



# Study of corneal endothelial cells in diabetic patients

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**Background:** The cornea is the anterior transparent part of the eye. In addition to its optical and refractive function, it is an important protective structure.

**Aim:** The aim of this study was to assess corneal endothelium (counts, morphology and structure) as well as corneal thickness of Type 2 diabetic participants.

**Setting:** This is a hospital-based case-control study and was carried out at Ibn Al Haitham tertiary eye hospital in Baghdad, Iraq.

**Methods:** The sample size was 240 eyes of 120 diabetic participants and 120 healthy participants. Non-contact specular microscopy was utilised to evaluate corneal endothelial cells, including endothelial cell density (ECD), coefficient of variation in cell area (CV), hexagonality (HEX) of cells as well as central corneal thickness (CCT).

**Results:** The ECD was lower in the diabetic corneas ( $2584.87 \pm 259.15$  cell/mm<sup>2</sup>) compared with the healthy corneas ( $2717.56 \pm 289.67$  cell/mm<sup>2</sup>) ( $p = 0.017$ , statistically significant). Coefficient of variance (CV) was greater in the diabetic group ( $40.8 \pm 4.17$ ) as opposed to the group with healthy corneas ( $37.3 \pm 2.89$ ) ( $p = 0.019$ , statistically significant). The corneas of the diabetic group showed lower hexagonality ( $44.36\% \pm 9.87\%$ ) compared with the healthy corneas ( $59.35\% \pm 9.67\%$ ) ( $p < 0.001$ , statistically significant). Furthermore, the corneas of the diabetic group had greater central thickness ( $581.1 \pm 32.4$   $\mu$ m) when compared with the control group ( $511.8 \pm 29.8$   $\mu$ m), ( $p < 0.001$ , statistically significant). No correlation was found between the severity level of diabetic retinopathy and corneal endothelial pathological alterations.

**Conclusion:** Long-term poorly controlled glycaemia has a remarkable impact on corneal endothelium (counts, morphology and structure) as well as corneal thickness.

**Keywords:** cornea; corneal endothelium; corneal thickness; specular microscopy; diabetes mellitus.

## Introduction

The corneal endothelial layer is composed of a single layer of securely arranged hexagonal cells, which is fundamental for the preservation of corneal health parameters like hydration status, thickness and clarity.<sup>1</sup> The hydration status of a cornea may be assessed by corneal thickness, which could be affected by endothelial cell count.<sup>2</sup> Progressive damage and loss of these cells result in an abnormal rise in corneal thickness, stromal oedema and decrease of vision as these cells are responsible for the relatively dehydrated status of the stroma through ionic pumps in basolateral plasma membranes.<sup>3</sup>

Globally, as the number of cases with diabetes mellitus rises, many of the previous studies had encountered the presence of diabetic retinopathy (DR); however, diabetes may also impact other parts of the eye like the cornea. Diabetes may involve any layer of the cornea, particularly epithelial and endothelial layers leading to significant pathological alterations in both these layers.<sup>4,5,6</sup> Structural and morphological alterations in the endothelium of a diabetic cornea have been reported previously in the literature.<sup>7,8,9</sup> Possible changes include a reduction in the endothelial cell count and polymorphism, defined as a reduction in the hexagonal arrangement of endothelial cells. Hexagonality of  $>60\%$  is regarded as normal. Also, polymegathism, defined as cells with varying shape, as a rise in values of the coefficient of variation of endothelial cell area above the normal range (between 21 and 32) and more than 40 is regarded as abnormal.<sup>7,8,9</sup>

Specular microscopy is very beneficial in providing detailed information regarding the structural and morphological status of the corneal endothelium.<sup>10</sup> This apparatus permits early awareness of endothelial alterations that might be hidden during evaluation with a slitlamp biomicroscope

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because of the higher magnification that is necessary to view these cells in detail.<sup>11</sup> Another important use of specular microscopy is the measurement of corneal thickness. This device is non-invasive, relatively simple to utilise, with acceptable operator-independent reproducibility.<sup>12</sup>

The aim of this study was to assess corneal endothelium (counts, morphology and structure) as well as corneal thickness of Type 2 diabetic participants and to estimate the influence of disease on the endothelial layer and overall corneal thickness.

