A Study of Corneal Endothelial Cells Related to Duration in Diabetes

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Background: *The corneal endothelial structure was abnormal in diabetic patients, but there were many factors affecting them.*
Objective: *To compare the corneal endothelial structure including endothelial cell density, hexagonality and polymegathism percentage, in diabetic related to diabetic duration and non-diabetic subjects.*
Material and Method: *The corneal endothelial structure of diabetic related to diabetic duration and non-diabetic subjects were examined by specular microscope (Confoscan4 CS4, Nidek) included endothelial cell density, hexagonality and polymegathism percentage. The data were analyzed using the descriptive statistics and the unpaired t-test.*
Results: *There were 271 eyes of 148 diabetic patients with the mean age of 61.04±9.51 years, and 82 eyes of 46 non-diabetic subjects with the mean age of 57.93±11.30 years. The diabetic patients were divided into 3 groups based on diabetic duration (<5 years, 5-10 years, and >10 years). All groups of diabetic endothelial cell density were less than non-diabetic group with statistical significance ($p<0.05$). In all groups of diabetic, the polymegathism percentage was more than while the hexagonality percentage was less than non-diabetic group with statistical insignificance ($p>0.05$). All endothelial parameters insignificantly differed between 3 groups of diabetic ($p>0.05$).*
Conclusion: *The diabetic corneal endothelial structure was abnormal, especially decreased cell density, compared to the non-diabetic subjects. The abnormalities and diabetic duration were unrelated.*

Keywords: *Corneal endothelium, Diabetes, Specular microscope, Polymegathism, Hexagonal cell*

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Diabetes mellitus is a chronic disease characterized by hyperglycemia and affects multiple organs including eyes. For ocular complications, diabetes causes diabetic retinopathy, corneal endothelial cell change, superficial punctate keratitis, recurrent corneal erosion, and persistent epithelial defects[1-3]. Corneal endothelium is a single layer of hexagonal cells and plays an important role to maintain corneal clarity. Results from studies related to diabetes and corneal endothelial abnormalities were debatable. Regarding endothelial cell density in diabetic patients, some studies showed it to be decreased[4-10], whereas others reported that it was similar to values in healthy subjects[2,11-17]. Also, diabetes may induce corneal endothelial morphological changes such as a high polymegathism and a reduction in the percentage of hexagonal cells[2,7-12,16,17]. However, others reported no differences in these parameters between diabetic and non-diabetic subjects[6,13]. In addition, one study reported that diabetic duration of over 10 years had more corneal morphological abnormalities in insulin dependent diabetes[7].

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

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Material and Method

Subjects

The present study enrolled 148 diabetic patients and 46 non-diabetic subjects at Thammasat University Hospital. The exclusion criteria consisted of previous eye trauma, intraocular surgery, previous

retinal photocoagulation, contact lens wearers, and corneal diseases. Participants with glaucoma or the intraocular pressure over 21 mmHg measured by Goldmann applanation tonometry were also excluded. The most recent fasting plasma glucose in one year of the non-diabetic subjects had to be lower than 126 mg/dl.

Methods

Ophthalmological examination included a complete medical history, slit-lamp microbioscopy, intraocular pressure measured by Goldmann applanation tonometry. The recorded corneal endothelial structure parameters comprised endothelial cell density, endothelial polymegathism percentage, and endothelial hexagonality percentage, were an average of three measurements by specular microscopy (Confoscan 4 (CS4), Nidek).

Statistical analysis

The two-tail unpaired t-test was applied to compare a variety of means between diabetic and non-diabetic groups, including age, fasting plasma glucose, corneal endothelial cell density, polymegathism percentage, and hexagonality percentage at the *p*-value <0.05 to be statistically significant. The diabetic duration was also divided in groups to compare with the non-diabetic group. The numbers of subjects were applied to the parameters of age and fasting plasma glucose, whereas the numbers of eyes were applied to the other parameters.

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 271 eyes of 148 diabetic patients (63 male and 85 female), and 82 eyes of 46 non-diabetic subjects (16 male and 30 female). The diabetic group contained four eyes of three diabetics with severe non-proliferative diabetic retinopathy (NPDR), 26 eyes of 16 diabetics with moderate NPDR, 47 eyes of 28 diabetics with mild NPDR, and 194 eyes of 105 diabetics with no diabetic retinopathy. The data of diabetic and non-diabetic subjects were presented in Table 1.

The compared data between diabetic and non-diabetic subjects were presented in Table 2. Fasting plasma glucose of diabetic group was more than non-diabetic group and the endothelial cell density of diabetic group was less than non-diabetic group with statistical significance ($p<0.05$). The polymegathism percentage of diabetic group was more than non-diabetic group while the hexagonality percentage of diabetic group was less than non-diabetic group, but these differences were statistical insignificance ($p>0.05$).

