• Original Article •

Density and morphology of corneal endothelial cell after phacoemulsification using ringer lactate versus balanced salt solution as irrigating solutions

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Abstract

• AIM: To compare the difference in corneal endothelial cell density and morphology after phacoemulsification using ringer lactate (RL) and balanced salt solution (BSS) irrigating solutions.

• METHODS: The prospective randomized controlled trial study was conducted between February 2017 and April 2017 in Dr. YAP Eye Hospital, Yogyakarta, Indonesia. There were a total of 52 subjects (52 eyes) who were senile cataract patients further grouped into two, 26 patients undergoing the phacoemulsification procedure using RL irrigating solution and the other 26 patients with BSS irrigating solution, both conducted by one operator. On the 1, 7, and 28d post operative, an evaluation was done to measure the density and corneal endothelial cell morphology, as well as the variable of inflammation in the two groups.

• RESULTS: Fifty-two eyes had undergone phacoemulsification with posterior intraocular lens implantation. Both groups were evaluated for the endothelial cell reduction and corneal endothelial cell morphology change, along with postoperative inflammation. On the 28d postoperative, endothelial cell reduction in the BSS group (173.96 cell/mm^2, 8.12%) was lower than the RL group (253.20 cell/mm^2, 10.25%), percentage of corneal endothelial cell variation coefficient increase in the BSS group (2.92%, 8.36%) was lower compared to the RL group (3.42%, 9.96%), decrease of hexagonal cells of corneal endothelium cells presentation percentage in the BSS group (4.30%, 8.17%) was lower compared to the RL group (4.84%, 8.97%), and the percentage increase of central corneal thickness in the BSS group (4.69 μm, 0.89%) was almost equal to the RL group (4.53 μm, 0.90%). All of the results regarding difference in density and corneal cell endothelium morphology between the two groups did not reveal any statistically significant difference (*P*>0.05). Inflammatory variable in the two groups were even.

• CONCLUSION: BSS and RL were equal in their capability of maintaining endothelial cell loss and endothelial cell morphologic change in senile cataract patients after phacoemulsification.

• KEYWORDS: ringer lactate; balanced salt solution; phacoemulsification; senile cataract; corneal endothelial cell

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INTRODUCTION

The number of people with age-related eye diseases is assumed to be on the rise with increasing life expectancy[1]. The most recent data published by World Health Organization (WHO) showed that the total number of persons with visual impairment worldwide in 2010 was estimated to be 285 million, including 39 million blind people, of whom around 80 per cent are above age of 50, with most of the causes being preventable[2].

The clear cornea is composed of multiple cell layers that form the primary refractive surface of the eye. The corneal endothelial cell layer lines the internal surface with a single layer of cells that functions to maintain corneal clarity by regulating corneal hydration. Unlike epithelial cells that divide to repair defects, human corneal endothelial cells do not proliferate in response to injury[3].

Endothelial damage is caused by surgical procedures affected by several pre-operative and intraoperative factors. Among the

pre-operative factors that contributes to corneal endothelial cell loss are age and grade of cataract. The age and history of diabetes mellitus of a patient becomes a significant factor that correlates to a higher corneal endothelial cell loss[4]. Many factors for postoperative endothelial cell loss have been evaluated after phacoemulsification, including surgery time, phacoemulsification time, and ultrasound power, IOL contact, instrument-related trauma, incision size, irrigation solution turbulence, type of IOL, and type of Ophthalmic Viscosurgical Devices (OVDs) can influence corneal endothelial cell loss after phacoemulsification procedures[5-6]. Clinical observations indicate than an endothelial cell density (ECD) of 400-600 cells/mm² is a crucial point at which endothelial decompensation develops. Therefore ECD is clinically an important parameter[7].

Alongside the advancing complexities of intraocular surgery techniques, there is an increase in demand of intraocular irrigation solutions that are capable of maintaining the integrity of corneal endothelial cells and other intraocular tissue even when used in large amounts and for lengthy periods of time[8]. The purpose of this study was to compare the difference in density and corneal endothelial cell morphology after phacoemulsification using ringer lactate and balanced salt solution as irrigating solutions.

