

# A Comparative Study of Corneal Endothelial Structure between Diabetes and Non-Diabetes

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**Background:** The corneal endothelial structure may be changed in diabetic patients, but the results were variable.

**Objective:** To compare the corneal endothelial structure including endothelial cell density, polymorphism, and pleomorphism, in diabetic and non-diabetic subjects.

**Material and Method:** The corneal endothelial structure of diabetic and non-diabetic subjects were measured by specular microscope (Confoscan4 (CS4), Nidek) for endothelial cell density, percentage of polymegathism, and percentage of hexagonal cells. The data were analyzed using the descriptive statistics and the unpaired t-test.

**Results:** There were 171 eyes of 90 diabetic patients with the mean age of  $58.49 \pm 9.78$  years, and 156 eyes of 90 non-diabetic subjects with the mean age of  $58.98 \pm 13.12$  years. Three parameters of measurement revealed no significant difference ( $p > 0.05$ ). The over one year diabetic patients, however, demonstrated a decreased percentage of hexagonal cells statistically significant ( $p < 0.05$ ), while the over two years diabetic patients demonstrated a decreased percentage of hexagonal cell and an increased percentage of polymegathism statistically significant ( $p < 0.05$ ).

**Conclusion:** The corneal endothelial structure is not different between diabetic and non-diabetic groups. When the disease progresses, however, the hexagonal cells decrease at first, followed by the polymegathism. No difference shows in endothelial cell density.

**Keywords:** Corneal endothelium, Diabetes, Specular microscope, Polymorphism, Pleomorphism

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Diabetes mellitus is a chronic disease that affects multiple organs including the eyes. It causes not only diabetic retinopathy, the common complication of the eyes, but also causes epithelial keratopathy<sup>(1)</sup>, increased central corneal thickness<sup>(2,3)</sup>, and altered corneal endothelial cells<sup>(4-6)</sup>. The corneal endothelial cell density is approximately 6,000 cell/mm<sup>2</sup> during the first month of life<sup>(7)</sup>, which reduces to 3,500 cell/mm<sup>2</sup> at age of five<sup>(8)</sup>. After age 18, the decrease slows to 0.6% per year and appears to remain at this rate for life<sup>(9)</sup>. With age, the coefficient of variation of cell area (polymegathism) gradually increases and the percentage of hexagonal cells (pleomorphism) gradually decreases<sup>(10)</sup>. These cells are susceptible to aging, glaucoma, contact lens wearers, trauma, intraocular surgery, corneal endotheliopathies, and systemic disease especially diabetes mellitus<sup>(11)</sup>.

There were conflicting results in most studies related to diabetes and corneal endothelial structure. Some studies reported increased polymegathism and decreased hexagonal cells, but no difference in endothelial cell density in diabetic patients compared with non-diabetic subjects<sup>(4,11-13)</sup>. However, one study showed decreased cell density and increased polymegathism, but no difference in hexagonal cells<sup>(14)</sup>. Furthermore, a study displayed all parameters were significantly different<sup>(15)</sup>, while another reported no parameters differed<sup>(16)</sup>.

The objective of the present study is to compare the corneal endothelial structure, including endothelial cell density, polymegathism, and pleomorphism in diabetic and non-diabetic subjects.

## Material and Method

### Subjects

Ninety diabetic patients and 90 non-diabetic subjects over or equal the age of 40 years were included at Thammasat University Hospital. The exclusion criteria for any eye of both groups consisted of corneal disease, glaucoma or the intraocular pressure over

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21 mmHg measured by Goldmann applanation tonometry, previous eye trauma, intraocular surgery, or retinal photocoagulation, and contact lens wearers. The most recent fasting plasma glucose (FPG) in one year of the non-diabetic group had to be lower than 126 mg/dl.

### Methods

All subjects were interviewed, examined by slit lamp microscope, and intraocular pressure measured by Goldmann applanation tonometry. The corneal endothelial structure was quantified by measuring a variety of factors, including cell density, percentage of polymegathism, and percentage of hexagonal cells by specular microscopy (Confoscan 4 (CS4), Nidek). An average of three measurements was recorded.

### Statistical analysis

The two-tail unpaired t-test was used to compare a variety of means between diabetic and non-diabetic groups, including age, corneal endothelial cell density, percentage of polymegathism, and percentage of hexagonal cells at the  $p$ -value  $<0.05$  to be statistically significant. The duration of diabetes was also divided in groups to compare with the non-diabetic group.

### Ethics

The present research has been approved by the Human Research Ethics Committee of Thammasat University (No. 1: Faculty of Medicine), Thailand. Informed written consent was obtained from all participants. The authors verified that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research, adhering to the tenets of the Declaration of Helsinki.

### Results

There were 171 eyes of 90 diabetic patients (41 male and 49 female), and 156 eyes of 90 non-diabetic subjects (34 male and 56 female). The diabetic group contained two eyes of one diabetic patient with severe non-proliferative diabetic retinopathy (NPDR), 11 eyes of 7 diabetic patients with moderate NPDR, 24 eyes of 13 diabetic patients with mild NPDR, and 134 eyes of 71 diabetic patients with no diabetic retinopathy. The compared data between two groups were presented in Table 1, in which the corneal endothelial structure displayed no statistically significant difference.

