Fluorophotometric Measurement of Corneal Autofluorescence in Diabetic and Normal Eyes

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Abstract

Introduction: Corneal autofluorescence (AF) has been recommended as an indicator of diabetic retinopathy. This study was conducted to evaluate corneal autofluorescence in non-insulin dependent diabetes subjects (with and without diabetic retinopathy) and compare the changes with healthy subjects.

Materials and methods: In this cross-sectional study, 145 eyes of diabetes mellitus subjects with and without diabetic retinopathy and 34 eyes of normal healthy subjects were included. Diabetic patients were subdivided by the severity of retinopathy based on international clinical diabetic retinopathy disease severity scale. The investigations included fluorophotometric determination of corneal autofluorescence, fasting glucose level (FBS) and glycosylated hemoglobin (HbA1c).

Results: The corneal autofluorescence values were significantly higher in moderate NPDR, severe NPDR and PDR when compared with healthy subjects (p<0.001). FBS values and HbA1c were significantly higher in all diabetic groups than the healthy subjects (p<0.001). In linear regression test, corneal autofluorescence seem to be related to duration of diabetes and FBS, but not with age and HbA1c.

Conclusion: We conclude that the level of corneal autofluorescence is dependent on the severity of diabetic retinopathy. Our study suggests that the formation of autofluorescent products in cornea depend upon the glucose concentration in the aqueous humor.

Key words: Ocular fluorophotometer, Corneal autofluorescence, Diabetic retinopathy, FBS, HbA1c.

Introduction

Diabetes mellitus (DM) is one of the fastest growing metabolic disorders in the world (Ting et al, 2016). The World Health Organization has estimated that the total number of people with DM will be around 366 million by the year 2030 (Wild et al, 2004). Increasing prevalence of this condition worldwide along with diabetes changes in the eye represent an increasing threat to sight. Diabetic retinopathy (DR) is a microvascular complication of DM,
remains the leading cause of acquired vision loss and blindness worldwide (Ting et al, 2016; Kobrin et al, 2007; Cheung et al, 2010). With the increasing number of DM, the number of vision threatening DR has been estimated to rise 56.3 million by 2030, which includes non-proliferative DR (NPDR), proliferative DR (PDR) and diabetic macular edema (DME) (Brussels & Belgium, 2018). Several risk factors for the development of retinopathy in diabetic patients have been established. It includes older age, longer diabetes duration, higher Hemoglobin A1c (HbA1c), hypertension, higher pulse pressure and higher total cholesterol levels (Yau et al, 2012; Wong et al, 2008). After 15 years approximately 15% will have developed PDR, whereas 75% will have developed other forms of DR (Van et al, 1998). Timely treatment and regular follow-up is essential to prevent sight threatening progression.

Corneal autofluorescence (AF) has been recommended as an indicator of diabetic retinopathy (Stolwijk et al, 1992). It originates from endogenous fluorophores that are physiologically present in the corneal structure. It results from impaired metabolism of corneal mitochondrial respiration (Rovati et al, 2004). For this reason, corneal AF is described as a good indicator of the metabolic health of the cornea (Calo-Maroto et al, 2016). The measurement of corneal AF has been shown to be a useful parameter in ophthalmology because an increased corneal autofluorescence was found in diabetic patients compared with healthy controls (Chang et al, 1995; Fantaguzzi et al, 1994; Ishiko et al, 1998; Janiec et al, 1994; Mori et al, 1997).

The aim of this study was to evaluate corneal autofluorescence in non-insulin dependent diabetes subjects (with and without diabetic retinopathy) and to compare the changes with healthy subjects.

**Materials and methods**

This cross-sectional study which was conducted in the Diabetic Retinopathy Project, Sankara Nethralaya for a period of one year (May 2008- April 2009) which included 145 eyes of diabetic mellitus subjects with and without diabetic retinopathy (DR) and 34 eyes of normal healthy subjects.

