± 7.3 years	27 ± 6.5 years
Gender	114 female 86 male	30 female 20 male
Cautions about sun-related danger for the eye (based on questionnaire)	167 subjects 83.5%	46 subjects * 92%
Routine use of sunglasses	130 subjects 65%	41 subjects * 82%
Selection of glasses with the best protection	18 subjects 11% (of the sunglasses users)	15 subjects * 30% (of the sunglasses users)

*Data extracted from the baseline questionnaire.

after the summer", 64 were right (RE) and 54 left (LE). However, the number of encountered cysts was very similar for both eyes (149 RE and 151 LE). The size of the cysts also increased from 12 to 78 µm at baseline to 14–174 µm after the summer exposure to sun. The summary of the results for the cyst parameters (number and mean diameter), is presented in Table 2. The cysts also had a specific topographic representation, with higher distribution in the horizontal meridian, which was asymmetrical for the right and left eyes. The topographic characteristics of the lesions are presented schematically on Fig. 2. The risk for cyst formation before and after summer sun exposure is presented as a diagram in Fig. 3. The total area of the lesions was calculated before and after the summer sun exposure and enlargement of 20 times the initial size was encountered. For the 50 test subjects, results were similar and are presented in Table 3. The total affected area after sun exposure in these longer-term follow-up eyes increased 11 times after the sun exposure and returned within statistically normal range in one year's time.

Another interesting observation was encountered on the conjunctiva of the upper lids. Those were specific, rounded lesions with dark centres and bright borders (Fig. 4), which increased significantly in size and number after the summer period as presented in Table 4. The total area of the cysts after the summer increased five times. The total affected area in the control group (50 eyes) increased six times after sun exposure and returned within the normal range in twelve months. The results obtained 12 months later did not demonstrate a statistically significant difference in the dynamics of the cyst size and number, but

the affected area was 18% larger in comparison to the baseline.

Spearman correlation was assessed separately for the right and left eyes in order to determine non-parametric rank correlation between sun protection habits and microstructural damage. Analyses highlighted a negative correlation between the habits to wear sunglasses (answer "YES/NO I wear sunglasses most of the time during the summer") and the number of cysts. The value of the coefficient was $-.542$ and $-.373$ for the right and left eyes, respectively, and negative correlation between sun protection of the eyes and number of cysts was established.

4. Discussion

There are a number of published interesting studies, highlighting the potential harmful effect of UV light on the ocular surface, anterior chamber, crystalline lens, and even the posterior segment [10,19–24]. This increasing interest is driven further by the relatively recent introduction of crosslinking into wider clinical practice [25]. Alternatively, there is some evidence that UV B exposure increases lutein blood levels and might be associated with a decreased rate of myopia progression [26]. The rationale for this is also a well-known observation that time spent outdoors in the sun is negatively linked with myopia progression [27]. Although, there are contradicting research discoveries regarding the effect of UV light on the human organism, there is an agreement that excessive UV exposure is harmful for the skin [2,4,28,29].

The current study demonstrates that, at microstructural level, UV exposure during the summer season at 43 °N latitude is associated with significant, progressive changes of the anterior ocular surface including cornea, bulbar and palpebral conjunctiva. In the test group (100 eyes), followed over a longer period of time, those changes had a reverse evolution. However, there was no complete morphological restoration to the baseline especially regarding conjunctiva. Although not statistically significant, those changes appear to accumulate even over as short period of time as 12 months. A possible cumulative effect of those changes over longer periods of time might contribute to the "solar ageing of the ocular surface" and may trigger disease such as dry eye, chronic conjunctivitis, chronic blepharitis, decreased corneal transparency, and limbal stem cell deficiency.