## Subjects and methods

The design of the study was a hospital-based case-control study. The sample included 240 eyes (120 diabetic patients and 120 healthy (control) patients). All participants were enrolled at the Ibn Al-Haitham Teaching Eye Hospital between January 2018 and October 2019. Ethical approval for the study was obtained from the relevant committee of the College of Medicine, University of Anbar, and informed consent was obtained from each participant.

Inclusion criteria included participants between 45 and 65 years of age who had been diagnosed with Type 2 DM according to the World Health Organization (WHO) criteria.<sup>13</sup> A control healthy non-diabetic group (approved by a random blood sugar reading test) of the same age range was included in this study.

Exclusion criteria excluded participants of age younger than 45 years; participants with TI DM; previous ocular surgery including trauma; previous or recent ocular infection and/or inflammation; pseudoexfoliation or glaucoma or corneal dystrophy, corneal disorders because of long-standing conjunctival or lid abnormalities like advanced pterygium, entropion, ectropion and trichiasis; use of contact lenses, any systemic diseases that affect tear components and/or its function like rheumatoid arthritis and chronic use eye drops. Previous retinal photocoagulation and pregnancy were also exclusion criteria.

A detailed case history was taken from all participants, including random blood sugar readings, uncorrected and best-corrected (near/far) visual acuities. Clinical evaluation was carried out with slitlamp biomicroscopy of anterior and posterior segments as well as the measurement of intraocular pressure by Goldman applanation tonometry. In the current study, only the right eyes of participants were assessed. Corneal endothelium parameters were measured with the non-contact NIDEK CEM-530 (Tokyo, Japan) microscope by well-trained medical staff. About  $100 \pm 20$  endothelial cells were captured in each exam to be analysed. To verifying the readings, this exam was repeated three times for each image, after which the mean value of the endothelial cell calculations were used for data analysis. In this study, the structure of the endothelium was assessed with different parameters, such as corneal endothelium density, coefficient of variation (CV),

cellular hexagonality in addition to central corneal thickness (CCT).

The diabetic participants were sub-categorised according to the level of DR present, for example, none or very mild DR, non-proliferative or proliferative DR.

Post-examination, data were collected and analysed using the Statistical Package of Social Science, version 24 (SPSS, US). A probability value ( $p$ -value)  $< 0.05$  was interpreted as statistically significant.

## Ethical considerations

Ethical clearance to conduct this study was obtained from the Ministry of Higher Education & Scientific Research, University of Anbar Ethical Approval Committee (No. 42). Informed consent was obtained from each participant.

## Results

The study involved 240 eyes of 120 diabetic participants and 120 healthy (control) participants. In the diabetic group, the mean age was 56.4 years, and the mean age of the healthy controls was 55.4 years. There was no statistically significant difference between the two groups. In the sample, male participants represented 45% of the diabetic group and 43% of the control group.

Investigation of the endothelial cell density (ECD) showed that a lower ECD was found in the corneas of the diabetic group ( $2584.87 \pm 259.15$  cell/mm<sup>2</sup>) as compared with the healthy control group ( $2717.56 \pm 289.67$  cell/mm<sup>2</sup>) ( $p = 0.017$ , statistically significant). The coefficient of variation (CV) was higher in the corneas of the diabetic group ( $40.8 \pm 4.17$ ) opposed to the healthy cornea group ( $37.3 \pm 2.89$ ) ( $p = 0.019$ , statistically significant). The corneas of the diabetic group showed lower percentages for hexagonal cells (HEX) ( $44.36\% \pm 9.87\%$ ) compared with the healthy cornea group ( $59.35\% \pm 9.67\%$ ) ( $p < 0.001$ , statistically significant). The thickness of the diabetic corneas was greater ( $581.1 \pm 32.4$   $\mu$ m) compared with the control group ( $511.8 \pm 29.8$   $\mu$ m) ( $p < 0.001$ , statistically significant). The statistical values of the above corneal endothelium and thickness of the two groups are shown in Table 1.

In this study, diabetic participants included 60% with proliferative DR (PDR) and 40% with non-proliferative DR (NPDR). Statistical analysis revealed no significant differences between these groups for all endothelial structure parameters as well as corneal thickness (Table 2).

