Corneal endothelium and central corneal thickness changes in type 2 diabetes mellitus

Amira El-Agamy1,2,*
Shams Alsubaie1,3,*
1Department of Optometry and Vision Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia; 2Mansoura Ophthalmic Center, Faculty of Medicine, Mansoura University, Mansoura, Egypt; 3Department of Optometry and Vision Sciences, Sulaiman Al Habib Hospital, Riyadh, Saudi Arabia
*These authors contributed equally to this work

Purpose: This study was conducted to compare the corneal endothelial cell density (ECD), morphological features, and central corneal thickness (CCT) in type 2 diabetes mellitus (DM) with age-matched, nondiabetic control subjects using EM-3000 Specular Microscope.

Study design: This was a prospective, hospital-based, nonrandomized, case–control, observational, and quantitative study.

Subjects and methods: The study included 57 patients (57 eyes) with type 2 DM and 45 control (nondiabetic) subjects (45 eyes). The corneal endothelial structure and CCT were examined in all eyes by noncontact specular microscopy using EM-3000 Specular Microscope. The endothelial structure was studied for ECD, coefficient of variation of cell area (CV), and percentage of hexagonal cells.

Results: The study included 36 eyes without diabetic retinopathy (DR), 14 eyes with nonproliferative DR, and 7 eyes with proliferative DR. There were 26 eyes with a duration of ≤10 years and 31 eyes with a duration of >10 years. Also, there were 24 eyes with HBAlc ≤7.5% and 33 eyes with HBAlc >7.5%. ECD was significantly lower in the diabetic cornea than in control group (P=0.014). CV was higher in diabetic cornea (P=0.008). The diabetic cornea group had lower percentage of hexagonal cells than the control group, but the difference was not statistically significant (P=0.603). Also, diabetic cornea was thicker than control group, but not statistically significant (P=0.301).

Conclusion: This study documented that type 2 DM causes a significant reduction of ECD and increased CV (polymegathism). Also, diabetic cornea has increased CCT and lower percentage of hexagonal cells than normal subjects, but without statistical significance.

Keywords: endothelial cell density, central corneal thickness, diabetes mellitus, diabetic retinopathy, noncontact specular microscopy

Introduction

Many studies have demonstrated considerable effects of diabetes mellitus (DM) on all the layers of the cornea. These studies have documented that diabetic cornea is vulnerable to many abnormalities such as corneal endothelial damage, recurrent corneal erosions, punctate epithelial keratopathy, persistent epithelial defects, impaired corneal sensitivity, ulcers, slowed wound repair, and superficial keratitis.1–3

Morphological features of the corneal endothelium in diabetic patients recognized by a number of studies include a decrease in endothelial cell density (ECD) and polymegathism (decrease in the percentage of hexagonal cells [the normal percentage >50%]) as well as polymegathism, which means increased coefficient of variation (CV) of cell area (CV values measured between 0.22 and 0.31 are considered normal and above 0.4 are abnormal) and increased central corneal thickness (CCT).4–6
EM-3000 Specular Microscope (CBD/Tomey, Phoenix, AZ, USA) is a noncontact specular microscope that avoids some disadvantages of the classic contact specular microscopes such as corneal ulceration and transmission of infectious diseases. It has the ability to assess corneal endothelium features and measure CCT at the same time with accuracy of $\pm 10 \text{µm}$.\(^7\)

The aim of this study was to compare corneal ECD, morphological features, and CCT in type 2 DM with age-matched, nondiabetic control subjects using EM-3000 Specular Microscope. In addition, correlation between these corneal changes and the duration of DM and severity of diabetic retinopathy (DR) and glycemic control (glycated hemoglobin $[\text{HbA}_1c]$ level) were also assessed.

**Study design**

This study was a prospective, hospital-based, nonrandomized, case–control, observational, and quantitative study. It got the approval of Research Ethics Committee of College of Applied Medical Sciences, King Saud University, and Security Forces Hospital, Riyadh, Saudi Arabia. It adhered to the tenets of the Declaration of Helsinki. All the participants signed comprehensive consent after explanation of the possible consequences of the study prior to investigations.