Subjects with diabetes mellitus were selected according to the following criteria: no metabolic disorders besides diabetes, no present or previous eye disease other than that caused by diabetes, no history of tobacco, no history of laser treatment and ocular surgery. Healthy control were recruited according to the following criteria: no metabolic disorders, no present or previous eye disease, no use of ocular medication, no history of ocular surgery, no history of tobacco and blood glucose level (≤110mg/dl). All subjects underwent a slit-lamp biomicroscopic examination to ensure the absence of corneal disease and blood samples were taken to assess the fasting glucose level (FBS) and percentage of glycosylated hemoglobin (HbA1c). Diabetic retinopathy was diagnosed on the basis of fundus examination and fluorescein angiography and the stage was classified based on international clinical diabetic retinopathy disease severity scale (Wilkinson et al, 2003). Diabetic retinopathy was classified into five groups which includes no diabetic retinopathy, mild non-proliferative diabetic retinopathy (NPDR), moderate non-proliferative diabetic retinopathy, severe non-proliferative diabetic retinopathy and proliferative diabetic retinopathy (PDR). The study was approved by the Institutional Review Board (Ethics committee, Vision Research Foundation) and a written informed consent was obtained from the subjects as per the Declaration of Helsinki.

Corneal autofluorescence was measured along the optical axis of the eye with the scanning fluorophotometer (Fluorotron TM Master,
OcuMetrics, Inc., Mountain View, CA, U.S.A.). It is a simple non-invasive technique and measures fluorescein within the eye. A blue excitation light (440 – 480 nm) is delivered through the optics of the system to the ocular cavity and the resulting emitted fluorescent light (531 – 634 nm) is collected and directed into a photodetector. Both the blue excitation light and the emitted fluorescent light pass through the same lens system and the area of measurement is called focal diamond. The focal diamond is moved along the optical axis of the eye by moving the lens system in steps of 0.25 mm. The focal diamond is 50µm wide, 1.9 mm high and 0.50mm deep. 148 sequential readings are made along an axis from retina to anterior of the cornea and the level of fluorescence is detected at each point. The measurements of the cornea were taken at about the same position to reduce the effect of non linearity of the scanning device. False measurements (eye movements, eyelids blink) were immediately rejected and the eye re-scanned before analysis and storage of data. All the subject corneal scans were taken between 9.00 am to 11.00 am to avoid diurnal variation. The peak values of corneal autofluorescence of three fluorophotometric scans were determined after correction for ocular lens tailing (Lohmann et al, 1995). Ocular lens tailing occurs when part of the ocular lens fluorescence is measured together with corneal fluorescence because of the limited axial resolution of the scanning fluorophotometer. The total autofluorescence along the corneal axis was calculated by multiplying the average autofluorescence by the central corneal thickness (0.543 mm) (Giasson et al, 1992).

Data analysis

The statistical software package SPSS for Microsoft Windows (v.15.0; IBM Corp., Armonk, NY) was used for analysis. Data are presented as mean ± standard deviation throughout and for categorical data, number and percentage were calculated. Kolmogorov-Smirnov was tested and the parameters were normally distributed. ANOVA with Bonferroni correction was performed. Correlation between variables, linear regression analysis was performed. \(P\) value less than 0.05 were considered statistically significant.

Results

The baseline measurements that include number of eyes, age of the subjects and duration of diabetes mellitus in each DR, no DR and healthy group are shown in Table 1.

Table 2 shows the comparison of parameters (age, FBS, HbA1c and corneal autofluorescence) among the groups (healthy subjects, no DR, mild NPDR, moderate NPDR, severe NPDR, PDR). A significant difference in FBS, HbA1c, corneal autofluorescence were noted between the groups (\(p<0.001\)) and no significant difference in age between the groups (\(p = 0.34\)) (ANOVA). The means of corneal autofluorescence values did not show any significant difference between no DR and mild NPDR (Mean±SD in ng.eq./ml: 13.11 ± 3.90 and 13.43 ± 2.98, respectively). The means of subjects with moderate NPDR, severe NPDR and PDR (Mean±SD in ng.eq./ml: 16.22 ± 3.15, 16.73 ± 4.26, 16.61 ± 4.75, respectively) were significantly higher than the means of no DR, mild DR and healthy subjects (\(p<0.001\)).

