Assessment of pinguecula and pterygium in the aging eye with ultraviolet fluorescence photography (UVFP): Data analysis of eye disease survey in Shanxi, China

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Abstract

Purposes: To quantify the fluorescence of both pinguecula and pterygium via ultraviolet fluorescence photography (UVFP) and to elucidate its clinical significance. Methods: In 690 participating farmers (aged ≥ 50 years old) of an ophthalmic survey conducted in Shanxi Province, China, 444 eyes of 222 farmers were examined for pinguecula and pterygium and each of which was graded as Grade 0 to 3. A UV filter with peak wavelength of 365 nm was used in UVFP. The receiving filter had a UV-infrared cutoff. The fluorescence intensity was rated from the images as Grade 0 to 3. For statistical analysis, Pearson’s χ²-test or logistic regression adjusting for age was used. And for degree comparison, Spearman’s rank correlation coefficient was calculated. Results: The overall prevalence of pinguecula and pterygium was 67.6% and 29.7%, respectively. Presence of both lesions (the mixed type), was found in 21.2% of all eyes. Fluorescence was detected in 70.0% of all eyes, 61.3% of normal eyes, 82.5% of eyes with only pinguecula, 55.3% of eyes with only pterygium, and 58.5% of eyes with the mixed type. Fluorescence intensity was positively correlated with pinguecula grading; although it was negatively correlated with that of pterygium. Further, the fluorescence detection rate in Grade 0 of both lesions declined with patient age. Conclusions: Fluorescence detected by UVFP showed significant association with pinguecula and its progression. The data further provide important clue to the mechanism of appearance and the growth of both pinguecula and pterygium. The applicability of UVFP as a clinical routine is supported.

Key words ultraviolet fluorescence photography (UVFP), pinguecula, pterygium
I. Introduction

Pinguecula and pterygium are common conjunctival lesions. Histologically, pinguecula is an epithelial nodule consisting of collagen fibers from connective tissue degeneration. It does not invade the cornea itself. On the other hand, the pterygium tissue extends from the conjunctiva into the peripheral cornea, and in more advanced cases, the extension can reach the central cornea, thereby impairing vision. Further, the two types of lesions can overlap. In developed countries, pterygium rarely causes low vision or blindness. In developing countries, however, the lack of knowledge that excessive ultraviolet radiation can lead to advanced pterygium may explain the widespread occurrence of many such cases. We have been involved in an ocular disease survey of low-income farmers in China, sponsored by the Japanese International Cooperative Agency (JICA), and have in fact found that in the rural areas of Hainan, a 73% prevalence of pterygium with many exhibiting the advanced forms. A report issued by the WHO has noted that UV can induce cataracts and other ocular disorders including pterygium. In developed countries, pterygium has been accorded more importance than pinguecula as a UV-related disease even though pinguecula should be given equal attention. Coroneo’s group reported that with the ultraviolet fluorescence photography (UVFP), fluorescence was detected in the active cellular sites of pterygium. It appears, however, difficult to correlate the overall pathological change of pterygium with the limited localization of fluorescence which can be at the tip of pterygium or the limbus. Further, without testing UVFP on a large patient population and examining the degree of disease correlation, it is difficult to ascertain the clinical applicability of UVFP as proposed by Coroneo et al. We have therefore constructed a UVFP device and, with it, imaged a number of patients in the rural areas of Taiyuan City in Shanxi Province, China, as part of the afore-mentioned JICA ocular disease survey project. Preliminary data showed significant correlation between increasing fluorescence intensity and progression of pinguecula (p<0.0001), while the intensity decreased with the extension of pterygium (p<0.0001). This study was expanded and further conducted on a total of 222 participants (444 eyes) and the final results reported here.

II. Subjects and Methods

1. Subjects

Farmers of ≥ 50 years old who resided in Liu Jia Bao County, Xiao Dian District, Taiyuan City of Shanxi Province, China, were invited to participate and 690 had responded (a consulting rate of 91.3%, randomly selected). Those eligible for UVFP included 103 male, 119 female for a total of 222 or 444 eyes (male aged 62.3 ± 9.5 and female, 59.8 ± 8.7 years). Before the examination, the project significance and the examination procedures were explained to the participants and informed consents obtained. Table 1 shows the gender and age distribution of the participants.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>male</th>
<th>female</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-59</td>
<td>44</td>
<td>71</td>
<td>115</td>
</tr>
<tr>
<td>60-69</td>
<td>29</td>
<td>21</td>
<td>50</td>
</tr>
<tr>
<td>70-</td>
<td>30</td>
<td>27</td>
<td>57</td>
</tr>
<tr>
<td>Grand total</td>
<td>103</td>
<td>119</td>
<td>222</td>
</tr>
</tbody>
</table>