In addition, the diabetic patients were also divided into 3 groups based on diabetic duration, including below 5 years, 5 to 10 years, and over 10 years. The compared data between the non-diabetic group and the diabetic patients whose diabetic duration were below 5 years, 5 to 10 years, and over 10 years were present in Table 3-5, respectively. The fasting plasma glucose and endothelial cell density of all tables revealed statistically significant differences ($p<0.05$), besides the polymegathism and hexagonality percentage of all tables were insignificant difference ($p>0.05$), which were similar to Table 2. However, only Table 5 showed the over 10 year-diabetic was more than non-diabetic group with statistical significance in the age parameter ($p< 0.05$).

Furthermore, Table 6-8 displayed compared data between diabetic duration below 5 years and 5 to 10 years, below 5 years and over 10 years, and 5 to 10 years and over 10 years, respectively. Endothelial cell density, polymegathism and hexagonality percentage of all tables were statistically insignificant difference ($p>0.05$), but the age parameter in Table 7 and 8 showed statistically significant difference ($p<0.05$).

Discussion

The results revealed a decreased endothelial cell density and hexagonality percentage and an increased polymegathism percentage in all groups of diabetic compared with non-diabetic group, whereas only cell density indicated statistically significant difference. However, the comparisons between diabetic groups based on diabetic duration displayed insignificant difference in endothelial cell structure. Though some tables showed significant difference in the age parameter, the other parameters demonstrated a similar trend with ignorance to the age factor. The present outcome was surprisingly contrary to the authors' previous report disclosed the hexagonal

Table 1. The data of diabetic and non-diabetic groups

Parameters	Diabetic duration			Total diabetics	Non-diabetics
	<5 years	5-10 years	>10 years		
Number of subjects	51	51	46	148	46
Male	22	22	19	63	16
Female	29	29	27	85	30
Number of eyes	96	95	80	271	82
Severe NPDR (eyes)	1	1	2	4	-
Moderate NPDR (eyes)	6	9	11	26	-
Mild NPDR (eyes)	10	14	23	47	-
No diabetic retinopathy (eyes)	79	71	44	194	-

NPDR = non-proliferative diabetic retinopathy

Table 2. The statistic parameters of all diabetic and non-diabetic groups

Parameters	Diabetics (n = 148 subjects, 271 eyes)		Non-diabetics (n = 46 subjects, 82 eyes)		*p*-value
	Mean ± SD	Range	Mean ± SD	Range	
Age (year)	61.04±9.51	40-87	57.93±11.30	41-78	0.066
FPG (mg/dl)	143.23±45.29	82-321	99.98±8.62	76-115	0.000
Diabetic duration (year)	9.13±7.43	1/12-36	-	-	-
Cell density (cell/mm^2)	2,345.60±255.06	1,539-2,887	2,443.90±191.07	1,978-2,960	0.000
Polymegathism (%)	59.09±10.80	38.4-90.9	57.12±9.59	37.9-91.9	0.139
Hexagonal cell (%)	33.45±7.05	20.0-53.0	34.76±6.27	23.0-54.0	0.134

FPG = fasting plasma glucose

Table 3. The statistic parameters of diabetic <5 years and non-diabetic groups

Parameters	Diabetics <5 years (n = 51 subjects, 96 eyes)		Non-diabetics (n = 46 subjects, 82 eyes)		*p*-value
	Mean ± SD	Range	Mean ± SD	Range	
Age (year)	57.88±10.87	41-87	57.93±11.30	41-78	0.982
FPG (mg/dl)	140.37±46.81	82-321	99.98±8.62	76-115	0.000
Diabetic duration (year)	2.23±1.29	1/12-4	-	-	-
Cell density (cell/mm^2)	2,366.77±242.84	1,539-2,887	2,443.90±191.07	1,978-2,960	0.019
Polymegathism (%)	59.20±10.38	41.2-87.3	57.12±9.59	37.9-91.9	0.169
Hexagonal cell (%)	33.98±7.34	21.0-51.0	34.76±6.27	23.0-54.0	0.453

FPG = fasting plasma glucose

cells decrease at first, following by the increased polymegathism and no difference in the endothelial cell density, as the same methods except the subjects and diabetic duration[16]. However, the present results were similar to the report of Sudhir et al with indistinct correlation with diabetic duration[6].

