Comparison of Endothelial Cell Density, Morphological Changes and Central Corneal Thickness after Phacoemulsification between Diabetic and Non-Diabetic Patients

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Abstract:
Background: Corneal endothelial tissues are susceptible to mechanical trauma from ultrasound energy during phacoemulsification. Several studies have reported various results of phacoemulsification effect on corneal endothelial cells between diabetic and non-diabetic patients.

Purpose: To compare endothelial density, hexagonality, Coefficient of Variance (CV) and Central Corneal Thickness (CCT) changes between diabetic and non-diabetic patients at four weeks after phacoemulsification.

Methods: Specular microscopy examinations prior to phacoemulsification and at four-weeks after phacoemulsification were performed on diabetic and non-diabetic groups with cataract. Later, patients in the diabetic group were divided based on their HbA1c level. The changes in endothelial density, the percentage of hexagonality, CV and CCT were evaluated.

Results: At a four-weeks follow up, there were no statistical differences in endothelial cells density, CV and CCT changes between diabetic and non-diabetic groups. Mean (SD) of hexagonality percentage at four weeks of follow-up was lower (p-value=0.001) in diabetic group [(14.6) 41.7%] compared to non-diabetic group [(14.5) 50.1%]. There were no differences in endothelial cells density, hexagonality, CV and CCT values between the diabetic group with HbA1c level <7.5% and HbA1c level ≥7.5% at 4-weeks after phacoemulsification.

Conclusion: There were no statistically significant differences in the endothelial loss, reduction of CV and CCT changes between the diabetic and non-diabetic group at four-weeks follow up after phacoemulsification. Diabetic group showed greater hexagonality decrease compared to non-diabetic group at four weeks after phacoemulsification. Different HbA1c levels did not affect the changes in endothelial density, the percentage of hexagonality, CV and CCT after phacoemulsification.

Keywords: Corneal endothelial cell, Hexagonality, Central corneal thickness, Cataract, Phacoemulsification, Diabetes mellitus.

1. INTRODUCTION

Cataract in diabetic patients can develop at an earlier age. Data from population studies such as Blue Mountains Eye Study, Beaver Dam Eye Study, and Visual Impairment Projects have reported a close correlation between cataract and diabetes mellitus [1, 2]. It was estimated that around 20% of all cataract surgeries were performed on diabetes mellitus patients [1]. Phacoemulsification with intraocular lens (IOL) implantation is one of the most common procedures for cataract extraction. Phacoemulsification can improve visual acuity significantly with low risk of complications. However, thorough and careful preoperative measures should be taken before carrying out phacoemulsification procedure on diabetes mellitus patients [1, 2].
Phacoemulsification surgery can cause corneal endothelial cell loss due to ultrasound energy during the surgery. Exaggeration of corneal endothelial cell loss can lead to postoperative complications such as impairment of corneal edema healing or even permanent edema of the cornea or bullous keratopathy [3, 4]. There are several studies that demonstrated the effects of cataract surgery on endothelial cell density, morphological changes and central corneal thickness in diabetic and non-diabetic patients. However, endothelial cell loss, morphological and central corneal thickness changes varied widely between diabetic and non-diabetic patients [5-11]. This study was conducted to evaluate the corneal endothelial loss, changes in endothelial morphology and central corneal thickness at four-weeks after phacoemulsification and to evaluate whether or not HbA1c levels affected the results.

2. MATERIALS AND METHODS

A prospective study was conducted with a follow-up visit at 4-weeks after phacoemulsification. The sample size was calculated with a formula to test for two means with 90% of power and 10% of drop-out numbers. Consecutive patients were chosen from Cataract and Refractive Surgery Unit at Cicendo National Eye Hospital from April to August 2018 after being granted an ethical clearance from Padjadjaran University Ethics Committee. Informed consent was obtained from participated patients.

Inclusion criteria in the diabetic group were patients with cataract grade II–IV, aged above 30 years old, having a history of diabetes with HbA1c < 10% and/or blood glucose<200 mg/dL. Inclusion criteria in the non-diabetic group were patients with cataract grade II–IV, aged above 30 years old without a history of diabetes mellitus. Patients with preoperative intraocular pressure (IOP) > 21 mmHg, endothelial cells density <1,500/mm², anterior chamber depth <2.5 mm, with a history of intraocular surgery, pseudo-exfoliative syndrome, zonular weakness, with a systemic history that can affect endothelial cells, and patients without IOL implantation were excluded from this study. Patients with complications during or after the surgery and those who did not attend the follow-up visit at four-weeks after surgery were dropped out from this study as well.

