Conjunctival Hypoxia in Diabetes Mellitus

Sherwin J. Isenberg, William E. McRee, and Michael S. Jedrznyski

A frequently cited theory for the pathogenesis of neovascularization in diabetic retinopathy is that retinal hypoxia and/or ischemia release a factor which stimulates neovascularization. To the authors' knowledge, there is no direct in vivo evidence in the human proving this theory. One hundred and twenty-two diabetic subjects were studied to see whether worsening retinopathy was associated with changes in conjunctival oxygen tension (pO₂). Diabetics without retinopathy had a conjunctival pO₂ which was similar to an age-matched normal population. Diabetics with only background retinopathy had a significantly lower conjunctival pO₂ than those without retinopathy (P < 0.01). Diabetics with proliferative retinopathy showed a conjunctival pO₂ that was significantly lower than either of the first two groups (P < 0.05). The lowest value of all was found in patients with rubeosis iridis. Duration of diabetes alone did not correlate significantly to conjunctival pO₂. These findings support the hypoxic theory of diabetic neovascular retinopathy.


The etiology of diabetic retinopathy is a controversial subject. It is important, however, because the development of therapeutic measures, such as laser photocoagulation,1 may be affected by what is determined to be the pathogenesis of this major cause of blindness. The most commonly accepted etiological theory is that diabetic vasculopathy engenders ocular hypoxia and ischemia, causing the release of a biochemical factor which stimulates neovascularization.2 Yet, there are no data in the literature directly showing that the human eye becomes hypoxic as diabetes mellitus progresses. We, therefore, studied the relationship between various degrees of diabetic retinopathy on the oxygen tension of the human conjunctiva to see whether this theory could be supported.

Materials and Methods

We studied 122 adult patients who presented with diabetes mellitus. Subjects with other systemic illnesses or a history of ocular surgery were excluded from the study. Since we have shown that topically administered 2.5% phenylephrine eyedrops significantly reduce conjunctival oxygen tension,3 we also excluded patients using this or any other topical ocular medication.

For analysis, patients were divided into three groups of about equal number on the basis of ophthalmoscopy and/or fluorescein angiography: those with no retinopathy, those with only non-proliferative retinopathy, and those with neovascular proliferation of the retina and/or optic nerve (Table 1). Generally, the assessment of the retinopathy status was obtained from examination of the patient performed on the day, or at least within a month, of the conjunctival oxygen determination. For analysis, the subjects were also divided into juvenile onset diabetics characterized by reduced or absent endogenous insulin production, ketosis without exogenous insulin, and age of onset below 40 yr old, and adult onset diabetics characterized by onset after 40 yr of age with no ketosis.4

After informed consent was obtained, each subject was tested with the previously described conjunctival oxygen monitor.5 This device continuously displays the oxygen tension and temperature of conjunctival tissue. This measurement was usually carried out after assessment of the retinopathy group. Because measurements of conjunctival oxygen tension are objective and not possibly influenced by observer bias, it was not felt necessary to mask the examiner to the subject's retinopathy group before every measurement. Before each determination, the sensor was calibrated to zero with anoxic solution and to the partial pressure of oxygen in room air. After administering a topical anesthetic agent, the sensor was placed within the conjunctival fornix until a stable reading was present for at least 2
min. This process lasted less than 5 min per eye. Aside from occasional transient conjunctival injection, the monitor caused no morbidity in this investigation.

Results

The means and standard deviations for age, conjunctival oxygen tension, conjunctival temperature, and years of diabetes for each group are shown in Table 1. Of the 122 subjects studied, 42 were male and 80 were female. Their age ranged from 25–84 yr (mean of 56 yr). The mean age of each group was in the same decade. Mean conjunctival temperature did not vary among the three groups.