The most prominent corneal microstructural change highlighted in one year of follow-up was the density of the epithelial cells. The outlined difference was statistically significant following the summer season and sun exposure at 43 °N latitude. Douth et al., in a very comprehensive study, proved that the central cornea absorbs 47% of the UV light with wavelength of 400 to 310 nm [30]. They also measured that the transmission is decreasing with the decreased

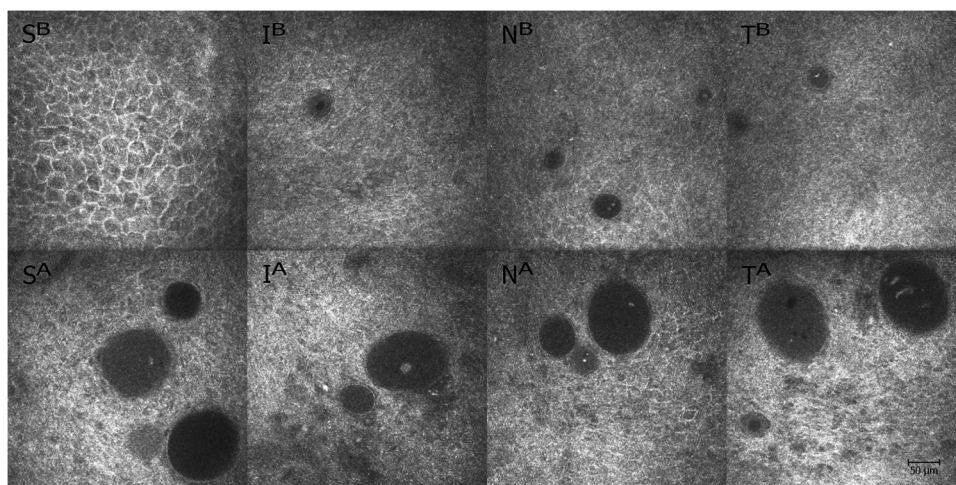


Fig. 1. Cystic lesions of the conjunctiva imaged by in vivo confocal microscopy (HRT II corneal module) from superior (S), inferior (I), nasal (N) and temporal (T) region, before (^B) and after (^A) sun exposure at 43° N latitude. Please note that before sun exposure none of the eyes had cysts in the superior region, and the epithelium had normal appearance (S^B).

Table 2

Summary of the conjunctival cyst parameters (number and mean diameter), assisted by in vivo confocal microscopy, before and after sun exposure (SE) during the summer season at 43° N latitude for the study group of 200 subjects (400 eyes). The results are presented per conjunctival region (superior (Sup), inferior (Inf), temporal (Temp), nasal (Nas), average per eye and as total sum.

Parameters	Right eye (n = 200)				Left eye (n = 200)				Total	Statistics
	Sup	Inf	Temp	Nas	Sup	Inf	Temp	Nas		
Number of cysts before SE	0	2	7	8	1	1	7	5	31	NA
Total number per eye before SE	17	(Affected 13 out of 200 eyes)				14	(Affected 12 out of 200 eyes)			
Number of cysts after SE	33	9	56	51	19	3	56	73	300	Change is significant to 'Before SE'
Total number per eye after SE	149	(Affected 64 out of 200 eyes)				151	(Affected 54 out of 200 eyes)			
Average cysts diameter (μm) before SE	32	14	18	12	34	15	16	11	19 ± 9.5 μm	NA
Mean cysts diameter (μm) before SE	19 ± 9 μm				19 ± 10 μm					
Average cysts diameter (μm) after SE	43	46	24	31	43	41	41	40	38.5 ± 5.5 μm	Change is significant to 'Before SE'
Mean cysts diameter (μm) after SE	36 ± 9 μm				41 ± 2 μm					P = 0.022
Total affected area (μm ²) before SE	1014 μm ²				835 μm ²				1849 μm ²	NA
Total affected area (μm ²) after SE	16 843 μm ²				19 400 μm ²				36 243 μm ²	NA