Table 1. Demographic status.

| Characteristic | Group       | Gender | Male | Female | Age (years) | Mean (SD) | HbA1c (%) | Mean (SD) |
|----------------|-------------|-------|------|--------|-------------|-----------|-----------|-----------|
| Diabetic (n=67) | 37          | 30    | 60.2 (9.4) | 7.3 (1.08) | -           | 61.6 (12.6) | -         |
| Non-Diabetic (n=86) | 56         |       |       |        |             |           |           |           |

SD: Standard Deviation.

A complete history taking, ophthalmology examinations such as visual acuity, slit lamp biomicroscopy, noncontact tonometer IOP, funduscopy and ultrasound (if needed) were performed at Cataract and Refractive Surgery Unit and diagnostic installation at Ciendo National Eye Hospital. All patients were evaluated using a specular microscope (Topcon SP-3000P) for endothelial density, morphology and Central Corneal Thickness (CCT); and biometry to measure IOL power.

The patients underwent phacoemulsification by two experienced cataract surgeons (BU and ES) with WhiteStar Signature PRO (Abbott Medical Optic [AMO], Santa Ana, CA) phacoemulsification machine. Prior to surgery, all patients received tropicamide 1% eye drop to dilate the pupil, and intracameral adrenaline could be added during surgery if needed. Phacoemulsification procedure was as follows: gave topical anesthesia on the cornea with tetracaine hydrochloride 2%; performed aseptic and antiseptic steps with povidone iodine; applied sterile drape and speculum; incised corneal three planes with 2.75 mm keratome; injected Healon-5 Viscoelastic into anterior chamber; performed continuous curvilinear capsulorhexis with cystotome, hydrodissection, and hydrodelineation; performed side port incision with super blade 15-degree; performed phacoemulsification using phaco-chop or stop-and-chop technique; performed aspiration and irrigation of epinucleus and cortex; injected Healon-5 Viscoelastic; implanted IOL within capsular bag; performed aspiration and irrigation of viscoelastic; hydrated the main incision; and applied 1 drop of antibiotic eye drop.

Postoperative treatment included ciprofloxacin tablet 2x500 mg per oral, levofloxacin eye drops 6 times daily, and prednisolone acetate eye drops 6 times daily. Ciprofloxacin was given to all patients as a preventive measure because there were increasing endophthalmitis incidents at the hospital by the time this study was conducted. Culture at the laboratory showed that gram-positive bacteria were the main pathogen of these endophthalmitis cases.

At 4-weeks after surgery, the patients were evaluated with the following examinations: visual acuity, IOP, slit lamp biomicroscopy, funduscopy and, specular microscope to assess endothelial cell density, hexagonality, CV and CCT.

Statistical analysis was performed using SPSS Version 22 (IBM, Inc., Chicago, IL) program. Normality of the data was tested using the Shapiro-Wilk test. Comparison of endothelial cell density, hexagonality, CV and CCT preoperative and postoperative between two groups were calculated using an unpaired t-test, t-test or Mann-Whitney test. A p-value less than 0.05 was considered as statistically significant.

3. RESULTS

One-hundred-fifty-three patients were included in this study. The diabetic group consisted of 67 patients, and the non-diabetic group consisted of 86 patients. Mean (SD) of age in the diabetic group was 60.2 (9.4) years, while in the non-diabetic group was 61.6 (12.6) years. Mean (SD) of HbA1c percentage in the diabetic group was 7.3% (1.08) (Table 1). There were no statistical differences in mean (SD) on endothelial cell density, hexagonality, CV and CCT between diabetic and non-diabetic groups prior to surgery (Table 2).

At the 4-weeks evaluation, there were no statistically significant differences in endothelial density, CV and CCT between diabetic and non-diabetic groups. Meanwhile, there was a statistical difference in hexagonality percentage at 4
weeks after surgery between diabetic and non-diabetic groups with p-value = 0.001 (Table 3). There were no statistically significant changes in endothelial cell density, CV and CCT at 4 weeks after surgery between diabetic and non-diabetic groups. However, the changes in hexagonality percentage at 4 weeks after surgery were statistically significant between diabetic and non-diabetic groups with p-value= 0.004 (Table 4).

