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Conjunctival ultraviolet autofluorescence imaging: an emerging diagnostic tool for monitoring the effects of ocular sun exposure

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ABSTRACT

Conjunctival ultraviolet autofluorescence is a relatively new diagnostic method that is utilized to diagnose and quantify the amount of ocular ultraviolet exposure. It has also been used to monitor the progression of myopia. This article describes the principles of this method and the technical aspects. We give some examples of diagnosis and describe the various factors that influence it.

KEY WORDS: conjunctiva; ultraviolet radiation; autofluorescence imaging; sun exposure; novel technique

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INTRODUCTION

The damaging effect of ultraviolet radiation (UV) on the human eye has been known for a long time and studied extensively. Various structures of the eye have been found to be impacted. Ultraviolet radiation damage has been discovered to affect both the anterior and posterior segments. The conjunctiva and cornea are by far the most affected structures of the eye. They are exposed to both direct and indirect ultraviolet radiation. The penetration of ultraviolet light within the cornea depends on its' wavelength [1]. Ultraviolet light in the spectral C region (UVC: 100–290 nm) has the lowest penetration. It is absorbed by the corneal epithelium. However, its' effects on the cornea are not seen as the ozone layer within Earth's atmosphere absorbs all of it, and therefore, humans are not exposed to it. Ultraviolet light in the spectral B region (UVB: 290–320 nm) penetrates the corneal epithelium and anterior stroma, while ultraviolet light in the spectral A region (UVA: 320–400 nm) penetrates all the corneal layers. As a result of these properties, ultraviolet light is known to induce a number of medical conditions, both acute and chronic [2, 3].

Photokeratitis is probably the most well-known condition affecting the cornea. It can be caused by exposure to ultraviolet light in a natural setting or from an artificial source. Initially, epithelial cells are shedded, and subsequently, corneal edema develops. Fortunately, healing usually occurs after 24 hours. However, in rare cases, high-energy exposure can lead to permanent endothelial damage [4].

Climatic droplet keratopathy is another well-known condition associated with prolonged ocular exposure to ultraviolet light. The disease

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is characterized by the appearance of translucent spherules or globules made of proteinaceous material in the anterior stroma, Bowman's membrane, subepithelium, and, in some cases, also the epithelium. The conjunctiva may also be affected. Similar deposits may appear in the interpalpebral regions at the nasal or temporal aspect. The pathophysiology of this condition has not yet been fully elucidated. However, it seems for the time being that one possibility might be that ultraviolet light may promote diffusion of serum proteins from the corneal limbus [5].

Pterygium is a common ocular disorder characterized by the triangular encroachment of the bulbar conjunctiva onto the cornea[6]. As in the case of climatic droplet keratopathy, its pathophysiology cannot be yet exactly established. However, numerous epidemiological studies have found that prolonged ocular sun exposure, particularly in the ultraviolet, is a definitive risk factor in its development.

Ocular surface squamous neoplasia is the most common ocular tumor affecting the conjunctival epithelium; however, it can also locally spread to the cornea. Clinically, these tumors present as pearly grey masses with a variable degree of pigmentation, vascularity, and leukoplakia. Ultraviolet B exposure, as well as infection with the human papilloma virus (HPV) and human immunodeficiency virus (HIV), are the main associated risk factors [7].

CONJUNCTIVAL ULTRAVIOLET AUTOFLUORESCENCE — CLINICAL USE

Conjunctival ultraviolet autofluorescence (CUVAF) is a reliable and tested biomarker of ultraviolet light exposure. It was first introduced to ophthalmology by Ooi et al. [8]. The clinical application of this method has been well established in such conditions as pterygium, pinguecula [9], or ocular surface squamous neoplasia [10]. Conjunctival ultraviolet autofluorescence has been recently found to be a good indicator of outdoor activity and has also been found to correlate with the progression of myopia [11–13]. Unfortunately, the scientific basis of conjunctival ultraviolet autofluorescence is not fully understood. Numerous causes have been suggested, including changes to collagen, elastin, lysosomes, mitochondria, cytokines, nicotinamide adenine dinucleotide (NADH), tryptophan, lipofuscin or matrix metalloproteinases [14, 15].