In addition, the diabetic patients whose duration of disease were under or equal one year were excluded and reanalyzed by comparing with the non-diabetic group. The second data analysis showed 130 eyes of 69 diabetic patients (32 male and 37 female) and contained, then, the percentage of hexagonal cells demonstrating statistically significant differences at the  $p$ -value  $<0.05$ , for which the mean of the diabetic group was lower than the non-diabetic group. The second data analysis between two groups was presented in Table 2.

Furthermore, the authors excluded the diabetic patients whose duration of disease was under or equal to two years and reanalyzed as the third analysis by comparing them with the same non-diabetic group. The third data analysis showed 118 eyes of 63 diabetic patients (29 male and 34 female) which contained, at this moment, both percentage of hexagonal cells and polymegathism and demonstrated statistically significant differences at the  $p$ -value  $<0.05$ , in which the diabetic group showed the mean of percentage of hexagonal cells was lower and the mean of percentage of polymegathism was higher than the non-diabetic group. The third data analysis between two groups was presented in Table 3.

**Table 1.** The statistic parameters of diabetic and non-diabetic group

Parameters	Diabetics (n = 171 eyes)		Non-diabetics (n = 156 eyes)		p-value
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	
Age (year)	58.49 $\pm$ 9.78	40-85	58.98 $\pm$ 13.12	41-91	0.777
FPG (mg/dl)	159.84 $\pm$ 58.59	95-386	94.40 $\pm$ 10.90	42-120	0.000
Duration of disease (year)	6.73 $\pm$ 6.13	1/12-30	-	-	-
Cell density (cell/mm <sup>2</sup> )	2,384.97 $\pm$ 254.44	1,539-2,887	2,413.37 $\pm$ 313.76	1,155-4,409	0.372
Polymegathism (%)	57.83 $\pm$ 10.97	33.6-88.4	55.81 $\pm$ 10.02	35.5-86.0	0.084
Hexagonal cell (%)	34.13 $\pm$ 6.98	20.0-54.0	35.52 $\pm$ 6.71	20.0-54.0	0.068

FPG = fasting plasma glucose

**Table 2.** The statistic parameters of diabetic >1 year and non-diabetic group

Parameters	Diabetics >1 year (n = 130 eyes)		Non-diabetics (n = 156 eyes)		p-value
	Mean ± SD	Range	Mean ± SD	Range	
Age (year)	60.26±9.85	40-85	58.98±13.12	41-91	0.483
FPG (mg/dl)	153.74±44.45	100-321	94.40±10.90	42-120	0.000
Duration of disease (year)	8.55±5.89	2-30	-	-	-
Cell density (cell/mm <sup>2</sup> )	2,360.37±245.65	1,628-2,862	2,413.37±313.76	1,155-4,409	0.110
Polymegathism (%)	58.26±11.06	39.0-88.4	55.81±10.02	35.5-86.0	0.051
Hexagonal cell (%)	33.83±7.03	20.0-53.0	35.52±6.71	20.0-54.0	0.039

**Table 3.** The statistic parameters of diabetic >2 year and non-diabetic group

Parameters	Diabetics >2 year (n = 118 eyes)		Non-diabetics (n = 156 eyes)		p-value
	Mean ± SD	Range	Mean ± SD	Range	
Age (year)	60.65±9.53	40-85	58.98±13.12	41-91	0.363
FPG (mg/dl)	155.46±45.82	100-321	94.40±10.90	42-120	0.000
Duration of disease (year)	9.17±5.79	2-30	-	-	-
Cell density (cell/mm <sup>2</sup> )	2,362.19±245.78	1,628-2,862	2,413.37±313.76	1,155-4,409	0.131
Polymegathism (%)	58.63±11.20	39.0-88.4	55.81±10.02	35.5-86.0	0.029
Hexagonal cell (%)	33.43±7.05	20.0-53.0	35.52±6.71	20.0-54.0	0.013

## Discussion

The present study displayed a decreased endothelial cell density and percentage of hexagonal cells and an increased percentage of polymegathism in diabetics compared with non-diabetics, but the results demonstrated no statistically significant differences. The results were similar to the report of Larsson et al, which concluded that there were no significant differences in corneal structure between type II diabetics and normal subjects<sup>(16)</sup>. However, these might be due to the duration of diabetes since the mean of duration in this study was just only 6.73 years. These were much different than the reports by Schultz et al that included type II diabetes for more than ten years from which they detected significant difference<sup>(4)</sup>. After excluding the initial diabetic patients whose duration of disease was under or equal to one year, the percentage of hexagonal cells were lower than the non-diabetic group and statistically significant. Moreover, when the under or equal to two years diabetic patients were excluded, the results demonstrated significant differences for the two parameters, the lower percentage of hexagonal cells and the higher percentage of polymegathism. These findings were similar to the report by Schultz et al in the case of type II diabetic groups<sup>(4)</sup>, the review by Bourne et al<sup>(11)</sup>, and Itoi et al<sup>(12)</sup>. The present results also resembled the report by Matsuda et al but

they did not find any correlation with the duration of diabetes<sup>(13)</sup>.