Table 1. Age and gender of participants
2. Procedures
i) Diagnosis of ocular diseases including pinguecula and pterygium was performed by the same examiner (H.S.) who was also responsible for the diagnosis in surveys conducted in Liaoning and Hainan as part of the JICA project.

ii) The grading of pinguecula and pterygium was done under a slit-lamp biomicroscope using the Kanazawa Medical University (KMU) classification system. Pinguecula was the slightly raised yellowish conjunctival lesion adjacent to the limbus with Grade 0: asymptomatic or absence of lesion; Grade 1: moderately elevated with slight hyperemia; and Grade 2: prominent elevation (Fig. 1).

And for pterygium,
Grade 0: asymptomatic or absence;
Grade 1: tip of pterygium reaching less than 1/3;
Grade 2: reaching 1/3 to 2/3 of corneal radius; and
Grade 3: reaching the central cornea (Fig. 2).

3. Fluorescence detecting device
UVFP of the anterior segment including the cornea and the conjunctiva employed a single-lens digital camera equipped with a UV-activating filter (UV-D36C, peak wavelength: 365 nm) fitted onto the flash unit and a UV-infrared cut-filter over the lens. The distance between the camera and the eye being imaged was fixed. Usually both normal and UV photos were taken from the nasal side. Grading of fluorescence intensity of the photographs was done by two examiners, Grade 0: no fluorescence; Grade 1: slight; Grade 2: moderate; and Grade 3: prominent intensity.
4. Statistical analyses
Data analyses were done according to Pearson's χ²-test or logistic regression adjusting for age. And for degree comparison, Spearman's rank correlation coefficient was calculated.

III. Results
1. Unique fluorescence detected by UVFP
A comparison between biomicroscopic observation and UVFP of pinguecula and pterygium is shown in Figs. 4 and 5, respectively.

![Fig. 4. Left: Biomicroscopy of pinguecula and right: UVFP-detected fluorescence](image1)

![Fig. 5. Left: Biomicroscopy of pterygium and right: UVFP-detected fluorescence](image2)

2. Prevalence of pinguecula and pterygium
In all 444 eyes, 23.9% (106 eyes) were without lesions (i.e., normal), 67.6% (300 eyes) showed pinguecula and 29.7% (132 eyes) pterygium. Pinguecula or pterygium alone was seen in 46.6% (206 eyes) and 8.6% (38 eyes) of the eyes, respectively. Both lesions were found in 21.2% of the eyes (the mixed type). 99.7% of pterygia were located on the nasal side, 1.5% lateral, and 0.8% on both sides.

3. Fluorescence
Fluorescence was detected in 70.0% of all eyes, in 61.3% of normal eyes, in 75.0% of eyes with pinguecula, and 57.6% with pterygium. Comparing to the normal, eyes with pinguecula had significantly higher (p=0.0004) and eyes with pterygium significantly lower (p=0.0005) fluorescence detection rate (based on logistic regression adjusted for age). Since fluorescence detection rate of pinguecula and pterygium had significant age correlations, adjusting for age was required (see also section 6 and Table 4 below). Further, fluorescence was detected in 82.5% of eyes with pinguecula alone, 55.3% of eyes with pterygium alone, and 58.5% in eyes with both lesions. And comparing to the normal, pinguecula alone had significantly higher detection rate (p=0.0065), pterygium alone and the mixed type significantly lower rate (p=0.0035 and 0.0379, respectively, based on Pearson's χ² analysis).

4. Pinguecula grading, fluorescence detection and intensity (Table 2)
Fluorescence was detected in 59.7% of Grade 0 (normal) eyes, 72.8% of eyes with Grade 1, and 100.0% of eyes with Grade 2 pinguecula. With increasing grading, fluorescence detection was significantly higher (p=0.012, age-adjusted logistic regression analysis). The fluorescence intensity and the grading also co-increased (p<0.0001 based on Pearson's χ² test and p<0.0001 based on Spearman's rank correlation)
Pinguecula

<table>
<thead>
<tr>
<th>grading</th>
<th>frequency (%) (eyes)</th>
<th>frequency (%) (eyes)</th>
<th>Frequency of the fluorescence intensity (%) (eyes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32.4 (144)</td>
<td>40.3 (58)</td>
<td>0 25.7 2.1 59.7</td>
</tr>
<tr>
<td>1</td>
<td>62.2 (276)</td>
<td>27.2 (107)</td>
<td>38.8 25.7 8.3 72.8</td>
</tr>
<tr>
<td>2</td>
<td>5.4 (24)</td>
<td>0.0 (8)</td>
<td>33.3 29.2 37.5 100.0</td>
</tr>
</tbody>
</table>

Table 2. Frequency of the fluorescence intensity by pinguecula grading in 444 eyes.

