Corneal autofluorescence in choroidal melanoma or in choroidal naevus

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Abstract

Aims—To investigate whether corneal autofluorescence is different in patients with choroidal melanoma or choroidal naevus.

Methods—Corneal autofluorescence was determined by fluorophotometry in both eyes of 32 patients with a unilateral choroidal melanoma, 32 patients with a unilateral choroidal naevus, and 32 age matched healthy controls. The corneal autofluorescence ratio between affected and contralateral eyes of patients or between randomly selected eyes of healthy controls was calculated.

Results—Mean corneal autofluorescence ratio of patients with a choroidal melanoma was significantly higher than that of healthy controls (mean ratio: 1.09 (SD 0.15) and 1.00 (0.09), respectively, ANOVA p=0.014), and than that of patients with choroidal naevus (mean ratio: 0.96 (0.09), p<0.001). Mean ratios of patients with choroidal naevus and healthy controls were not significantly different (p=0.27).

Conclusions—Corneal autofluorescence ratio of patients with a unilateral choroidal melanoma is increased. This is probably due to an increased flow of glucose through the impaired blood-aqueous barrier in the affected eye, resulting in additional glycation of corneal proteins and hence in increased autofluorescence. The corneal autofluorescence is not increased in patients with a choroidal naevus, because the blood-aqueous barrier is not impaired in the affected eye in these patients. Measurement of corneal autofluorescence is simple, fast, and non-invasive, and might be helpful to distinguish between patients with choroidal melanoma and those with choroidal naevus.

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Even though choroidal melanoma is the most common primary malignant tumour of the eye in adults, it remains a rare condition, with an incidence of eight per million per year in the Netherlands. For this reason most ophthalmologists see only a few patients with a choroidal melanoma in their working life. In approximately 50% of patients with localised tumours, the choroidal melanoma is treatable, and preservation of vision is possible with current treatment techniques. Prognostic factors are, for instance, the size of the tumour, tumour location, the cell type, nucleus size, pigmentation, age of onset, number of mitosis, and extraocular extension. Of these factors, size is the most important determinant of treatment—that is, enucleation or eye conserving therapy. The symptoms of choroidal melanoma are loss of vision, flashes, or a decreased field of vision. Often no symptoms are present and the melanoma or its possible precursor, the choroidal naevus, is detected during routine ophthalmological examination. The diagnosis of choroidal melanoma is generally based on a combination of funduscopy, transillumination, fundus fluorescein angiography, and perimetry. These routine techniques need to be performed and interpreted by an ophthalmologist with experience in ocular oncology. It is important to distinguish between choroidal melanoma and naevus, but this can be difficult, especially when a small melanoma is concerned. Corneal autofluorescence values in patients with diabetes correlate with the severity of their diabetic retinopathy, although the mechanism underlying this relation remains uncertain. This study was conducted to investigate whether corneal autofluorescence is different in patients with a melanoma or naevus in the choroid.

Materials and methods

Patients were selected from the oncology outpatient clinic of the department of ophthalmology of the Leiden University Medical Center (LUMC). They were diagnosed with a unilateral choroidal melanoma or naevus. The clinical diagnosis of melanoma or naevus rested on thorough examination of the patient in the ocular oncology service of the Leiden University Medical Center. Standard examination included direct and indirect ophthalmoscopy, ultrasonography (A and B mode), transillumination, fundus fluorescein angiography, and perimetry. In difficult cases, magnetic resonance imaging, fine needle aspiration biopsy, indocyanine green angiography, and electro-oculography were performed as well. Patients were excluded if they had diabetes mellitus type 1 or 2, ocular disease other than ocular melanoma or naevus, if they...
had undergone ocular surgery (except cataract surgery), or if they had received intravenous or intraocular fluorescein less than 36 hours before examination. Healthy controls where selected from among friends and colleagues. They were excluded if they had a history of ocular or systemic disease. Patients and healthy controls were informed about the study and gave their consent in accordance with the Helsinki treaty. The ethics committee of the Leiden University Medical Center approved the study.

Both corneas were inspected by slit lamp examination. Fluorophotometry was carried out with a scanning fluorophotometer (Fluorotron Master, Ocumetrics Inc, Palo Alto, CA, USA, Fig 1). Six fluorophotometric scans of the anterior segment of each eye were made for each participant to reduce the effect of spread in the values. The corneal autofluorescence values were determined after correction for lens tailing11 (Fig 1) and noise (a moving average over three scan points was used). In each patient with a choroidal melanoma or a choroidal naevus, the ratio between mean corneal autofluorescence values of affected and healthy eyes was calculated. In healthy controls a random selection of left-right and right-left ratios was made.

A ratio between the corneal autofluorescence of the selected eye and the control eye of each individual was used to reduce the effect of the large spread in the mean corneal autofluorescence values of both eyes on the results (about a factor 2)5 since the corneal autofluorescence values of the right and the left eyes in a group of individuals are strongly correlated (correlation coefficient >0.9, p <0.001).8

Before the average ratio of each of the three groups (melanoma patients, naevus patients, and healthy controls) was calculated, a weight factor was introduced in order to take the variation in the autofluorescence values of each cornea into account. The corresponding mathematical procedure is described in the appendix. The corneal autofluorescence ratio distribution of each of the three groups was tested for a normal distribution using d’Agostino’s test for departure of normality. A single factor analysis of variance was performed to test the null hypothesis that the values did not differ among groups.12 13 If the null hypothesis was rejected, then the Tukey multiple comparison procedure was applied for each pair of groups. The Kramer approximation for unequal numbers of values was used when necessary. For comparison, the non-parametric Kruskal-Wallis test was performed as well. Software specially developed at the laboratory of the ophthalmology department of the Leiden University Medical Center and written in Borland Pascal 7.0 and in Delphi 3 was used for all calculations.