## Discussion

The corneas of the diabetic participants may appear to be normal and healthy upon examination; however, in fact, they may be subjected to considerable morphological alterations influencing their function and health later.<sup>7,8,9,14,15,16</sup> Endothelial cell assessment provides the ophthalmologist a paramount

**TABLE 1:** Comparison of corneal endothelial cell parameters and thickness between diabetic cases (T2 DM) and controls.

Variable	Diabetic cases (n = 120)	Healthy participants (n = 120)	t-Test value	p	Sig.
<b>CD (cell/mm<sup>2</sup>)</b>					
Mean ± s.d.	2584.87 ± 259.15	2717.56 ± 289.67	15.7	0.017	S
Range	2268–3175	2294–3265			
<b>CV</b>					
Mean ± s.d.	40.8 ± 4.17	37.3 ± 2.89	11.3	0.019	S
Range	33–44	30–39			
<b>HEX (%)</b>					
Mean ± s.d.	44.36±9.87	59.35 ± 9.67	5.7	< 0.001	S
Range	29–53	35–65			
<b>CCT (μm)</b>					
Mean ± s.d.	581.1±32.4	511.8±29.8	14.2	< 0.001	S
Range	527–637	467–575			

CCT, central corneal thickness; CV, coefficient of variation; CD, cell density; HEX, hexagonal cells percentage; Sig., significance; S, significant.

**TABLE 2:** Characteristics of corneal endothelial cell parameters and thickness of diabetic cases according to the diabetic retinopathy status.

Variable	Proliferative DR (n = 72, 60%)	Non proliferative DR (n = 48, 40%)	t-Test value	p	Sig.
<b>CD (cell/m<sup>2</sup>)</b>					
Mean ± s.d.	2562.91 ± 254.25	2663.54 ± 269.67	4.7	0.638	NS
Range	2269–3079	2284–3235			
<b>CV</b>					
Mean ± s.d.	39.8 ± 3.97	38.8 ± 3.49	5.3	0.364	NS
Range	34–45	32–44			
<b>HEX (%)</b>					
Mean ± s.d.	45.76 ± 8.97	46.65 ± 9.68	3.7	0.304	NS
Range	35–54	35–55			
<b>CCT (μm)</b>					
Mean ± s.d.	578.2 ± 39.7	567.6 ± 36.5	14.2	0.356	NS
Range	536–622	529–605			

CCT, central corneal thickness; CV, coefficient of variation; CD, cell density; DR, diabetic retinopathy; HEX, hexagonal cells percentage; Sig., significance; NS, non significant.

clinical awareness regarding corneal health and activity. Study and analysis of the endothelial cell morphological parameters provide both quantitative (cell count) and qualitative (variance in cell area and shape) corneal features. So the decrease in cells may not be observed through cell density readings alone but may be discovered through evaluation of other endothelial parameters like the CV, HEX in addition to central corneal thickness.<sup>17</sup>

In the current study, corneal endothelial parameter measurements were significantly dissimilar between diabetic and healthy participants. In the diabetic group, corneas were significantly thicker, and the mean endothelial cell count was significantly lower in comparison with the control participants ( $p < 0.001$ ,  $p = 0.017$ , respectively).

Similar results were reported by previous studies,<sup>9,18,19,20</sup> which assessed the corneal morphological changes in corneas of diabetic patients compared with healthy controls. These studies revealed that diabetic corneas were thicker in structure and have lower endothelial cell counts and increased thickness possibly because of an increase in water content in the stroma, which can be attributed to the malfunction of endothelial cells. Choo et al.<sup>7</sup> and Inoue et al.<sup>21</sup> revealed a significant reduction in

endothelial cells; however, no statistically significant difference was observed in corneal thickness between the studied groups.

Other studies demonstrated no differences between the corneas of diabetic and non-diabetic patients. Schultz et al.<sup>22</sup> mentioned that endothelial cell counting of diabetic corneas was comparable with healthy corneas. Similar results were observed by other related studies.<sup>23,24,25</sup> However, most of these studies had fewer numbers of participants compared with the current study.