In healthy eyes, conjunctival ultraviolet autofluorescence is absent (Fig. 1). However, in pinguecula (Fig. 2) and pterygium (Fig. 3) usually, these changes show prominent hyperautofluorescence. Pingueculae often appear larger than viewed in visible light. Interestingly, in the case of pterygia, the head usually displays the most intense hyperautofluorescence compared to any other part. Of note is that Vogt's white limbal girdles also display pronounced hyperautofluorescence (Fig. 4). This may be due to the fact that histologically, they are similar to pterygia.

CONJUNCTIVAL ULTRAVIOLET AUTOFLUORESCENCE — IMAGE ACQUISITION AND EVALUATION METHODS

Conjunctival ultraviolet autofluorescence is performed using a camera, external ultraviolet light source, and appropriate filters. In most studies, digital single-lens reflex (DSLR) cameras have been utilized, although smartphones have also been adapted [16]. Outside the ultraviolet spectrum blue light autofluorescence has also been used to monitor the conjunctiva with similar effects [11]. Most

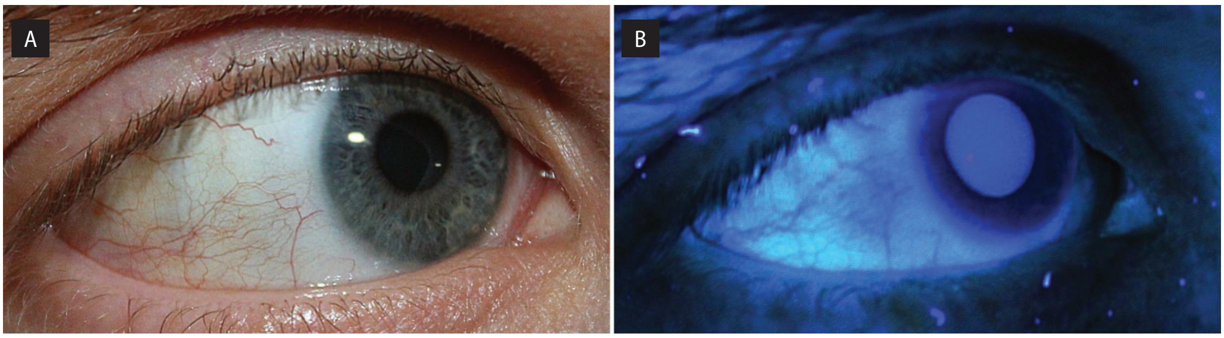

FIGURE 1. A. Visible image of the right eye in a healthy 42 year old male; **B.** Conjunctival ultraviolet autofluorescence image of the same person