Inoue et al, however, reported a decreased corneal endothelial cell density and increased coefficient of variation of cell area in type II diabetic patients, but the percentage of hexagonal cells was not significantly different from the non-diabetic subjects<sup>(14)</sup>. Roszkowska et al reported in type II diabetic patients had decreased endothelial cell density, increased polymegathism, and decreased percentage of hexagonal cells<sup>(15)</sup>.

The present results purport that the corneal endothelial structure had a tendency to be unaffected at the initial stages of diabetes, though they showed insignificant, unfavorable change. When the disease progressed, however, the percentage of hexagonal cells decreased at first, and then later the percentage of polymegathism increased. There was no significant difference in endothelial cell density. In addition, the age parameter demonstrated insignificant differences at every step of analysis.

## Conclusion

The corneal endothelial structure is no different between diabetic and non-diabetic groups. When the disease progresses, however, the hexagonal cells decrease at first, followed by increased polymegathism. No difference appeared in endothelial cell density.

**What is already known on this topic?**

The corneal endothelial structure may change in diabetic patients, but the results were variable.

**What this study adds?**

The corneal endothelial structure changes according to the duration of diabetes, during which the hexagonal cells decrease at first, followed by increased polymegathism, except with endothelial cell density.

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**Potential conflicts of interest**

None.

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การศึกษาเปรียบเทียบเซลล์ endothelium ของกระจกตาระหว่างกลุ่มผู้ป่วยเบาหวานกับกลุ่มที่ไม่เป็นเบาหวาน

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**ภูมิหลัง:** ความสมบูรณ์ของเซลล์ endothelium ของกระจกตาอาจมีการเปลี่ยนแปลงไปในผู้ป่วยเบาหวาน แต่ผลการศึกษายังมีความหลากหลาย

**วัตถุประสงค์:** เพื่อเปรียบเทียบความสมบูรณ์ของเซลล์ endothelium ของกระจกตาโดยดูจากความหนาแน่นของเซลล์ ความแตกต่างทางขนาดของเซลล์ และความแตกต่างทางรูปร่างของเซลล์ระหว่างผู้ป่วยเบาหวานกับผู้ที่ไม่เป็นเบาหวาน

**วัสดุและวิธีการ:** แบ่งกลุ่มตัวอย่างเป็นกลุ่มเบาหวานและกลุ่มที่ไม่เป็นเบาหวาน ทำการตรวจเซลล์ endothelium ของกระจกตาด้วยเครื่อง specular microscope (Confoscan 4 (CS4), Nidek) และบันทึกผลเป็นค่าความหนาแน่นของเซลล์ ค่าร้อยละของ polymegathism และค่าร้อยละของเซลล์รูปหกเหลี่ยม เปรียบเทียบผลโดยใช้สถิติเชิงพรรณนา และ unpaired t-test

**ผลการศึกษา:** ผู้ป่วยกลุ่มเบาหวานจำนวน 90 ราย มีจำนวนตาที่อยู่ในการศึกษา 171 ตา อายุเฉลี่ย  $58.49 \pm 9.78$  ปี และกลุ่มที่ไม่เป็นเบาหวานจำนวน 90 ราย มีจำนวนตาที่อยู่ในการศึกษา 156 ตา อายุเฉลี่ย  $58.98 \pm 13.12$  ปี ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติ ( $p > 0.05$ ) จากการตรวจด้วย specular microscope ทั้งสามตัวแปร แต่ในกลุ่มเบาหวานที่เป็นโรคมมากกว่า 1 ปี พบค่าร้อยละของเซลล์รูปหกเหลี่ยมน้อยกว่ากลุ่มที่ไม่เป็นเบาหวานอย่างมีนัยสำคัญทางสถิติ ( $p < 0.05$ ) ในขณะที่กลุ่มเบาหวานที่เป็นโรคมมากกว่า 2 ปี พบค่าร้อยละของเซลล์รูปหกเหลี่ยมน้อยกว่า และค่าร้อยละของ polymegathism มากกว่ากลุ่มที่ไม่เป็นเบาหวานอย่างมีนัยสำคัญทางสถิติ ( $p < 0.05$ )

**สรุป:** ความสมบูรณ์ของเซลล์ endothelium ของกระจกตาในผู้ป่วยเบาหวานไม่มีความแตกต่างจากผู้ที่ไม่เป็นเบาหวาน แต่เมื่อระยะเวลาของการเป็นโรคนานขึ้นจะทำให้ค่าร้อยละของเซลล์รูปหกเหลี่ยมของเซลล์ endothelium ลดลงก่อน และหลังจากนั้นก็จะมีผลต่อขนาดของเซลล์ endothelium ทำให้มีขนาดใหญ่ขึ้น โดยที่ค่าความหนาแน่นของเซลล์ endothelium ไม่มีความแตกต่างจากผู้ที่ไม่เป็นเบาหวาน

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