Table 2. Mean endothelial density, hexagonality, CV and CCT before surgery.

| Group                  | Endothelial density (cells/mm²) | Hexagonality (%) | CV (%) | CCT (µm) | Mean (SD) |
|------------------------|---------------------------------|------------------|--------|----------|-----------|
| Diabetic (n=67)        | 2525.7 (338.2)                  | 514.0 (26.8)     | 51.9 (9.0) | 38.3 (6.0) | 711.9 (31.5) |
| Non-Diabetic (n=86)   | 2481.7 (341.7)                  | 511.9 (31.5)     | 52.1 (10.6) | 37.1 (5.8) | 711.9 (31.5) |

Comparison of Endothelial Cell Density

| Group                  | Endothelial density (cells/mm²) | Hexagonality (%) | CV (%) | CCT (µm) | Mean (SD) |
|------------------------|---------------------------------|------------------|--------|----------|-----------|
| Diabetic (n=67)        | 2525.7 (338.2)                  | 514.0 (26.8)     | 51.9 (9.0) | 38.3 (6.0) | 711.9 (31.5) |
| Non-Diabetic (n=86)   | 2481.7 (341.7)                  | 511.9 (31.5)     | 52.1 (10.6) | 37.1 (5.8) | 711.9 (31.5) |

*unpaired t-test; CV: Coefficient of Variance; CCT: Central Corneal Thickness; SD: Standard Deviation.

Table 3. Four-week evaluation after phacoemulsification.

| Group                  | Endothelial density (cells/mm²) | Hexagonality (%) | CV (%) | CCT (µm) | Mean (SD) |
|------------------------|---------------------------------|------------------|--------|----------|-----------|
| Diabetic (n=67)        | 1667.3 (553.8)                  | 521.4 (31.1)     | 40.1 (9.3) | 38.1 (7.5) | 517.2 (34.6) |
| Non-Diabetic (n=86)   | 1773.3 (542.2)                  | 50.1 (14.5)      | 41.7 (14.6) | 38.1 (7.5) | 517.2 (34.6) |

Comparison of Endothelial Cell Density

| Group                  | Endothelial density (cells/mm²) | Hexagonality (%) | CV (%) | CCT (µm) | Mean (SD) |
|------------------------|---------------------------------|------------------|--------|----------|-----------|
| Diabetic (n=67)        | 1667.3 (553.8)                  | 521.4 (31.1)     | 40.1 (9.3) | 38.1 (7.5) | 517.2 (34.6) |
| Non-Diabetic (n=86)   | 1773.3 (542.2)                  | 50.1 (14.5)      | 41.7 (14.6) | 38.1 (7.5) | 517.2 (34.6) |

*unpaired t-test; CV: Coefficient of Variance; CCT: Central Corneal Thickness; SD: Standard Deviation.

Table 4. Changes in endothelial density, hexagonality, CV and CCT 4 weeks after surgery.

| Group                  | Endothelial density (cells/mm²) | Hexagonality (%) | CV (%) | CCT (µm) | Mean (SD) |
|------------------------|---------------------------------|------------------|--------|----------|-----------|
| Diabetic (n=67)        | 33.9 (20.1)                     | 167.3 (553.8)    | 41.7 (14.6) | 521.4 (31.1) | 11.4 (7.1) |
| Non-Diabetic (n=86)   | 28.6 (21.0)                     | 1773.3 (542.2)   | 50.1 (14.5) | 517.2 (34.6) | 13.2 (7.9) |

Comparison of Endothelial Cell Density

| Group                  | Endothelial density (cells/mm²) | Hexagonality (%) | CV (%) | CCT (µm) | Mean (SD) |
|------------------------|---------------------------------|------------------|--------|----------|-----------|
| Diabetic (n=67)        | 33.9 (20.1)                     | 167.3 (553.8)    | 41.7 (14.6) | 521.4 (31.1) | 11.4 (7.1) |
| Non-Diabetic (n=86)   | 28.6 (21.0)                     | 1773.3 (542.2)   | 50.1 (14.5) | 517.2 (34.6) | 13.2 (7.9) |

*unpaired t-test; **Mann-Whitney test; CV: Coefficient of Variance; CCT: Central Corneal Thickness; SD: Standard Deviation.