Bonferroni correction was used within the groups and we found overall significance in corneal autofluorescence, FBS, HbA1c and duration of diabetes between the groups. The corneal autofluorescence values were significantly higher in moderate NPDR, severe NPDR and PDR when compared with healthy subjects (\(p<0.001\)); no significant difference in no DR and mild NPDR when compared with healthy subjects (\(p=0.664\) and \(p=0.368\)). The FBS values were significantly higher in all diabetic groups than in healthy subjects (\(p<0.001\)); moderate NPDR group showed higher FBS values than no DR group (\(p=0.03\)). The HbA1c values were significantly higher
in all diabetic groups than healthy subjects (p<0.001) Among the DR groups, the HbA1c values didn’t show any significant difference. There was a statistically significant difference in the duration of diabetes in moderate NPDR than no DR in diabetes (p=0.004). Among the DR groups, duration of diabetes didn’t show any significant difference.

Using linear regression test, in the healthy controls, the corneal autofluorescence (y; ng.eq./ml) values were independent of age (y=10.11 +0.002 * age, R² = 0.001, p = 0.813), HbA1c (y=−9.08+0.52 * HbA1c, R² = 0.01, p = 0.545) and FBS (y=8.32+0.03 * FBS, R²=0.01, p = 0.529). In diabetes group we calculated the relationship between (a) age (years), duration of diabetes (DD; months), HbA1c (%), degree of diabetic retina (DR), FBS (mg/dl) and (b) corneal autofluorescence (y; ng.eq./ml). The result showed corneal autofluorescence seems to be related to duration of diabetes and FBS but not with age and HbA1c. The formula is

\[
y = 6.18 + 0.22* \text{age} + 0.005*\text{DD} + 0.647*\text{HbA1c} + 0.683*\text{DR} - 0.001*\text{FBS}.
\]

Overall there seems to be a relation but diabetes with no DR group shows to have a better ‘Fit of the linear curve’ than the DR group.

Table 1: Clinical characteristics

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of Subjects (n) (%)</th>
<th>Number of Eyes (n) (%)</th>
<th>Age (years) (Mean± SD)</th>
<th>Duration of diabetes (years) (Mean± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td>19 (18.62%)</td>
<td>34 (18.99%)</td>
<td>62.0 ± 5</td>
<td>------</td>
</tr>
<tr>
<td>No DR*</td>
<td>29 (28.43%)</td>
<td>49 (27.37%)</td>
<td>58.2 ± 11.0</td>
<td>8 ± 1.25</td>
</tr>
<tr>
<td>Mild NPDR†</td>
<td>19 (18.62%)</td>
<td>36 (20.11%)</td>
<td>59.1 ± 7.4</td>
<td>9.6 ± 5.1</td>
</tr>
<tr>
<td>Moderate NPDR</td>
<td>14 (13.72%)</td>
<td>23 (12.84%)</td>
<td>59.3 ± 9.2</td>
<td>13.1 ± 6.1</td>
</tr>
<tr>
<td>Severe NPDR</td>
<td>11 (10.78%)</td>
<td>16 (8.93%)</td>
<td>58.2 ± 5.4</td>
<td>9.8 ± 4.2</td>
</tr>
<tr>
<td>PDR‡</td>
<td>10 (9.80%)</td>
<td>21 (11.73%)</td>
<td>57.3 ± 8.1</td>
<td>11.1 ± 8.3</td>
</tr>
</tbody>
</table>

*DR - Diabetic retinopathy; †NPDR - Non Proliferative diabetic retinopathy; ‡PDR - Proliferative diabetic retinopathy

Table 2 : Comparison of parameters between the groups (Healthy and stages of DR)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy subjects (Mean± SD)</th>
<th>No DR* (Mean± SD)</th>
<th>Mild NPDR† (Mean±SD)</th>
<th>Moderate NPDR (Mean±SD)</th>
<th>Severe NPDR (Mean± SD)</th>
<th>PDR‡ (Mean± SD)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62± 5</td>
<td>58 ± 11</td>
<td>59 ± 7</td>
<td>59 ± 9</td>
<td>58 ± 5</td>
<td>57 ± 8</td>
<td>0.34</td>
</tr>
<tr>
<td>FBS† (mg/dl)</td>
<td>93.91 ± 7.99</td>
<td>139.43± 50.43</td>
<td>157.47 ± 53.12</td>
<td>186.1 ± 114.9</td>
<td>164.63 ± 22.66</td>
<td>183.24 ± 67.96</td>
<td>0.001</td>
</tr>
<tr>
<td>HbA1c‡ (%)</td>
<td>4.69 ± 0.52</td>
<td>6.99 ± 1.47</td>
<td>7.20 ± 1.59</td>
<td>8.18 ± 2.12</td>
<td>8.12 ± 1.18</td>
<td>7.74 ± 1.94</td>
<td>0.001</td>
</tr>
<tr>
<td>Corneal Autofluorescence (ng/ml)</td>
<td>11.49 ± 2.48</td>
<td>13.11 ± 3.90</td>
<td>13.43 ± 2.98</td>
<td>16.22 ± 3.15</td>
<td>16.73 ± 4.26</td>
<td>16.61 ± 4.75</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*DR - Diabetic retinopathy; †FBS - Fasting blood sugar; ‡HbA1c - Hemoglobin A1c; †NPDR - Non Proliferative diabetic retinopathy; ‡PDR - Proliferative diabetic retinopathy
Corneal Autofluorescence in Diabetic and Normal Eyes