Lee et al concluded that diabetic duration of over 10 years had more corneal morphological

Table 4. The statistic parameters of diabetic 5-10 years and non-diabetic groups

Parameters	Diabetics 5-10 years (n = 51 subjects, 95 eyes)		Non-diabetics (n = 46 subjects, 82 eyes)		*p*-value
	Mean ± SD	Range	Mean ± SD	Range	
Age (year)	60.86±8.42	40-81	57.93±11.30	41-78	0.155
FPG (mg/dl)	142.45±37.24	100-321	99.98±8.62	76-115	0.000
Diabetic duration (year)	7.73±2.03	5-10	-	-	-
Cell density (cell/mm^2)	2,336.04±263.77	1,628-2,862	2,443.90±191.07	1,978-2,960	0.002
Polymegathism (%)	58.51±11.12	38.4-90.9	57.12±9.59	37.9-91.9	0.377
Hexagonal cell (%)	33.20±6.98	20.0-49.0	34.76±6.27	23.0-54.0	0.123

FPG = fasting plasma glucose

Table 5. The statistic parameters of diabetic >10 years and non-diabetic groups

Parameters	Diabetics >10 years (n = 46 subjects, 80 eyes)		Non-diabetics (n = 46 subjects, 82 eyes)		*p*-value
	Mean ± SD	Range	Mean ± SD	Range	
Age (year)	64.74±7.76	50-85	57.93±11.30	41-78	0.001
FPG (mg/dl)	147.26±51.95	100-321	99.98±8.62	76-115	0.000
Diabetic duration (year)	18.33±5.58	11-36	-	-	-
Cell density (cell/mm^2)	2,331.54±260.36	1,639-2,829	2,443.90±191.07	1,978-2,960	0.002
Polymegathism (%)	59.65±11.01	39.0-90.9	57.12±9.59	37.9-91.9	0.121
Hexagonal cell (%)	33.12±6.84	20.0-53.0	34.76±6.27	23.0-54.0	0.115

FPG = fasting plasma glucose

Table 6. The statistic parameters of diabetic <5 years and diabetic 5-10 years

Parameters	Diabetics <5 years (n = 51 subjects, 96 eyes)		Diabetics 5-10 years (n = 51 subjects, 95 eyes)		*p*-value
	Mean ± SD	Range	Mean ± SD	Range	
Age (year)	57.88±10.87	41-87	60.86±8.42	40-81	0.125
FPG (mg/dl)	140.37±46.81	82-321	142.45±37.24	100-321	0.805
Diabetic duration (year)	2.23±1.29	1/12-4	7.73±2.03	5-10	-
Cell density (cell/mm^2)	2,366.77±242.84	1,539-2,887	2,336.04±263.77	1,628-2,862	0.403
Polymegathism (%)	59.20±10.38	41.2-87.3	58.51±11.12	38.4-90.9	0.658
Hexagonal cell (%)	33.98±7.34	21.0-51.0	33.20±6.98	20.0-49.0	0.453

FPG = fasting plasma glucose

abnormalities, especially the coefficient of variation in cell size, compared with the normal subjects, but the results of endothelial cell density and hexagonality percentage did not differ statistically significant between below or equal 10 years and over 10 years of diabetic duration[7]. Parekh et al found a significant inverse correlation between endothelial cell density and the diabetic duration, whereas no displayed

Table 7. The statistic parameters of diabetic <5 year and diabetic >10 years

Parameters	Diabetics <5 years (n = 51 subjects, 96 eyes)		Diabetics >10 years (n = 46 subjects, 80 eyes)		*p*-value
	Mean ± SD	Range	Mean ± SD	Range	
Age (year)	57.88±10.87	41-87	64.74±7.76	50-85	0.001
FPG (mg/dl)	140.37±46.81	82-321	147.26±51.95	100-321	0.494
Diabetic duration (year)	2.23±1.29	1/12-4	18.33±5.58	11-36	-
Cell density (cell/mm^2)	2,366.77±242.84	1,539-2,887	2,331.54±260.36	1,639-2,829	0.352
Polymegathism (%)	59.20±10.38	41.2-87.3	59.65±11.01	39.0-90.9	0.780
Hexagonal cell (%)	33.98±7.34	21.0-51.0	33.12±6.84	20.0-53.0	0.423

FPG = fasting plasma glucose

Table 8. The statistic parameters of diabetic 5-10 years and diabetic >10 years

Parameters	Diabetics 5-10 years (n = 51 subjects, 95 eyes)		Diabetics >10 years (n = 46 subjects, 80 eyes)		*p*-value
	Mean ± SD	Range	Mean ± SD	Range	
Age (year)	60.86±8.42	40-81	64.74±7.76	50-85	0.021
FPG (mg/dl)	142.45±37.24	100-321	147.26±51.95	100-321	0.605
Diabetic duration (year)	7.73±2.03	5-10	18.33±5.58	11-36	-
Cell density (cell/mm^2)	2,336.04±263.77	1,628-2,862	2,331.54±260.36	1,639-2,829	0.910
Polymegathism (%)	58.51±11.12	38.4-90.9	59.65±11.01	39.0-90.9	0.498
Hexagonal cell (%)	33.20±6.98	20.0-49.0	33.12±6.84	20.0-53.0	0.939

FPG = fasting plasma glucose

hexagonality and polymegatism percentage[5].