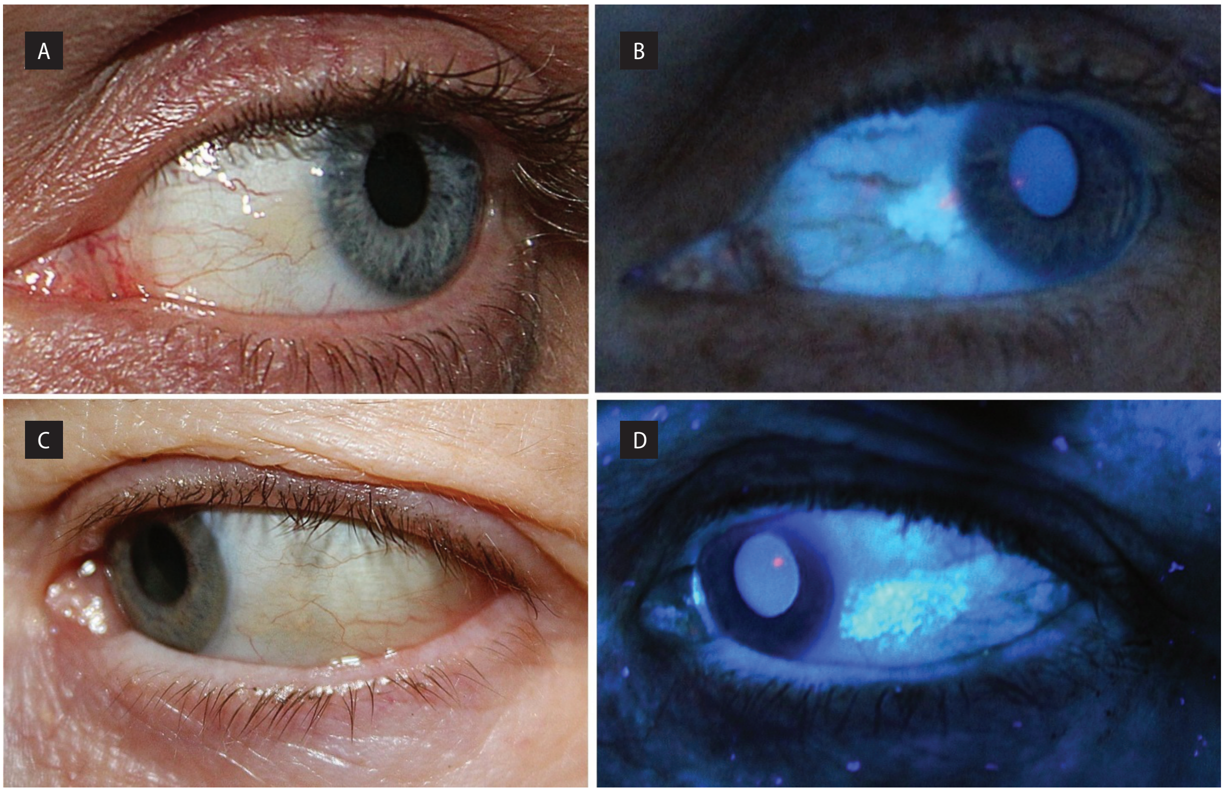

FIGURE 2. A. Visible image of a pinguecula in the left eye of a 41 year old male; **B.** Conjunctival ultraviolet autofluorescence image displaying hyperautofluorescence of the pinguecula; **B.** visible image of a pinguecula in the left eye of a 65-year-old female; **D.** Conjunctival ultraviolet autofluorescence image displaying hyperautofluorescence of the pinguecula

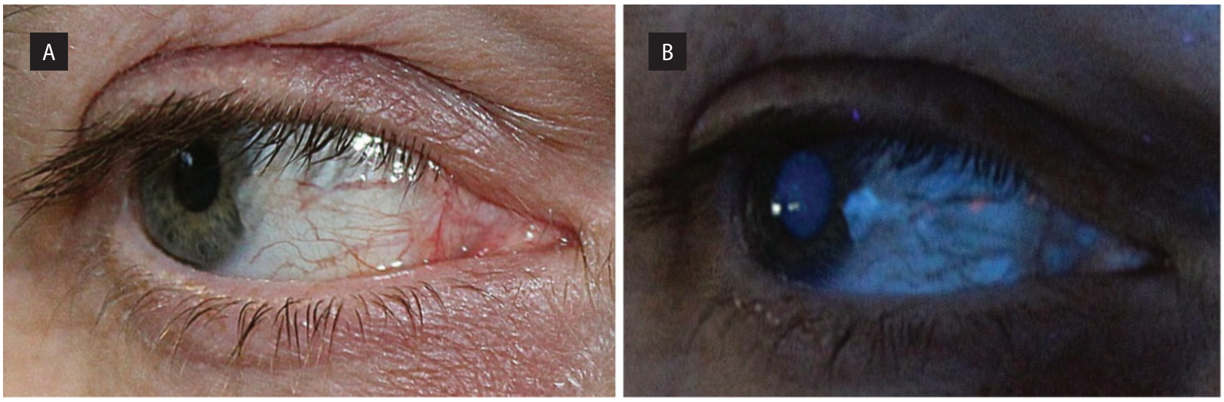

FIGURE 3. A. Visible image of a pterygium in the right eye of a 51-year-old male; **B.** Conjunctival ultraviolet autofluorescence image displaying prominent hyperautofluorescence of the head of the pterygium

CUVAF imaging systems have been integrated into slit lamp mounts for ease of operation. For most cameras, it is obligatory to use ultraviolet filters to block out the ultraviolet light that reaches the camera from the artificial light source.