Figure 1: Linear relationship between corneal autofluorescence and duration of diabetes in 3 groups which includes all the groups (Fig.1A; y=12.86+0.01\*duration, R^2=0.04, p=0.015), diabetes with no DR group (Fig.1B; y=10.61+0.003\*duration, R^2=0.12, p=0.012) and DR group (Fig.1C; y=14.26+0.001\*duration, R^2=0.01, p=0).

Figure 2: Linear relationship between corneal autofluorescence and HbA1c.

Discussion

Corneal autofluorescence was significantly increased in diabetic patients especially in cases with moderate and severe NPDR and PDR when compared to healthy subjects. This is similar to previous reports, (van Best et al, 1995; Stolwijk et al, 1992) it suggests the vascular component of diabetes mellitus causing specific microangiopathy and consequently leads to progressive diabetic retinopathy which is related to the metabolic disorder. This can also affect the cornea, resulting in an increase in corneal AF. Laing et al (1980) suggested that the corneal autofluorescence in diabetes originates from flavoproteins and pyridine nucleotides due to metabolically impaired corneal mitochondrial respiration. In our study although the corneal autofluorescence was measured with the higher wavelength of light, advanced Maillard product or cornea advanced glycation end product (AGEs) may in part be responsible for this fluorescence. AGEs are
produced by the non enzymatic reaction of glucose and other aldoses with protein through Maillard reaction and accumulate in the cornea of subjects with diabetes. AGEs have specific fluorescence characteristics and were observed in corneal basement membrane. The means of subjects with moderate NPDR, severe NPDR and PDR were significantly higher than the means of no DR, mild DR and healthy subjects. This finding is consistent with the previous study (Stolwijk et al, 1992).

The accumulation of fluorophores in diabetic cornea related to the duration of diabetes has not adequately explained the increased autofluorescence in subjects with retinopathy. Some previous studies found a significant correlation between the corneal autofluorescence and duration of diabetes (Stolwijk et al,1992;Fantaguzzi et al,1994; Stolwijk et al,1990; Sato et al, 2001), whereas others failed to show the difference (Kessel et al, 2003). In our study the duration of diabetes was correlated in the no DR group but did not show the correlation among the DR groups. This suggested that increased corneal autofluorescence in DR groups depends on metabolic disorder not only caused by chronic hyperglycemia but also the vascular component of diabetes causing blood aqueous barrier breakdown.

Glucose levels play an important role in the development of DR, prolonged high serum glucose levels usually increases the severity of retinopathy with the increased level of advanced glycosylation end products in collagen (Brownlee et al, 1988) and also increase in corneal autofluorescence (Stolwijk et al, 1992; Fantaguzzi et al, 1994; Janiec et al, 1994). In our study, corneal autofluorescence seems to be related to FBS in the diabetes group but in the healthy group it is independent of FBS.

In our study corneal AF was found to be independent with HbA1c level in normal and diabetic groups. In some studies chronic hyperglycemia seems to account for the increased corneal autofluorescence in diabetes (Sato et al, 2001; McNamara et al, 1998) and others it was found to be independent (Stolwijk et al, 1992).

**Figure 3:** Linear relationship between corneal autofluorescence and age.
Conclusion

The level of corneal autofluorescence is dependent on severity of diabetic retinopathy. It shows a statistically increase in corneal autofluorescence in diabetic retinopathy group (moderate, severe NPDR and PDR) when compared to subjects with no DR and healthy group. We conclude that the formation of autofluorescent products in the cornea depend upon the glucose concentration in the aqueous humor.

References


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