Pterygium

<table>
<thead>
<tr>
<th>grading</th>
<th>frequency (%) (eyes)</th>
<th>frequency (%) (eyes)</th>
<th>Frequency of the fluorescence intensity (%) (eyes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>70.3 (312)</td>
<td>24.7 (77)</td>
<td>36.5 29.2 9.6 75.3</td>
</tr>
<tr>
<td>1</td>
<td>18.2 (81)</td>
<td>38.3 (31)</td>
<td>30.9 24.7 6.2 61.7</td>
</tr>
<tr>
<td>2</td>
<td>9.9 (44)</td>
<td>45.5 (20)</td>
<td>45.5 9.1 0.0 54.5</td>
</tr>
<tr>
<td>3</td>
<td>1.6 (7)</td>
<td>71.4 (6)</td>
<td>28.6 0.0 0.0 28.6</td>
</tr>
</tbody>
</table>

Table 3. Frequency of the fluorescence intensity by pterygium grading in 444 eyes.

5. Pterygium grading, fluorescence detection and intensity (Table 3)
The frequency of pterygium was 70.3% in Grade 0 (normal), 18.2% in eyes with Grade 1, and 9.9% Grade 2, and 1.6% Grade 3 pterygium. Increasing grading was accompanied by a decreasing fluorescence detection rate (p=0.0015, based on age-adjusted logistic regression analysis). Fluorescence intensity decreased significantly with increasing pterygium grading (p=0.0012, based on Pearson's χ² test, and p<0.0001, based on Spearman's rank correlation).

6. Fluorescence detection rate of pinguecula and pterygium in relation to age (Table 4)
Table 4 shows the fluorescence grading and detection rate in relation to age. The fluorescence detection rate decreased significantly with aging in pinguecula Grade 0 (p=0.0252) and Grade 1 (p=0.0021). There was no difference in Grade 2 as fluorescence was detected in all eyes. In addition, the fluorescence detection rate decreased with aging in Grade 0 (p<0.0001) pterygium. There was no correlation with aging as far as Grade 1 (p=0.1890), Grade 2 (p=0.0977), and Grade 3 (p=0.8901).
pterygium (based on nominal logistic model analysis).
Analyses of changes in fluorescence intensity with aging in pinguecula and pterygium alone and in the
on health effects have been global. And the main
diseases are skin cancer followed by ocular damages.
From the 1980s on, the WHO on a global level and

<table>
<thead>
<tr>
<th>Fluorescence detection rate (%)</th>
<th>Pinguecula</th>
<th>Pterygium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>50-59</td>
<td>69.2</td>
<td>81.6</td>
</tr>
<tr>
<td>60-69</td>
<td>55.2</td>
<td>68.3</td>
</tr>
<tr>
<td>70-</td>
<td>43.2</td>
<td>59.7</td>
</tr>
</tbody>
</table>

Table 4. Fluorescence detection rate of pinguecula and pterygium in relation to age

mixed type could not be done owing to insufficient
data.

7. Location of fluorescence in pinguecula and
pterygium
The pinguecula itself showed high fluorescence,
followed by the conjunctiva and limbus.
Fluorescence appeared in pterygium itself, the
conjunctiva, limbus, and the tip of extension into the
cornea.

IV. Discussion
Clinical diagnosis of pinguecula and pterygium is
quite straightforward. Special diagnostic methods to
support new hypotheses were not formulated until
the end of the 20th century. Exposure to solar
ultraviolet as a risk factor for cataract formation and
progression was put forth in the 1970s, especially
with the discovery that chlorofluorcarbons from air
pollution destroyed the ozone layer thereby
increasing UV radiation. The interest and discussion
the National Environment Agency (now the
Ministry of the Environment) of Japan have both
encouraged research and prevention. Other
developed countries also have vigorously begun
pursuing in this field. In Japan, there are
relatively few cases of skin cancer, research on
ultraviolet-induced health issues has been mostly
ophthalmological and most investigators
concentrated on a major topic, cataract formation.
We have actively conducted experimental, clinical,
and epidemiological research in this field, both at
home and abroad even today. We have, however,
also continued examining pinguecula and pterygium
in relation to solar UV exposure. This area of
research has not attracted much attention until
recently when Coroneo et al proposed UVFP for the
examination of solar ultraviolet radiation-induced
eye disease, specifically the early signs of pterygium.
This method when perfected will have wide
applications including the study of
sunglasses/shields, hats, and other protective measures against solar UV radiation.