Additionally, infrared blocking filters may be used to remove unwanted near-infrared radiation. Polarizing filters can be helpful to enhance image quality through a reduction in reflections and glare from the conjunctival surface [17]. The examination is conducted in a dark room. It is advisable to adjust the focus of the camera prior to extinguishing the light in the room. Also, remember that taking a more prolonged exposure might be necessary. Problems with focus and eye movement during the exposure may be addressed with additional

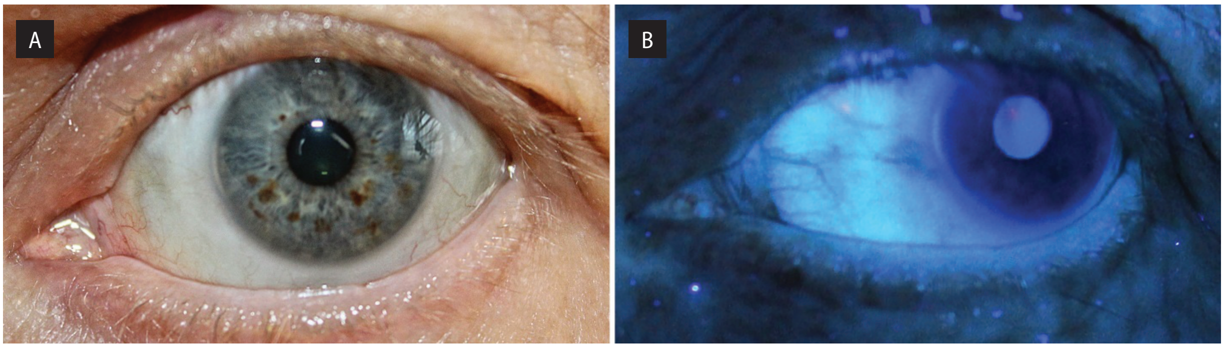

FIGURE 4. A. Visible image of a limbal girdle of Vogt in the left eye of a 70-year-old male; **B.** Conjunctival ultraviolet autofluorescence image displaying hyperautofluorescence of the limbal girdle of Vogt

computer processing of the image after capture. Following image capture evaluation of previous ocular ultraviolet exposure mainly involves measuring the area of conjunctival ultraviolet autofluorescence. Traditionally, most studies have reported this value in square millimeters. To achieve this, a millimeter ruler image using the same settings should be performed. Once the image is obtained, a conversion of pixel size to area measured in square millimeters is possible. Manual and semi-automated methods of CUVAF area measurement have been described [18]. One important thing to keep in mind is that ultraviolet light is a high-energy source. Therefore, using a UVA light source for conjunctival ultraviolet autofluorescence is advisable. The energy of a photon at 365 nm is 3.4 eV, whereas the energy of a photon of blue light at 380 nm is 3.27 eV. This means there is a difference of 4% of the energy per photon between both wavelengths.

ENVIRONMENTAL FACTORS INFLUENCING CONJUNCTIVAL EXPOSURE TO ULTRAVIOLET RADIATION

Several factors can influence a person's ultraviolet exposure, such as geographical location, time of day and season, atmospheric conditions, air pollution, and proximal individual factors. Ultraviolet radiation exposure regarding the examined person's geographic location is relatively easy to predict. This translates to latitude, longitude, and altitude. Longitude does not play a role. However, latitude and altitude do. The amount of ultraviolet light reaching the surface of the Earth changes throughout the year depending on the latitude [19]. Near the June solstice, the most excellent ultraviolet radiation levels can be found throughout a wide belt ranging from the Equator to the Tropic of Cancer and slightly northward. The opposite happens during the December solstice. The greatest ultraviolet radiation levels can be found throughout a wide belt ranging from the Equator to the Tropic of Capricorn and even slightly more southward. Altitude is also important. With increasing altitude, less atmosphere is available to absorb UV radiation. A rule of thumb is that with every 1000 meters of altitude, UV levels increase by 10%. Time of year and time of date impacts UV levels. For example, during the summer, at mid-latitudes, the Sun's altitude in the sky is highest during a 4-hour period around solar noon. The Sun's rays take the most direct path to the Earth. In contrast, the Sun's rays have to pass at a greater angle through the atmosphere in the early morning or late afternoon. Much more UV radiation is absorbed, and less of it reaches the surface [20].