SUBJECTS AND METHODS

Ethical Approval The study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the Faculty of Medicine Gadjah Mada University- Dr. Sardjito General Hospital. After detailed explanation, informed consent was obtained from each patient prior to examination.

Subjects This research was the prospective randomized controlled trial design. The inclusion criteria for this research were all patients with senile cataract who underwent cataract surgery with phacoemulsification, aged 40-80 years old, level of cataract density was II-III, and willing to provide consent to contribute to the research and sign the informed consent form. The sample size was calculated using two independent means comparison formula and revealed 26 subjects for each group. Exclusion criteria for this research were patients with previous history of intraocular surgery, patients with diabetes mellitus, patients with lens luxation and exfoliation syndrome, complications during surgery that include posterior capsule rupture or vitreous prolapse, pre-operative corneal endothelial cell count <1500 cell/mm^2, surgery duration >15min, ultrasound time >1min. The drop out criteria for this research were patients that were not present at postoperative follow up, emergence of complications such as endophthalmitis, persistent corneal edema, and patients that are not compliant in their medication schedule.

Surgical Technique The surgical procedure was carried out by one operator using the following steps: eyes were anaesthetized with topical anesthesia pantocaine 0.5% on a maximally dilated eye, irrigated with povidone-iodine 5%, eye lids and area around the eye were aseptic and antiseptically prepared with povidone-iodine 10%, cornea were incised with a keratome, intracamera injection with lidocaine, HPMC OVDs were injected into anterior chamber followed by capsulotomy using the continuous curvelinear capsulorrhexis (CCC) technique, lens was hydrodissected, Infiniti Vision System was applied for phacoemulsification with the vertical chop technique, residual cortex were irrigated and aspirated until clean, implantation Rohto neo eye IOL foldable acrylic hydrophilic (Rohto Laboratories, Indonesia) in the bag, irrigation and aspiration of viscoelastic until clean, and injected dexamethasone and levofloxacin diluted with BSS in to anterior chamber.

Measurement of Corneal Endothelial Density and Morphology Follow-up were done on the 1, 7, and 28d post phacoemulsification. This length of follow-up was similar with previous study that showed no additional endothelial cell loss did not occur after 1mo[9]. The primary result was visual acuity measurement, biomicroscopy of the anterior and posterior segment, and tonometry (Shin-nippon non-contact Tonometry, Shin-Nippon, Japan), density and morphology of the corneal endothelium using Topcon SP-3000 (Topcon, Japan), central corneal thickness, and inflammation variables such as pain, blepharospasm based on the Jancovic Rating Scale, conjunctival hyperemia based on the Cornea and Contact Lens Research Unit (CCLRU), and flare and cell in the front chamber of the eye using the grading system from Standardization of Uveitis Nomenclature (SUN) Working Group.

Statistical Analysis Statistical analysis was performed using the SPSS 22.0 for Windows software. Data were expressed as the mean±SD and range, normality of all data samples was first confirmed by the Kolmogorov-Smirnov test. Difference in subject research characteristic is analyzed using the Chi-square test for categorical data and unpaired *t* test for numerical data if the distribution is normal or Mann-Whitney *U* test if the distribution is not normal. Difference in density and corneal endothelial cell morphology between the two groups is analyzed using unpaired *t* test. It was followed with pairwise comparison between the follow-up days using the independent samples *t* test. Inflammation parameters were analyzed using the chi square test.

RESULTS

Fifty-two eyes of 52 patients were enrolled in this study for undergoing cataract surgery using the phacoemulsification during the research period in Dr. YAP Eye Hospital Yogyakarta, that was conducted from February 2017 until April 2017.