Coroneo et al. suggested that UVFP could provide clinical diagnosis based on specific fluorescence of pinguecula and pterygium, i.e., the onset of the symptoms could be indicated by applying excitation of specific wavelengths of light to elicit specific fluorescence. Mechanisms causing this fluorescence, however, are still unknown. Nonetheless, switching from the conventional slit-lamp biomicroscopic observation to fluorescence photography, the latter even with only prototypes, for the analysis of pinguecula and pterygium in situ certainly is a distinct possibility. This has been confirmed in the present study. Our results have shown that:

(1) UVFP can detect fluorescence not only in pinguecula and pterygium but also in the eyes without these lesions. The latter result suggests the presence of fluorescence-generating chromophores which maybe precursors to future pinguecula formation. In addition, eyes over 50 years of age without pinguecula and pterygium, or with Grade 1 pterygium, tended to have decreasing fluorescence with aging. All eyes with Grade 2 pinguecula, however showed the fluorescence. Fluorescence intensity is therefore a good indicator for disease progression.

(2) It is clear that the fluorescence detection rate of pinguecula was significantly higher and pterygium significantly lower than the normal eye.

(3) It is also clear that, in the aging eye, fluorescence intensity increased with increasing grading of pinguecula and which generally decreased with increasing grading of pterygium. It is possible that with growth, pterygium thickens to block the expression of fluorescence, or the growth actually promotes degradation of fluorescence chromophores. Additional studies are needed to differentiate these two as well as other possibilities.

Pinguecula, in the absence of pterygium but the presence of fluorescence, can be regarded as being in a precursor state. Since it has been associated with excess exposure to UV, examination of conjunctival abnormalities in children and young people with UVFP may be useful in documenting the potential and the extent of injury from exposure to UV. However, as in skin tanning, the conjunctival sensitivity to UV of each individual also will be different. Even with the same exposure, the same degree of fluorescence is unlikely to occur. On the other hand, fluorescence can be a reliable index of UV-induced conjunctival disturbance.

It is interesting to note an age-related decline of fluorescence in eyes without or with Grade 1 pinguecula. It is tempting to speculate that the elderly might have curtailed outdoor activities somewhat hence a decrease to UV exposure, and previous injuries may have been repaired - including that of the conjunctiva - even under age-related degeneration. Our results in fact showed an age-related decrease of fluorescence detection suggesting that aging is not a risk factor, at least, in cases of age over 60s.

The long-wavelength UV generating fluorescent chromophores may include elastin, collagen, tryptophan and its derivatives, NAD, and the precursors of melanin. In addition, racemization of amino acids also has been proposed. Unfortunately, there has been no experimental evidence to date. Introduction of UVFP into large-scale epidemiological studies and extensive clinical observations are still needed. And accurate
quantitation of fluorescence intensity also must be established. For us, the development of an improved UVFP device will be a logical next step. Because of its simplicity and potential efficacy, UVFP may become a valuable routine procedure in ophthalmological practice.

V. Conclusions
UVFP can be a useful tool for the examination of the mechanisms of pathogenesis and/or progression of pinguecula and pterygium.

VI. References


Acknowledgments
We wish to thank the support for this study by the Grassroot Technological Assistance Program of Japan International Cooperative Agency (JICA), administered through Kanazawa Medical University, Uchinada, Japan.
紫外線蛻光撮影による中高齢者の顕裂斑，翼状片の評価
：中国山西省地区眼疾患調査における解析から

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要旨
【目的】紫外線蛻光撮影法（UVFP）で顕裂斑，翼状片を伴う眼より検出される蛻光の臨床的意義を評価する。【対象・方法】中国人农民層を対象に行われた眼疾患疫学調査に参加した50歳以上の一般地域住民中の222名，444眼を対象とした。UVFPは紫外線励起フィルター（UV-D36C，ピーク波長：365 nm）を介したフラッシュを1眼のデジタルカメラに装着し，紫外線および赤外線カットフィルターを介し顕裂部結膜を撮影した。統計解析に，Pearsonの$\chi^2$検定，名義ロジスティックモデルを用いた多変量解析を行い，程度比較にはSpearman's順位相関を用いた。【結果】顕裂斑，翼状片の有り見率は67.6%と29.7%，両者併発は21.2%であった。蛻光は全対象眼で70.0%，正常眼で61.3%，顕裂斑单独眼で82.5%，翼状片单独眼で55.3%，両者合併眼で58.5%にみられた。蛻光強度は顕裂斑の程度と有意な正の相関があり，翼状片の程度，年齢とは有意な負の相関があった。【結論】UVFPは顕裂斑，翼状片の発現，進行メカニズムを考察する上で有用な手段になり得る。

【キーワード】紫外線蛻光撮影法（UVFP），顕裂斑，翼状片