Table 5. Mean corneal endothelial density, hexagonality, CV and CCT 4 weeks after surgery based on HbA1c level.

| Group                  | HbA1c < 7.5% (n=32) | HbA1c ≥ 7.5% (n=30) |
|------------------------|---------------------|---------------------|
| Endothelial density (cells/mm²) | 1640.7 (585.5) | 1709.9 (548.9) |
| Mean (SD)               | 40.3 (16.2)         | 43.0 (13.4)         |
| Hexagonality (%)        | 522.0 (30.0)        | 523.3 (31.9)        |

Comparison of Endothelial Cell Density

| Group                  | HbA1c < 7.5% (n=32) | HbA1c ≥ 7.5% (n=30) |
|------------------------|---------------------|---------------------|
| Endothelial density (cells/mm²) | 1640.7 (585.5) | 1709.9 (548.9) |
| Mean (SD)               | 40.3 (16.2)         | 43.0 (13.4)         |
| Hexagonality (%)        | 522.0 (30.0)        | 523.3 (31.9)        |

*unpaired t-test; CV: Coefficient of Variance; CCT: Central Corneal Thickness; SD: Standard Deviation.

Table 6. Mean effective phaco time (EPT) and duration of operation in diabetic and non-diabetic groups.

| Group                  | Effective Phaco Time (EPT) | Surgery Time (minutes) |
|------------------------|---------------------------|------------------------|
| Diabetic (n=67)        | 77.4 (74.3)               | 13.2 (7.9)             |
| Non-Diabetic (n=86)   | 86.8 (79.5)               | 13.2 (7.9)             |

Comparison of Endothelial Cell Density

| Group                  | Effective Phaco Time (EPT) | Surgery Time (minutes) |
|------------------------|---------------------------|------------------------|
| Diabetic (n=67)        | 77.4 (74.3)               | 13.2 (7.9)             |
| Non-Diabetic (n=86)   | 86.8 (79.5)               | 13.2 (7.9)             |

*unpaired t-test; EPT: Effective Phaco Time (seconds); Surgery Time (minutes).

Effective phaco time (EPT) in the diabetic group and non-diabetic group was 77.4 (74.3) and 86.8 (79.5), respectively. There was no statistical difference between both the groups (p = 0.586). While surgery time in the diabetic and non-diabetic groups was 11.4 (7.1) and 13.2 (7.9), respectively. There was no statistical difference between both the groups (p = 0.683) (Table 6).

4. DISCUSSION

In this study, there were no statistically significant differences in endothelial cell density, hexagonality, CV and CCT prior to surgery between diabetic and non-diabetic groups Table 2. These results were similar to several studies which reported that there were no differences in endothelial cell density, hexagonality, CV and CCT prior to surgery between diabetic and non-diabetic groups [6, 9 - 11]. In contrast, Choo et al. reported that mean (SD) of endothelial cell density in type 2 diabetic patients was significantly lower compared to that of patients without diabetes in Malaysian population [12]. Meanwhile, Storr-Paulsen et al. reported that there was an increase in central corneal thickness in diabetic patients compared to that of non-diabetic patients. However, these results could be affected by glycemic control [6].

Khan et al. and Elbasiounny et al. reported parallel results with our study which showed that there were no statistically significant differences in corneal endothelial density after
phacoemulsification between diabetic and non-diabetic groups [10, 11]. Those results were contrary to Hugod et al. and Yang et al., who reported greater endothelial cell loss in diabetic patients compared to that of non-diabetic patients until 3 months after surgery [9, 13]. Trauma due to ultrasound energy during phacoemulsification plays a great role in endothelial cell loss. The pupil in diabetic patients could be smaller in size which can lead to a higher risk of surgery complications; the need for more irrigation solution and greater ultrasound energy which could lead to greater endothelial cell loss in diabetic patients [9, 14].

In this study, there was a statistically significant difference in mean hexagonality percentage at 4 weeks after surgery in the diabetic group. Hugod et al. reported a similar result that there was a greater hexagonality decrease in diabetic patients compared to non-diabetic patients [9, 13]. Endothelial density measurement alone was not sufficient to evaluate the function of corneal endothelial cells after intraocular surgery. Polymegathism represented by the coefficient of variation and polymorphism represented by hexagonality are more sensitive to evaluate whether the corneal endothelium is in a state of stress or not.

Endothelial cell loss after intraocular surgery is approximately around 0–30%. The loss of endothelial cells is the direct result of the phacoemulsification procedure itself, while the decrease of hexagonality is the parameter of corneal endothelial wound healing due to trauma [9, 15, 16].