Moreover, some studies presented diabetes correlated with varied factors. Shenoy et al reported that endothelial cell density was significantly lower in eyes with diabetic retinopathy than those without diabetic retinopathy[8]. Storr-Paulsen et al disclosed type II diabetes had no impact on corneal cell density or morphology in subjects with good glycemic status; however, higher HbA1c was associated with lower endothelial cell density[15].

With regard to the present study, the diabetic corneal endothelial structure tended to be abnormal, especially decreased endothelial cell density, compared with the non-diabetic subjects.The division ingroups based on diabetic duration purported that diabetic duration was unrelated to the endothelial abnormalities.

Conclusion

The diabetic corneal endothelial structure was abnormal, especially decreased endothelial cell density, compared with the non-diabetic subjects. The abnormalities and diabetic duration were unrelated.

What is already known on this topic?

The corneal endothelial structure may be abnormal in diabetic patients, but the affected factors were variable.

What this study adds?

The diabetic corneal endothelial structure is abnormal, especially decreased endothelial cell density. The abnormalities and diabetic duration are unrelated.

Potential conflicts of interest

None.

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การศึกษาเซลล์เอ็นโดธีเลียมของกระจกตาในผู้ป่วยเบาหวานที่ระยะเวลาแตกต่างกัน

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ภูมิหลัง: เซลล์เอ็นโดธีเลียมของกระจกตามีความผิดปกติในผู้ป่วยเบาหวานแต่มีปัจจัยต่างๆ มากมายที่มีผลต่อความผิดปกตินี้

วัตถุประสงค์: เพื่อเปรียบเทียบเซลล์เอ็นโดธีเลียมของกระจกตาโดยดูจากความหนาแน่นของเซลล์ ร้อยละของเซลล์รูปหกเหลี่ยมและความแตกต่างทางขนาดของเซลล์ระหว่างผู้ป่วยเบาหวานที่เป็นโรคที่ระยะเวลาต่างๆ กันกับคนที่ไม่เป็นเบาหวาน

วัสดุและวิธีการ: ศึกษาในกลุ่มเบาหวานที่เป็นโรคที่ระยะเวลาต่างๆ กันกับกลุ่มที่ไม่เป็นเบาหวาน โดยทำการตรวจเซลล์เอ็นโดธีเลียมของกระจกตาด้วยเครื่อง specular microscope (Confoscan4 CS4, Nidek) และบันทึกผลเป็นค่าความหนาแน่นของเซลล์ ค่าร้อยละของเซลล์รูปหกเหลี่ยมและ polymegathism เปรียบเทียบผลโดยใช้สถิติเชิงพรรณนาและ unpaired t-test

ผลการศึกษา: กลุ่มเบาหวานจำนวน 148 คน มีจำนวนตาที่อยู่ในการศึกษา 271 ตาอายุเฉลี่ย 61.04±9.51 ปี กลุ่มที่ไม่เป็นเบาหวานจำนวน 46 คน มีจำนวนตาที่อยู่ในการศึกษา 82 ตา อายุเฉลี่ย 57.93±11.30 ปี ซึ่งกลุ่มเบาหวานถูกแบ่งออกเป็น 3 กลุ่ม โดยอิงตามระยะเวลาของการเป็นโรค (<5 ปี, 5-10 ปี, และ >10 ปี) พบว่ากลุ่มเบาหวานทุกกลุ่มมีความหนาแน่นของเซลล์เอ็นโดธีเลียมน้อยกว่ากลุ่มที่ไม่เป็นเบาหวานอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) กลุ่มเบาหวานทุกกลุ่มมีค่าร้อยละของ polymegathism มากกว่าและค่าร้อยละของเซลล์รูปหกเหลี่ยมน้อยกว่ากลุ่มที่ไม่เบาหวานแต่ไม่มีนัยสำคัญทางสถิติ ($p>0.05$) และไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของทุกค่าของเซลล์เอ็นโดธีเลียมระหว่างกลุ่มผู้ป่วยเบาหวานทั้ง 3 กลุ่ม ($p>0.05$)

สรุป: ความสมบูรณ์ของเซลล์เอ็นโดธีเลียมของกระจกตา ในผู้ป่วยเบาหวานมีความผิดปกติไปจากคนปกติ โดยเฉพาะความหนาแน่นของเซลล์เอ็นโดธีเลียม แต่อย่างไรก็ตามความผิดปกติของเซลล์และระยะเวลาที่เป็นเบาหวานไม่มีความสัมพันธ์กัน