Results

The personal data and corneal autofluorescence ratios of patients and healthy controls are presented in Table 1. The study included small, medium, and large sized melanomas (mean prominence 4.16 mm (SD 2.85), mean diameter 10.15 (2.62) mm). The naevi were generally smaller compared with the melanomas, according to their nature. Naevi with a prominence in excess of 3 mm are quite rare. The ratios were normally distributed in each of the three groups. The analysis of variance revealed that the values differed among the groups (p<0.0005). The results of the non-parametric Turkey multiple comparison procedure between pairs of groups are presented in Table 2, together with the results of the non-parametric Kruskall-Wallis test. The ratios of
Table 1  Personal data and corneal autofluorescence ratio

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Sex (F:M)</th>
<th>Age (years) (mean (SD))</th>
<th>Ratio† (mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with choroidal melanoma</td>
<td>32</td>
<td>15:17</td>
<td>58.0 (12.3)</td>
<td>1.09 (0.15)</td>
</tr>
<tr>
<td>Patients with choroidal naevus</td>
<td>32</td>
<td>24:8</td>
<td>60.4 (11.7)</td>
<td>0.96 (0.09)</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>32</td>
<td>18:14</td>
<td>58.8 (12.1)</td>
<td>1.00 (0.09)</td>
</tr>
</tbody>
</table>

†Ratio between corneal autofluorescence of selected and control eye.

Table 2  Difference of corneal autofluorescence ratio between groups

<table>
<thead>
<tr>
<th>Groups compared</th>
<th>Mean difference*</th>
<th>Difference range†</th>
<th>pparam‡</th>
<th>pnon-par§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma and naevus</td>
<td>+0.13</td>
<td>+0.068 to +0.192</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Naevus and healthy control</td>
<td>−0.04</td>
<td>−0.085 to +0.005</td>
<td>0.27</td>
<td>0.27</td>
</tr>
<tr>
<td>Melanoma and healthy control</td>
<td>+0.09</td>
<td>+0.028 to +0.152</td>
<td>0.014</td>
<td>0.020</td>
</tr>
</tbody>
</table>

*Difference between mean ratios of two groups.
†95% confidence range of the difference.
‡Significance according to the parametric Turkey multiple comparison procedure.
§Significance according to the non-parametric Kruskal-Wallis test.

Discussion

Corneal autofluorescence was determined in both eyes of patients with a unilateral choroidal melanoma, patients with a unilateral choroidal naevus, and healthy controls. The mean corneal autofluorescence ratio between the affected eye and the healthy eye was significantly higher in patients with a choroidal melanoma than in patients with a choroidal naevus or healthy controls. Because no correlation was found between tumour prominence or tumour diameter and the corneal autofluorescence ratio, and because even the six smallest melanomas in the study had corneal autofluorescence ratios in excess of 1.08 (mean prominence, diameter and ratio 2.29 (SD 0.74) mm, 1.80 (1.87) mm, 1.23 (0.11) mm). The autofluorescence ratios of the choroidal melanoma patients were correlated with tumour diameter or tumour prominence (Pearson correlation coefficients r = 0.19, p = 0.34 and 0.15, p = 0.45, respectively). In both cases, the ratios were distributed as a horizontal cloud.

The authors are indebted to Dr A H Zwinderman of the Department of Medical Statistics of the Leiden University Medical Center for help with the statistical procedures.

Appendix

The mean of six corneal autofluorescence values of the selected eye of an individual (M1), the corresponding standard deviation (SD1), the mean of six values of the control eye (M2) and the corresponding standard deviation (SD2) were calculated for each individual (i).

Then the corneal autofluorescence ratio was calculated for each individual:

\[ R_i = \frac{M_i}{M_2} \]

and the corresponding standard deviation:
$$SD_1 = \sqrt{\frac{SD_{M_1}^2}{M_1} + \frac{SD_{M_2}^2}{M_2}}$$

The expression for the standard deviation of a ratio corresponds to equation 15 in Mood et al assuming that the ratio is about one, and that the covariance between both corneas is about zero. The latter assumption is based on the fact that, in an individual, the deviations from the mean fluorescence value of the selected eye do not correlate with those of the control eye, when fluorescence is not measured simultaneously.

A weighting factor \((w_i)\) was introduced for each individual in order to take into account that an individual with a large standard deviation in one or both corneas will probably provide a less reliable ratio than an individual with small standard deviations in both corneas. The normalised inverse square root of the ratio was used as such a weighting factor, with

$$\sum_{i=1}^{N} w_i = N$$

where \(N\) is the number of individuals in the group considered. The weighted standard deviation for the group was defined as

$$SD_w = \sqrt{\frac{1}{N-1} \sum_{i=1}^{N} \left( w_i (R_i - M_2)^2 \right)}$$