In this study, a greater decrease of hexagonality percentage in diabetic group 4-weeks after surgery indicates corneal endothelial healing response, which could be longer than 4 weeks after surgery in diabetic patients. It might be necessary to follow up diabetic patients longer than 4 weeks after phacoemulsification. When structural changes occur in endothelial cells, the process to regain the cell to its normal and stable hexagonal shape is not easy. Hexagonality repair in diabetic patients may take more than 3 months. Uniform hexagonal patterns in corneal endothelial cells create a stable structure. The changes in hexagonality could lead to an unstable layer of corneal endothelial cells [9, 13, 16].

The comparison of CV after phacoemulsification between diabetic and non-diabetic patients did not show any differences. Due to their inability to regenerate, corneal endothelial cells stretch-and cover surrounding areas after surgery. That is the reason why CV percentage usually increases and hexagonality is usually decreased after surgery. However, after the endothelial cells are fully recovered, the CV and hexagonality percentages return to their former values. CV percentage increases in 1 day and reaches a peak at 1 week after surgery and then decreases slowly until 3 months after surgery. In diabetic patients, this process could be longer. Duration of diabetes and glycemic control can affect the results [17 - 20].

The results of this study were similar to studies conducted by Hugod et al., Khan et al. and Elbassiouny et al. that there were no differences in CCT between diabetic and non-diabetic patients after surgery. Several studies have reported the same results up to 3 months of follow up [9 - 11]. However, the diabetic effect on CCT is not clear yet. However, there are several possible mechanisms that might play a role, such as a decrease in endothelial pump function, swelling of stroma, and an increase in permeability due to diabetic metabolism. Corneal endothelial cells keep the hydration status and the clarity of the cornea by keeping their integrity to prevent stromal edema. Apical tight junctions in endothelial cells have a role as physical barriers. Meanwhile, the water movements from cornea into the anterior chamber are maintained by the endothelial ionic pump. In other words, corneal edema after surgery that leads to increased central corneal thickness is the result of anatomical barrier impairments or a decreased function of the endothelial ionic pump. These conditions depend on the degree of endothelial cell trauma [9, 19, 20].

Corneal endothelial cells are delicate tissues and they are more vulnerable in diabetic patients as a result of several mechanisms such as polyol pathway activity and sorbitol accumulation in endothelial cells due to prolonged hyperglycemic state, and also an increasing of osmotic pressure. Accumulation of advanced glycation end products (AGEs) in corneal endothelial cells generates oxidative stress in the DNA core of diabetic patients. Diabetes also decreases Na-K-ATPase activities in endothelial cells that play a role in maintaining the endothelial structure [9, 13]. Yang et al. reported that there were no statistical differences in corneal endothelial cell density, hexagonality and CV between groups with different fasting blood glucose levels prior to surgery [13]. The HbA1c level could represent glycemic control in diabetic patients more precisely compared to fasting blood glucose. However, HbA1c levels do not affect corneal density, CT, nor CCT [21].

The study by Ganesan et al. showed that there was no significant difference in endothelial cell density post phacoemulsification. Ganesan et al. also suggested that hexagonality was significantly decreased in the diabetic group [22]. These results were parallel with our study. Delayed corneal healing in diabetic patients and inflammation might play an important role in decreased hexagonality as found in this study.

Several factors that could affect endothelial cells after phacoemulsification in diabetic patients such as pupil size and irrigation volume were not measured in this study, hence this may become the limitation of this study along with a short duration of follow-up time (4 weeks).

CONCLUSION

There was a statistically significant decrease in hexagonality at 4-weeks after surgery in diabetic patients. No significant differences in endothelial density, CV and CCT were found at 4-weeks after phacoemulsification between diabetic and non-diabetic patients. In this study, HbA1c levels in the diabetic group did not affect corneal endothelial cells after phacoemulsification. Corneal endothelial cells are susceptible to trauma caused by phacoemulsification procedure, especially in diabetic patients. Maintaining endothelial cells health is very important in diabetic patients since they have endothelial wound repair impairment.
ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The ethical clearance was granted by the Padjadjaran University Ethics Committee, Indonesia. Ethics Approval Number: 409/UN6.KEP/EC/2018.

HUMAN AND ANIMAL RIGHTS

No Animals were used in this research. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was obtained from the participants before they were enrolled in this study.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author [B.B] upon request.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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