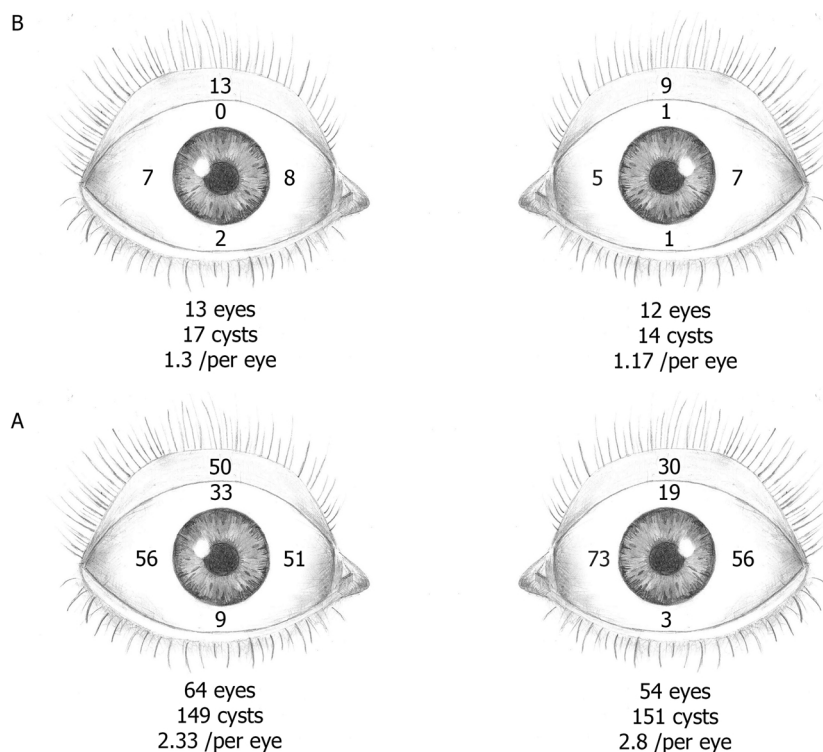


Fig. 2. Schematic demonstration of the distribution of the cysts before (A) and after (B) sun exposure at 43° N latitude.

wavelength of the UV light. This means that the superficial cornea is damaged by the UV light with the highest energy and this is also the explanation why epithelial changes are detectable even after one season of sun exposure. Previously, other studies proved that a decreased number of epithelial cells per area is a phenomenon related to ageing [31,32].

The most prominent observations of the ocular surface after the summer sun exposure were conjunctival changes. Those were cysts, which are a non-specific observation of the conjunctiva, previously having been described in a variety of “stress” situations for the anterior ocular surface, including surgery with involvement of the conjunctiva [33,34]. Interestingly, similar cysts were previously described as

features of skin damage after UV exposure [28]. [35] The described “microvesicles” in the skin structure and their development after UV exposure appear to be very similar to findings of the present study. The conjunctival cysts described in the current study had specific predilections, as expected within the inter-palpebral fissure. However, after sun exposure they had a greater diameter under the upper lid. The cysts in the test group decreased in number and size within a year. Pitts et al. in 1978, utilising electron microscopy, demonstrated significant microstructural damage of the primate cornea after acute UV exposure, interestingly the cornea restored to the normal architecture within a week [36]. The observations by in vivo confocal microscopy are not comparable with those ultrastructural studies, however, the technique

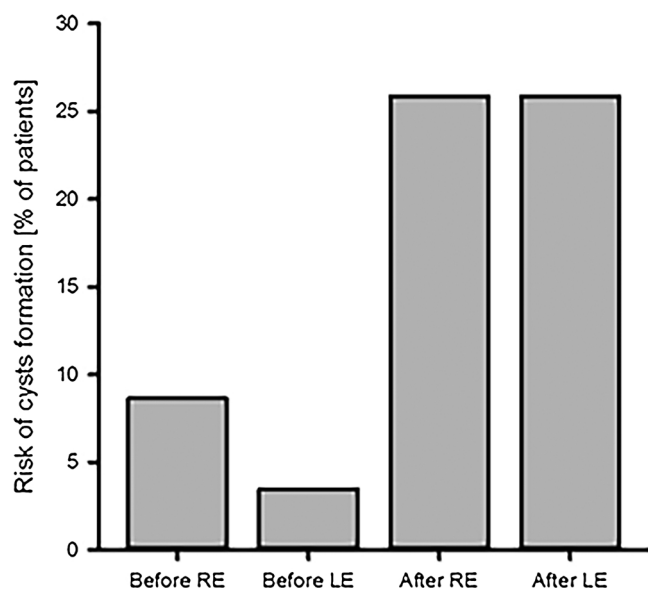


Fig. 3. Diagram presenting the risk of conjunctival cyst formation before and after the summer season for the 200 subjects (400 eyes).

utilised by the current study also highlighted significant reduction of the structural alterations over time.

Interestingly, there was a negative correlation between the sun protection habits regarding the eyes and the number of cysts, which proves that regardless of diverse styles of sunglasses, protection habits are producing results – less damage at cellular level.