In this study, the results revealed that the coefficient of variation of endothelial cells was significantly higher ( $p = 0.019$ ) in diabetic corneas. The rise in CV may indicate a higher prevalence of polymegathism which is present when endothelial cells tend to expand to close a gap between adjacent cells caused by endothelial cell loss to keep integrity with adjacent cells. This study also revealed that the cell hexagonality was significantly lower in diabetic corneas, reflecting the existence of pleomorphism ( $p < 0.001$ ). Similar results were reported by other related studies.<sup>18,19,21,26,27</sup> These studies revealed that diabetic corneas had a higher coefficient of variation and cell size and lower hexagonality percentage and explained these changes as diabetic corneas suffer from an increased rate of endothelial cells loss, which, in turn, affects both coefficient of variation and hexagonality. However, Larsson et al.<sup>24</sup> mentioned that there were no differences in hexagonality and CV between diabetic and healthy corneas.

This study shows that there was no linkage between the severity of retinopathy and corneal endothelial structural and morphological changes, as well as corneal thickness ( $p = 0.638$ ,  $0.364$ ,  $0.304$  and  $0.356$ , respectively). Larsson et al.<sup>24</sup> observed that the appearance of DR had no impact on the ECD; in addition, there were no significant variances among different grades of DR. However, Roszkowska et al.,<sup>19</sup> McNamara et al.,<sup>28</sup> Weston et al.<sup>29</sup> and Gekka et al.,<sup>30</sup> reported that there was a relationship between pathological alterations of corneal endothelium and thickness and severity level of retinopathy. Other studies<sup>9,20,24</sup> observed that cases of long-term diabetes had thicker corneas compared with cases of shorter duration of diabetes because of dysfunction of endothelial pumping activity and progressive hydration of the corneal stroma; however, the grade of DR was not included in their studies. Wiemer et al.<sup>31</sup> and Busted et al.<sup>32</sup> concluded that corneal endothelial cell status and thickness in diabetic cases showed no differences from that of the healthy cornea and also revealed no relationship between diabetes duration, grade of retinopathy and corneal changes.

Several studies<sup>7,33,34,35</sup> attempted to find the reasons behind the morphological and structural alterations in the cornea of diseased patients and postulated three main reasons: (1) abnormalities of the sorbitol–aldose reductase pathway in the cornea of the diabetic patient, which result in an excess of

sorbitol inside corneal cells and works like an osmotic factor and results in swollen endothelium. (2) Diabetes inhibits  $\text{Na}^+ - \text{K}^+$  ATPase action of endothelial cells, which affects endothelial pumping activity. (3) Cellular cytoskeleton abnormal changes, which have an essential role in the preservation of the endothelial cell volume, shape and barrier system. Corneal swelling increases light back-scattering, which results in the reduction of contrast sensitivity and increased susceptibility to glare.<sup>36</sup> This adverse event is of particular concern among postoperative patients because it directly influences the final postoperative corneal transparency, which, in turn, can affect visual acuity.<sup>37</sup>

Diabetic patients are more liable to have more intraocular surgeries than healthy persons; faster recovery of visual acuity after these surgeries is important.<sup>38</sup> Faster visual recovery may be attributed to minimal endothelial damage or stress.<sup>39</sup> In most cases, it is a short-lived phenomenon that presents as early and transient postoperative corneal oedema, but if endothelial cell loss persists and the cell count falls below a threshold of 500  $\text{mm}^2$ , corneal stromal swelling increases light scattering, which results in the reduction of contrast sensitivity and increased susceptibility to glare.<sup>40</sup> This adverse event is of particular concern among postoperative patients because it directly influences final postoperative corneal transparency, which, in turn, can affect visual acuity.<sup>41</sup>

In conclusion, diabetes can affect the corneal parameters discussed and investigated in this study. These effects on the cornea and especially the corneal endothelium suggest the need to carry out specular microscopy routinely for every diabetic patient who undergoes intraocular surgery. This is to avoid as much as possible the stress induced by surgical procedures, particularly cataract surgeries; these surgeries usually performed by phacoemulsification procedures in the anterior chamber and are close to the endothelium and Descemet's membrane.

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Y.F.D. is the sole author of this article.

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### Data availability

Data are available from the corresponding author, Y.F.D.

## Disclaimer

The views and opinions expressed in this article are those of the author and do not necessarily reflect the official policy or position of any affiliated agency of the author.

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