**Subjects and methods**

The study included 57 patients (27 males and 30 females [57 eyes]) with type 2 DM and 45 control (nondiabetic) subjects (45 eyes [22 males and 23 females]). The age range of diabetic group was 42–80 years (mean: 57.08±8.37 years), and the age range of the control group was 40–76 years (mean: 50.80±8.39 years). All patients were recruited from ophthalmology department at Security Forces Hospital, Riyadh, Saudi Arabia, by nonrandomized convenience sampling method from February to April 2015. The diagnosis of type 2 DM was based on the criteria of the World Health Organization (WHO).\(^6\) Control patients did not have diabetes confirmed by random blood sugar test. The mean duration of DM was 12.87±8.03 years (range: 1–40 years), and the mean value of $\text{HbA}_1c$ was 8.57%±2.09% (range: 6.3%–17.2%). Demographics of control and diabetic patients (eyes) are shown in Table 1.

### Table 1 Demographics of control and diabetic patients (eyes)

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>Control group</th>
<th>DM patients (eyes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (eyes) (n)/percent</td>
<td>45/44.1 %</td>
<td>57/55.9 %</td>
</tr>
<tr>
<td>Mean age ± SD/range (years)</td>
<td>50.80±8.39–40–76</td>
<td>57.08±8.37±42–80</td>
</tr>
<tr>
<td>Sex (males/females) (n)</td>
<td>22/23</td>
<td>27/30</td>
</tr>
</tbody>
</table>

**Statistical analysis**

The data were analyzed using SPSS 21 for windows software (IBM, Armonk, NY, USA). The results were expressed as
mean ± standard deviation. An independent sample t-test was performed to compare the means of CCT and corneal endothelium features in diabetic and control groups. The differences among 3 or more groups were analyzed by one-way analysis of variance. Pearson correlation test was performed to find the relationship between corneal changes and DM duration, HbA$_1c$, and DR status. A value of $P \leq 0.05$ was considered statistically significant.

### Results

The study included 36 eyes without DR, 14 eyes with NPDR, and 7 eyes with PDR. There were 26 eyes with DM duration of $\leq 10$ years and 31 eyes with DM duration of $>10$ years. Also, there were 24 eyes with HbA$_1c$ $\leq 7.5\%$ and 33 eyes with HbA$_1c > 7.5\%$. Classification of groups of diabetic eyes is shown in Table 2.

ECD was significantly lower in the diabetic cornea group (2,491.98±261.08 cell/mm$^2$) than in the control group (2,629.68±293.45 cell/mm$^2$) ($P=0.014$). CV was higher in the diabetic cornea group (0.41±0.07) than in the control group (0.37±0.08) ($P=0.008$, which was considered statistically significant). Diabetic cornea group had lower percentage of hexagonal cells (33.24±10.25%) than control group (34.24±8.73%), but the difference was not statistically significant ($P=0.603$). Also, cornea was thicker in the diabetic group (54.61±30.39 µm) than control group (53.94±29.22 µm), but not statistically significant ($P=0.301$). A comparison of the mean values of CCT, ECD, CV, and hexagonality between control and diabetic eyes is shown in Table 3. Specular microscope pictures taken from control and diabetic eyes are shown in Figures 1–4.

Eyes with DM duration of $\leq 10$ years had higher ECD (2,509.38±303.49 cell/mm$^2$) than eyes with DM duration of $>10$ years (2,477.38±223.61 cell/mm$^2$) ($P=0.658$), and they also had more hexagonality (35.61±9.87%) than those with DM duration of $>10$ years (31.26±10.29%) ($P=0.111$), but the differences were not significant. Also, there were no significant differences in CCT (P=0.431) and CV (P=0.927) between the 2 groups. A comparison of the mean values of CCT, ECD, CV, and hexagonality between the diabetic groups according to the duration of DM is shown in Table 4. Specular microscope pictures taken from diabetic eyes with DM duration of $>10$ years and $\leq 10$ years are shown in Figures 2 and 4.