One of the most interesting observations was the cystic change of the superior palpebral conjunctiva. Those cysts were similar, but not identical, to the bulbar conjunctiva, probably due to firmer tarsal adhesion, as visible on Fig. 4. Again in the control group those changes return close to the baseline within a year. The principal question is why

those changes were concentrated on the superior palpebral conjunctiva?

The concept that “peripheral light focusing” is affecting nasal conjunctiva is universal in ophthalmology. After the brilliant experiment of Prof. M. Coroneo, and scientific and clinical evidence of nasal location of the pinguecula and pterygium, the area most affected by light (and UV in particular), is believed to be nasal conjunctiva [37,38]. However, the interpalpebral (exposed) conjunctiva is prone to a variety of external and internal traumatic events, such as dust, wind, constant palpebral movement, etc., which have life-long effects, independent of UV exposure. In fact, considering the eye anatomy and the function of the lids, the most unprotected part of the limbus might be the lower limbus. Especially in the summer, people are looking up to the sky, relaxing with slightly closed eyes and the reflected light easily reaches the lower limbus, even below the sunglasses. Theoretically, the energy is neutralised faster by the intensive blood supply and movement associated with blinking, but the long-term effect could be microstructural damage, part of which is described by this study. One may question this theory highlighting the position of the pinguecula and pterygium. However, those formations might be related to the mechanical effect of the lid. Alternatively, superior limbic keratoconjunctivitis, limbal form of vernal keratoconjunctivitis, or superior limbal neovascularization might be explained with the contribution of the superior paralimbal damage. The phenomenon might also contribute to the chronic damage of the lid margins and Meibomian glands. This is further supported by the topography, clinical and histological characteristics of aforementioned conditions [39]. In this context, the argument that autofluorescence (accepted as an early sun damage sign) is located in interpalpebral conjunctiva [8,10,11], might be explained simply by the wipe-like effect of the lids and mechanically moved nasal and temporal cholesterol as a waste product of the anterior ocular surface damage.

Finally, there is an interesting phenomenon in nature. The eyes of most animals are given a limbal pigmentation, especially in the polar regions. This could well be a natural protective mechanism for light defocusing. Although, some darkly pigmented humans have pigmented

Table 3

Summary of the conjunctival cyst parameters (number and mean diameter), assisted by in vivo confocal microscopy, before, after sun exposure (SE) during the summer season at 43° N latitude and one year later, over the 50 controls (100 eyes). The results are presented per region superior (Sup), inferior (Inf), temporal (Temp), nasal (Nas), average per eye and as total sum.

Parameters	Right eye (n = 50)				Left eye (n = 50)				Total	Statistics
	Sup	Inf	Temp	Nas	Sup	Inf	Temp	Nas		
Number of cysts before SE	0	1	2	3	0	1	3	2	12	NA
Total number per eye before SE	6				6				(Affected 9 out of 100 eyes)	
Number of cysts after SE	5	3	9	12	2	2	10	17	60	Change is significant to 'Before SE'
Total number per eye after SE	29				31				(Affected 30 out of 100 eyes)	P = 0.013
Number of cysts one year later	0	2	3	3	1	1	2	2	14	
Total number per eye one year later	8				6				(Affected 10 out of 100 eyes)	
Average cysts diameter (μm) before SE	18	11	19	16	29	16	15	17	17.7 ± 5.5 μm	NA
Mean cysts diameter (μm) before SE	16 ± 4 μm				19.3 ± 7 μm					
Average cysts diameter (μm) after SE	39	41	26	29	33	43	49	41	38 ± 7 μm	Change is significant to 'Before SE'
Mean cysts diameter (μm) after SE	33.8 ± 7 μm				41.5 ± 7 μm					P = 0.043
Average cysts diameter (μm) one year later	17	14	21	14	23	19	17	19	18 ± 3 μm	
Mean cysts diameter (μm) one year later	16.5 ± 3 μm				19.5 ± 3 μm					
Total affected area (μm ²) before SE	301 μm ²				358 μm ²				659 μm ²	NA
Total affected area (μm ²) after SE	3 096 μm ²				4 088 μm ²				7 184 μm ²	NA
Total affected area (μm ²) one year later	427 μm ²				377 μm ²				804 μm ²	NA