Table 1 Research subjects characteristic

Subjects characteristic	RL (*n*=26)	BSS (*n*=26)	*P*
Age (a)	63.19±8.70	61.73±9.11	0.871
Gender (*n*, %)			
M	14 (53.8)	13 (50)	0.781
F	12 (46.2)	13 (50)	
Lens density (*n*, %)			
Grade 2	7 (50)	9 (52.7)	0.548
Grade 3	19 (50)	17 (47.3)	
Surgery duration (s)	405.38±103.62	450±104.95	0.781
Phacoemulsification time (s)	30.11±13.30	29.38±17.65	0.279
Irrigation fluid volume (mL)	63.84±21.92	62.69±23.07	0.988
CDE	6.85±3.09	6.89±4.39	0.199
Initial IOP (mm Hg)	15.00±2.91	14.84±3.10	0.775
IOP at 1d postop. (mm Hg)	16.07±5.77	18.15±4.62	0.282
IOP at 7d postop. (mm Hg)	14.03±3.69	13.96±3.39	0.621
IOP at 28d postop. (mm Hg)	12.53±2.43	12.65±2.01	0.324
Initial VA (logMAR)	1.05±0.25	1.03±0.29	0.079
VA at 1d postop. (logMAR)	0.54±0.11	0.55±0.31	0.814
VA at 7d postop. (logMAR)	0.27±0.19	0.30±0.18	0.955
VA at 28d postop. (logMAR)	0.16±0.13	0.15±0.13	0.712

RL: Ringer lactate; BSS: Balanced salt solution; CDE: Cumulative dissipated energy; IOP: Intraocular pressure; VA: Visual acuity.

Subject characteristic was provided on Table 1, consisting of age distribution, gender, initial intraocular pressure (IOP), initial visual acuity, lens opacity level, phacoemulsification time, duration of surgery, amount of fluid used and amount of energy used. Average age, gender, grade of lens density, duration of surgery, duration of phacoemulsification, volume of irrigation fluid used, cumulative dissipated energy (CDE), initial visual acuity, initial IOP, post-surgery IOP, and post-surgery visual acuity between the two groups are proportionate, there was no statistically significant difference in day one, seven, and twenty-eight postoperative examination (P>0.05).

Table 2 shows the difference in density decrease, variation coefficient, corneal endothelium cell hexagonality, and central corneal thickness on the 7 and 28d postoperative. There was no statistically significant difference for all variables between the two groups (P>0.05).

The change of density and corneal endothelial cell morphology, the mean cell density and corneal endothelial cell morphology showed similar results. The percentage decrease in corneal endothelial cell density for the BSS group on day 7 (6.6%) and day 28 (8.12%) was lower when compared to the RL group on day 7 (8.57%) and day 28 (10.25%), though there was no statistically significant difference between the two groups, (P=0.419) and (P=0.458). Corneal endothelial cell variance coefficient increase for the BSS group on day 7 (6.04%) and day 28 (8.36%) was lower when compared to the RL group on day 7 (7.08%) and day 28 (9.96%), however there was no statistically significant difference between the two groups (P=0.314) and (P=0.190).

Percentage decrease of the corneal endothelium's hexagonal cell percentage in the BSS group during day 7 (5.63%) and day 28 (8.17%) was lower when compared to group RL on day 7 (6.76%) and day 28 (8.97%), though there was no statistically significant difference between the two groups, (P=0.205) and (P=0.410). Central corneal thickness increase percentage for the BSS group on day 7 (3.60%) was lower to that of the RL group (4.33%), however the central corneal thickness on day 28 was almost similar for the BSS group (0.89%) and RL group (0.90%). There was no statistically significant difference between the two groups (P=0.273) and (P=0.873).

Injury of the corneal endothelial cell post cataract surgery leads to corneal edema, decrease of cell density, alteration of the hexagonal shape of the cells[9].

Table 3 and 4 show a comparison of conjunctival hyperemia, blepharospasm, flare, and cell proportions on day one and seven postoperative. All variables reveal no significant difference between the two groups (P>0.05).