At the time of testing, diabetes was present in each successive group for a progressively longer period of time (Table 1). By t-test, this duration of diabetes was significantly increased in each successive group at the 0.01 level of significance for the entire study population, and at the 0.05 level when the adult onset diabetics were studied individually. However, comparing the conjunctival oxygen tension of all subjects to the duration of diabetes yielded a correlation coefficient of 0.093, which was not significant ($P = 0.32$). Insulin was used to control serum glucose in 38% of the nonretinopathy group, 71% of the non-proliferative group, and 85% of the proliferative group. Table 2 shows the juvenile onset diabetics at the time of testing to be significantly younger, have had diabetes longer, and have a significantly higher conjunctival oxygen tension and temperature than the adult onset diabetics. Comparing the different retinopathy groups for adult onset diabetics showed no significant difference in age or conjunctival temperature.

Mean conjunctival oxygen tension was 53.3 mm Hg in the nonretinopathy group, 45.1 in the nonproliferative group, and 40.1 in the proliferative group (Table 1) for the entire study population. These values for the adult onset diabetics were 52.7, 44.2, and 38.0 mm Hg, respectively. By t-test, the difference between the first two groups was significant at the 0.01 level, and between the last two groups at the 0.05 level. The same significance values were also found in comparing the retinopathy groups for adult onset diabetics alone. When extracted from the proliferative retinopathy group, the four patients with rubeosis iridis had a mean conjunctival oxygen tension that was lower (37.3 mm Hg) than that of any other group.

Discussion

To best study the relationship between retinal hypoxia and diabetic retinopathy, one should study the oxygen tension of the diabetic human retina in vivo. Since that measurement is currently technically impossible, the problem must be investigated in another manner. The human conjunctiva is more accessible than the retina, and, since the advent of the conjunctival oxygen monitor, conjunctival oxygen tension can be easily measured.

One might ask if the conjunctival hypoxia shown by this study resulted mainly from the increased duration of diabetes found in each subsequent stage of retinopathy (Table 1). However, when statistically evaluating all subjects, the correlation coefficient (0.093) comparing conjunctival oxygen tension to duration of diabetes was not significant ($P = 0.32$). One might also ask if the decreased conjunctival oxygen tension that we found in advanced diabetics is caused by a reduced arterial oxygen tension in diabetics when compared with a normal population. There is no evi-

Table 1. Conjunctival oxygen tension in diabetes mellitus (mean ± SD)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Diabetes—No Retinopathy</th>
<th>Background Retinopathy Only</th>
<th>Proliferative Retinopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>39</td>
<td>42</td>
<td>41</td>
</tr>
<tr>
<td>Juvenile onset</td>
<td>2</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Adult onset</td>
<td>37</td>
<td>39</td>
<td>31</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>57 ± 12</td>
<td>60 ± 11</td>
<td>52 ± 14</td>
</tr>
<tr>
<td>Conjunctival oxygen (mm Hg)</td>
<td>53.3 ± 8.0</td>
<td>45.1 ± 8.6</td>
<td>40.1 ± 11.5</td>
</tr>
<tr>
<td>Conjunctival temp (°C)</td>
<td>34.7 ± 0.6</td>
<td>34.6 ± 0.7</td>
<td>34.7 ± 0.7</td>
</tr>
<tr>
<td>Yr of diabetes</td>
<td>7.7 ± 6.6</td>
<td>14.1 ± 6.9</td>
<td>18.5 ± 7.5</td>
</tr>
</tbody>
</table>