Diabetic patients with HbA$_1c$ $\leq 7.5$ had higher, but not statistically significant, ECD (2,537.62±311.86 cell/mm$^2$) than those with HbA$_1c$ $> 7.5$ (2,458.78±216.06 cell/mm$^2$) ($P=0.293$) and higher hexagonality (35.58±10.09%) than the other group (31.54±10.17%) ($P=0.144$). The mean of CV was significantly lower ($P=0.017$) in diabetic patients with HbA$_1c$ $\leq 7.5$ (0.39±0.05) than in the other group (0.43±0.07). Also, there was no statistically significant difference in CCT between the 2 groups (0.789). A comparison of the mean values of CCT, ECD, CV, and hexagonality between the diabetic groups according to HbA$_1c$ % is shown in Table 5.

In addition, there were no significant differences between CCT, ECD, CV, and hexagonality percentage of the corneal endothelial cells in diabetic patients without DR, with NPDR, and with PDR ($P=0.344, 0.806, 0.028$, and $0.500$, respectively) (Table 6).

Pearson correlation analysis showed that DM duration, HbA$_1c$ levels, and severity of DR had no significant correlations with CCT, CV, hexagonality, or ECD (Table 7).

### Table 2 Classification of groups of diabetic eyes

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>DR</th>
<th>DM duration (years)</th>
<th>HbA$_1c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No DR</td>
<td>NPDR</td>
<td>PDR</td>
</tr>
<tr>
<td>Eyes (n)</td>
<td>36</td>
<td>14</td>
<td>7</td>
</tr>
</tbody>
</table>

Abbreviations: DM, diabetes mellitus; DR, diabetic retinopathy; HbA$_1c$, glycated hemoglobin; NPDR, nonproliferative DR; PDR, proliferative DR.

### Table 3 A comparison of the mean values of CCT, ECD, CV, and hexagonality between control and diabetic eyes (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>CCT (µm)</th>
<th>ECD (cell/mm$^2$)</th>
<th>CV</th>
<th>Hexagonality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>539.42±29.22</td>
<td>2,629.68±293.45</td>
<td>0.37±0.08</td>
<td>34.24±8.73</td>
</tr>
<tr>
<td>DM</td>
<td>545.61±30.39</td>
<td>2,491.98±261.08</td>
<td>0.41±0.07</td>
<td>33.24±10.25</td>
</tr>
</tbody>
</table>

P-value: 0.301, 0.014, 0.008, 0.603

Abbreviations: CCT, central corneal thickness; CV, coefficient of variation of cell area; DM, diabetes mellitus; ECD, endothelial cell density; SD, standard deviation.
Discussion

Diabetic cornea may give the impression that is healthy, but actually it may suffer from many morphological features affecting its function later on. This fact was evidenced by several studies.1–6

In this study, we found that diabetic corneas showed a statistically significant reduction in mean corneal ECD of 5.24% (P=0.014) compared to the corneas in the control group. This was similar to the results of Choo et al4 of 4.5% significant reduction in ECD (P,0.01) in Malaysian type 2 DM patients. Also, the results of Inoue et al’s9 study of the corneal endothelium and CCT in type 2 DM in Japan showed similar results of 4.1% (P=0.016) significant reduction in ECD in the diabetic group. However, results of Storr-Paulsen et al’s6 and Siribunkum et al’s10 studies of corneal endothelium morphology in type 2 DM according to ECD were not consistent with our findings. The CV of corneal endothelial cells was found to be significantly increased in diabetic patients (P=0.008). This increase indicated the presence of polymegathism, in which endothelial cells enlarge to fill the gaps between adjacent cells. This result was concordant with those obtained by Shenoy et al11 and Lee et al,12 but not similar to those of Sudhir et al5 and Chen et al.13