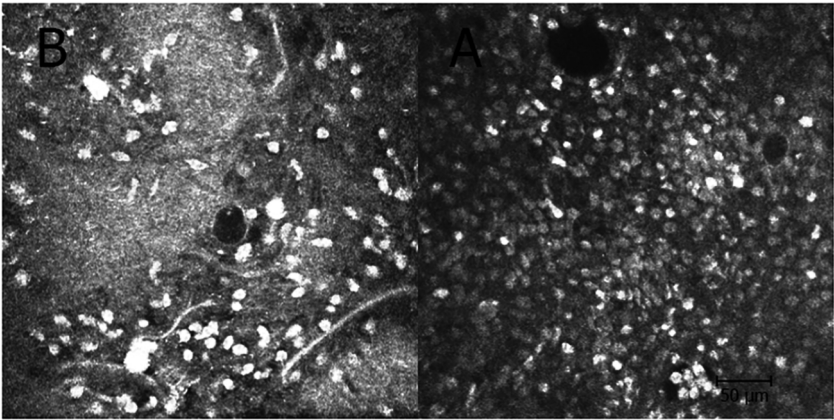


Fig. 4. Cystic lesions (arrowheads) of the conjunctiva of the upper lid imaged by in vivo confocal microscopy (HRT II corneal module) after lid eversion, before (B) and after (A) sun exposure at 43° N latitude.

Table 4
Summary of the superior palpebral conjunctival cyst parameters (number and mean diameter), assisted by in vivo confocal microscopy, before and after sun exposure (SE) during the summer season at 43° N latitude, for the study group of 200 subjects (400 eyes). The results are presented as average per eye and as total sum.

Parameters	Right eye (n = 200)	Left eye (n = 200)	Total	Statistical significance
Number of cysts before SE	13 (Affected 7 out of 200 eyes)	9 (Affected 5 out of 200 eyes)	22 (Affected 12 out of 400 eyes)	NA
Number of cysts after SE	50 (Affected 37 out of 200 eyes)	30 (Affected 21 out of 200 eyes)	80 (Affected 58 out of 400 eyes)	Change is significant to 'Before SE' P = 0.012
Average cysts diameter (μm) before SE	26.5 ± 6.5 μm	29 ± 6.5 μm	27.8 ± 6.5 μm	NA
Average cysts diameter (μm) after SE	34.8 ± 13 μm	39 ± 11 μm	36.9 ± 12 μm	Change is significant to 'Before SE' P = 0.023
Total affected area (μm ²) before SE	1082 μm ²	820 μm ²	1902 μm ²	NA
Total affected area (μm ²) after SE	5464 μm ²	4776 μm ²	10 240 μm ²	NA

perilimbal palisades, most of the individuals have no pigment depositions around the limbus. In this context, contact lenses with UV A and B protection might be the best option for individuals exposed to sun for long periods of time.

Nevertheless, UV damage of the eye is a fact, and when researchers try to explain exact pathological changes and their correlation with recognized clinical pathology, eye care practitioners should take heed and be meticulous in educating and making each patient aware of the needed UV protection for their eyes. Currently, there are glasses, contact lenses and even eye drops available. The eye drops are the subject of many negative comments as their effect is temporary, however, in certain activities eye drops could be an option. Soft contact lenses with a diameter of over 14.00 mm and proper UV blocking (including UV A and B) will certainly prevent limbal damage and peripheral corneal defocusing regardless of which part of the limbus might be responsible for them. More attention must also be paid to frame design focused on sun protection and possibilities for elimination of reflected light in consideration with face anatomy and other morphometric features.

In conclusion, summer sun exposure for one season at 43°N latitude leads to clinically undetectable, microstructural changes affecting the cornea, bulbar and palpebral conjunctiva with partially reversible nature. The long-term (cumulative) effect of those changes would lead to “solar ageing of the anterior ocular surface”, which appears to be similar to the well-known skin damage. Development of methods for anterior ocular surface protection, together with an increase in the public awareness of sun-related damage of the anterior eye, would have a significant health and social impact in the future. Eye care practitioners must pay clinical attention to the potential causative factors of ocular surface disease and educate their patients about the various methods of protection.

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