Table 2. Comparison of juvenile to adult onset diabetics (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Juvenile</th>
<th>Adult</th>
<th>Significance Level*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>15</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>31 ± 6</td>
<td>60 ± 10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Measured conjunctival oxygen (mm Hg)</td>
<td>51.8 ± 13.5</td>
<td>45.7 ± 10.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Normal conjunctival oxygen for age (mm Hg)</td>
<td>63.1</td>
<td>49.2</td>
<td></td>
</tr>
<tr>
<td>Conjunctival temp (°C)</td>
<td>35.1 ± 0.6</td>
<td>34.6 ± 0.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Yr of diabetes</td>
<td>18.8 ± 6</td>
<td>12.5 ± 8</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* by t-test.
A causal relationship between hypoxia and diabetic retinopathy was first suggested by Michaelson in 1948, but has never been definitely proven. However, there are a number of mechanisms which could explain this relationship. Vracko noted that capillaries of diabetics developed thickened basement membranes with the accumulation of fibrillary material in nodular deposits. Ditzel found changes in the erythrocytes of diabetics which shift the oxyhemoglobin dissociation curve and decrease tissue oxygen availability. Hemoglobin A1C, which has an increased affinity for oxygen, normally comprises about 5% of human hemoglobin, but increases up to 20% in diabetes. A varying level of 2,3-diphosphoglycerate found in erythrocytes of diabetics can diminish oxygen release to tissues. Among other hematological causes of tissue hypoxia in diabetics reviewed by Little were increased erythrocyte aggregation and rigidity, increased platelet aggregation with increased release of thromboxane and prostaglandin E, altered plasma proteins, and changes in the fibrinolytic response. All of these mechanisms can be operative in the conjunctiva, explaining the progressive conjunctival hypoxia we found as diabetic retinopathy advanced.

While it would be incorrect to assume that oxygen tension measurements from the conjunctiva would be identical to an intraretinal measurement, there should be some relationship. Both of these tissues are primarily vascularized by the internal carotid artery, and have an intricate system of capillaries. Studies in animals and humans showed a definite relationship between arterial and conjunctival oxygen tensions in hemodynamically stable subjects. Diabetic proliferative vasculopathy occurs in some ocular capillary systems (such as the iris or retina), but not in the conjunctiva, possibly because the conjunctiva has a large supply of oxygen on its external surface (especially when the eyelids are open), while the nearest reservoir of oxygen to the retina, the choroid, is separated from the retina by the retinal pigment epithelium.

Although not often appreciated, there are changes in the conjunctiva that are induced by diabetes. Ditzel and Duckers described the vascular changes in the conjunctiva of diabetic children to consist of either a generalized dilatation of larger venules, or constriction of all conjunctival vascular elements. Most of these changes were reversible as the systemic condition came under better control. However, Dexel et al found no correlation between the morphology of the bulbar conjunctiva in diabetics and the degree of retinopathy or duration of diabetes. Using photographic morphometry to compare diabetic and normal conjunctivae, Worthen et al showed diabetics to have a 25% decrease in capillary vascularity and a 15% increase in venular vascularity, which they felt was consistent with retinal changes. They also found diabetic conjunctivae to have an increased background density compatible with greater interstitial edema, and an increased diffusion distance to capillary networks. These changes in the diabetic conjunctiva can explain tissue hypoxia.

In this study, the mean conjunctival oxygen tension found for diabetics without retinopathy (53 mm Hg) would be considered normal for subjects at that mean age (Table 1) as shown by Isenberg and Green (51 mm Hg at 57 yr old). In normal subjects, they also reported conjunctival temperature and oxygen tension to significantly decrease with advancing age. Thus, the significant disparity in age between juvenile and adult onset diabetics (Table 2) can explain at least some of the differences in conjunctival oxygen tension and temperature. Other factors, such as the greater proportion of proliferative retinopathy in the juvenile group (P = 0.015 by chi square test) shown in Table 1, may also have contributed. When comparing the mean conjunctival oxygen tension of all juvenile and all adult onset diabetics to normals of the same mean age as listed in Table 2, the diabetics had a significantly lower value (P < 0.01 by t-test). However, when studying both types of diabetics together, age was not an important variable in analyzing the three stages of diabetic retinopathy, because the mean age in each of the three groups was similar (Table 1).

In this investigation, we have shown a relationship between worsening of diabetic retinopathy and progressive conjunctival hypoxia. The two occurrences could both be related to the severity of diabetes. This linking of hypoxia of an ocular structure to diabetic retinopathy supports the theory that hypoxia is the etiology of retinal neovascularization in diabetes mellitus.

Key words: conjunctiva, hypoxia, diabetes mellitus, diabetic proliferative retinopathy, conjunctival oxygen monitor

References