Also, our study demonstrated no significant difference in the percentage of hexagonal cells between the diabetic patients and the controls, which was in agreement with the results of Sudhir et al,5 Storr-Paulsen et al,6 and Inoue et al,9 and in disagreement with those of Choo et al4 and Lee et al.12

Table 4 A comparison of the mean values of CCT, ECD, CV, and hexagonality between the diabetic groups, according to duration of DM (mean ± SD)

<table>
<thead>
<tr>
<th>DM duration (years)</th>
<th>CCT (µm)</th>
<th>ECD (cell/mm²)</th>
<th>CV</th>
<th>Hexagonality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤10</td>
<td>549.11 ± 30.57</td>
<td>2,509.38 ± 303.49</td>
<td>0.41 ± 0.09</td>
<td>35.61 ± 9.87</td>
</tr>
<tr>
<td>&gt;10</td>
<td>542.67 ± 30.43</td>
<td>2,477.38 ± 223.61</td>
<td>0.42 ± 0.04</td>
<td>31.26 ± 10.29</td>
</tr>
<tr>
<td>p-value</td>
<td>0.431</td>
<td>0.658</td>
<td>0.927</td>
<td>0.111</td>
</tr>
</tbody>
</table>

Abbreviations: CCT, central corneal thickness; CV, coefficient of variation of cell area; ECD, endothelial cell density; HbA1c, glycated hemoglobin; SD, standard deviation.
Many studies have explained the morphological features of diabetic cornea. This was established by the assessment of polyol (sorbitol–aldose reductase) pathway in diabetic cornea. These studies documented that high glucose levels lead to increased activity of the aldose reductase, causing sorbitol buildup in the corneal epithelial and endothelial cells. This sorbitol acts as an osmotic agent and leads to swelling of endothelial cells. Also, DM diminishes Na⁺–K⁺ ATPase activity of the corneal endothelium, resulting in morphological and permeability changes in diabetic cornea, thereby leading to corneal destruction. Furthermore, endothelial pump function was proven to be affected by decreased ATP production as a result of slowing down of the Krebs cycle in diabetic cornea.

Regarding CCT in our study, there was no significant difference between diabetic and control groups. This result was in concordance with those of Choo et al and Sudhir et al. Other studies, for instance, Storr-Paulsen et al and Lee et al, demonstrated a statistically significant increase in CCT of diabetic compared to control subjects.

As regards the comparison of the mean values of CCT, ECD, CV, and cell hexagonality in patients with DM duration of ≤10 years and those with DM duration of >10 years, no statistically significant difference was detected in all these morphological features in this study. This finding was in agreement with Altay et al, who reported absence of statistically significant difference in CCT between the 2 groups. However, Lee et al documented that CCT and CV were significantly higher in patients with DM duration of >10 years compared with those with DM duration of ≤10 years, but there was no statistically significant difference regarding ECD and hexagonality between the 2 groups.

Concerning the comparison of the mean values of CCT, ECD, CV, and cell hexagonality in diabetic patients with HbA₁c ≤7.5% and those with HbA₁c >7.5% in our study, no significant differences were detected between the 2 groups in CCT, ECD, and hexagonality, but CV showed a statistically significant difference (P=0.017). Storr-Paulsen et al found significantly lower ECD in patients with elevated HbA₁c but without any influence on CCT. However, Altay et al documented significantly thicker CCT in hyperglycemic condition than in euglycemic condition in the same patient before and after successful control of HbA₁c.

Regarding the comparison of the mean values of CCT, ECD, CV, and hexagonality between the 3 diabetic groups according to the status of DR, our study found no significant differences between the 3 groups. This finding was not in accordance with that of Shenoy et al, who demonstrated significantly lower ECD in eyes with DR than those without DR.