Interestingly, in a 2011 study in Japan, Sasaki et al. noted that in summer, ultraviolet B levels at the cornea were twice as high during 8:00-10 a.m. and between 2:00 and 4:00 p.m. as during solar noon [21]. They conclude that geometric features of the face related to the orbital anatomy and natural protective mechanism, including squinting and pupil constriction, cause this result. When the Sun is at high altitude in the sky, the upper orbital rim and the brow cast a shadow on the eye. Medially, the eye is protected by the nose, while temporally, direct and reflected radiation has broad access. Another effect in ocular anatomy that plays a significant role is the peripheral light-focusing effect described by Coroneo. Ultraviolet light coming from the temporal direction is refracted in the eye and focused on the nasal limbus. On average, the ultraviolet levels at the nasal limbus may be 22 times higher than at the temporal side. This may explain why pterygia and pingueculae are typically located next to the nasal limbus of the cornea [22].

Atmospheric conditions have a significant impact, too. The thickness of the ozone layer on UV levels at the surface has already been described in the introduction. It should be noted that in some areas of the world, ozone levels have fallen for a long time. A 2012 study in Poland carried out by Krzysztof and Anna Błażejczyk observed a drop in stratospheric ozone levels from about 350 DU in 1980 to about 320 DU in 2008 throughout Europe [23]. In the southern hemispheres, the thickness of the stratospheric ozone layer dropped from about 280 DU to 240-250 DU in 2008. At this time, a sharp rise in the number of skin cancers diagnosed in Poland rose from 7000 cases in 1999 to 12000 cases in 2009. Whether a similar effect occurs in the conjunctival ultraviolet autofluorescence area remains to be examined. Similar to ozone, clouds also interact with the incoming solar ultraviolet light.

Clouds have the properties of scattering ultraviolet light that passes through them, and so are able to attenuate the amount reaching the surface of the Earth. Mean cloud attenuation has been studied globally, with results ranging from 25% to 30%. Attenuation depends on different cloud properties. Surprisingly, in some instances, ground-level ultraviolet radiation levels may be higher in cloudy conditions than under cloudless conditions. This phenomenon is known as cloud enhancement [24]. Closely correlated with cloud coverage are aerosol levels in the lower atmosphere. Aerosols form in natural processes and through human activity, primarily industrial emissions. Aerosols suspended in the air cause ultraviolet light to be reflected back into space. Since the 1970s, with less aerosol emissions into the atmosphere from industrial processes, there has been an increase in surface ultraviolet light reaching the surface of the Earth. This has led to a rise in global warming. Finally, the natural surroundings on the ground also play a role [25]. Many surfaces reflect ultraviolet radiation and, in doing so, can increase levels of exposure. Several studies have been conducted to evaluate different materials. In a natural setting, the highest albedo of UV light has been noted to occur in snow — 85.5%. Water at a lakeside has been found to have an albedo of 3.65%. Dry sand has an albedo of 14.04%, while grass has an albedo of 2.1%. Man-made surfaces tend to have higher albedo rates for UV light. Concrete, for example, has an albedo of 12.44%, while asphalt has an albedo of 5.90%, and red bricks have an albedo of 5.75% [26].

CONCLUSIONS

Conjunctival ultraviolet autofluorescence imaging is an exciting new tool that has been demonstrated to be valuable in diagnosing and quantifying ultraviolet ocular damage. Additionally, studies have revealed it to be also helpful in monitoring myopia progression. One drawback, for the time being at least, is a lack of dedicated equipment available commercially. Fortunately, the components needed to construct such an apparatus are pretty easy to obtain. Another positive development is that most studies have adopted the same standard concerning the measurement of the area of conjunctival ultraviolet autofluorescence. This made it easy to compare results across studies. More data is needed to understand better what is responsible for autofluorescence and how the various environmental and individual factors influence it.

Author contributions

M.C. — design, data collection, data interpretation, literature search, manuscript preparation; D.C. — design, manuscript preparation

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Conflict of interest

Authors declare no conflict of interests.

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