Pearson correlation analysis reported absence of significant correlations between DM duration, HbA₁c levels, and severity of DR and morphological features of diabetic cornea such as CCT, CV, hexagonality, or ECD. These results were similar to those of Choo et al. However, Siribunkum et al found a significant correlation between DM duration and pleomorphism, polymegathism, and CCT. Severity of DR was correlated with ECD, but these correlations were low. However, the corneal morphological abnormalities were not correlated with HbA₁c levels.

### Table 5 A comparison of the mean values of CCT, ECD, CV, and hexagonality between the diabetic groups, according to HbA₁c% (mean ± SD)

<table>
<thead>
<tr>
<th>HbA₁c%</th>
<th>CCT (µm)</th>
<th>ECD (cell/mm²)</th>
<th>CV</th>
<th>Hexagonality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤7.5</td>
<td>544.3±32.89</td>
<td>2,537.6±111.86</td>
<td>0.39±0.05</td>
<td>35.58±10.09</td>
</tr>
<tr>
<td>&gt;7.5</td>
<td>564.5±31.84</td>
<td>2,458.7±216.06</td>
<td>0.43±0.07</td>
<td>31.5±10.17</td>
</tr>
</tbody>
</table>

### Table 7 Correlation between corneal changes and DM duration, HbA₁c%, and DR status

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>CCT (µm)</th>
<th>ECD (cell/mm²)</th>
<th>CV</th>
<th>Hexagonality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM duration, years</td>
<td>R value</td>
<td>0.163</td>
<td>0.069</td>
<td>0.051</td>
</tr>
<tr>
<td>P-value</td>
<td>0.226</td>
<td>0.610</td>
<td>0.702</td>
<td>0.320</td>
</tr>
<tr>
<td>HbA₁c%</td>
<td>R value</td>
<td>0.156</td>
<td>0.218</td>
<td>0.125</td>
</tr>
<tr>
<td>P-value</td>
<td>0.245</td>
<td>0.103</td>
<td>0.351</td>
<td>0.234</td>
</tr>
<tr>
<td>DR status</td>
<td>R value</td>
<td>0.175</td>
<td>0.085</td>
<td>0.158</td>
</tr>
<tr>
<td>P-value</td>
<td>0.192</td>
<td>0.527</td>
<td>0.240</td>
<td>0.554</td>
</tr>
</tbody>
</table>

### Table 6 A comparison of Altay et al, who demonstrated significantly lower ECD in patients with elevated HbA₁c but without any influence on CCT. However, Altay et al documented significantly thicker CCT in hyperglycemic condition than in euglycemic condition in the same patient before and after successful control of HbA₁c.

Concerning the comparison of the mean values of CCT, ECD, CV, and hexagonality in diabetic patients with HbA₁c ≤7.5% and those with HbA₁c >7.5% in our study, no significant differences were detected between the 2 groups in CCT, ECD, and hexagonality, but CV showed a statistically significant difference (P=0.017). Storr-Paulsen et al found significantly lower ECD in patients with elevated HbA₁c but without any influence on CCT. However, Altay et al documented significantly thicker CCT in hyperglycemic condition than in euglycemic condition in the same patient before and after successful control of HbA₁c.

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Conclusion
This study documented that type 2 DM resulted in a significant reduction of ECD and increased CV (polymegathism). Also, diabetic cornea had increased CCT and lower percentage of hexagonal cells than in the cornea of normal subjects, but this is not statistically significant. In addition, our study demonstrated that DM duration, HbA1c levels, and severity of DR had no significant correlations with CCT, CV, hexagonality, or ECD.

In this study, patients with a history of laser photocoagulation were excluded due to its influence on the corneal structures. This exclusion produced a small sample size because most of diabetic patients who came to the clinic had undergone laser treatments. Therefore, further studies with a large sample size are recommended to verify the extent of corneal damage caused by type 2 DM.

Disclosure
Dr Amira El-Agamy is financially supported by the Research Center of College of Applied Medical Sciences, King Saud University, Saudi Arabia. The authors report no other conflicts of interest in this work.

References