DISCUSSION

With an excessive amount of US energy, collision of lens nucleus fragments with the corneal endothelium, air bubbles, or a localized temperature rise have been reported and are well known as damaging factors to the corneal endothelium[10-11]. In earlier days, phacoemusification was done in anterior chamber and was associated with 20 to 30% loss of endothelial cells,

Table 2 Corneal endothelial density and morphology between 2 groups

Parameters	Group I (RL)	Group 2 (BSS)	*P*
Corneal endothelial cell (cell/mm^2)			
Preop.	2498.63±214.82	2625.36±381.64	0.416
D+7	2284.46±235.70	2451.40±419.10	-
Endothelial cell loss	214.17±161.17 (8.57%)	173.96±193.24 (6.6%)	0.419
D+28	2245.42±246.52	2412.13±426.55	-
Endothelial cell loss	253.20±175.65 (10.25%)	213.23±208.65 (8.12%)	0.458
Endothelial CV (%)			
Preop.	34.32±3.09	34.90±2.08	0.438
D+7	36.75±3.20	37.01±2.76	-
CV increase	2.43±1.16 (7.08%)	2.11±1.06 (6.04%)	0.314
D+28 (Mean ±SD)	37.75±3.37	37.82±2.81	-
CV increase	3.42±1.46 (9.96%)	2.92±1.20 (8.36%)	0.190
Hexagonal cell percentage (%)			
Preop.	53.92±7.66	52.57±7.23	0.518
D+7	50.27±6.76	49.61±6.87	-
Hexagonal cell decrease	3.65±2.15 (6.76%)	2.96±1.70 (5.63%)	0.205
D+28	49.07±6.71	48.26±6.58	-
Hexagonal cell decrease	4.84±2.46 (8.97%)	4.30±2.20 (8.17%)	0.410
Central corneal thickness (μm)			
Preop.	500.73 ±34.18	521.53±40.14	0.051
D+7	522.46±34.54	540.34±43.01	-
CCT increase	21.73±8.15 (4.33%)	18.80±10.69(3.60%)	0.273
D+28	505.26±34.15	526.23±40.78	-
CCT increase	4.53±2.85 (0.90%)	4.69±3.93 (0.89%)	0.873

RL: Ringer lactate; BSS: Balanced salt solution; CCT: Central corneal thickness; CV: Coefficient of variability.

Table 3 Conjunctival hyperemia and blepharospasm proportions *n* (%)

Conjungtival hyperemia	Grade 1	Grade 2	Grade 3	Grade 4	*P*
D+2					
RL	21 (80.7)	5 (19.3)	0	0	0.442
BSS	23 (88.4)	3 (11.6)	0	0	
D+7					
RL	26 (100)	0	0	0	1.0
BSS	26 (100)	0	0	0	
Blepharospasm	Grade 0	Grade 1	Grade 2	Grade 3	*P*
D+2					
RL	7 (26.9)	16 (61.6)	3 (11.5)	0	0.786
BSS	9 (34.6)	15 (57.8)	2 (7.6)	0	
D+7					
RL	23 (88.5)	3 (11.5)	0	0	0.685
BSS	22 (84.8)	4 (15.2)	0	0	

RL: Ringer lactate; BSS: Balanced salt solution.

now, it is performed within capsular bag. Moreover, with advent of torsional ultrasound and higher retention OVDs, phacoemulsification was now more endothelial friendly[12].

The results of this study was similar to that conducted by Lucena *et al*[13] that reported endothelial cell loss post phacoemulsification with RL on the day 60th being approximately 13.1%±2.0% compared to BSS Plus at approximately 9.2%±1.9%, but without significant difference. On the 60th day, the variance coefficient in patients after phacoemulsification with RL was 23.0%±3.0% and with

Table 4 Cell and Flare anterior chamber proportions *n* (%)

Cell	0	+0.5	+1	+2	+3	*P*
D+2						
RL	6 (23.1)	15 (57.7)	5 (19.2)	0	0	0.475
BSS	7 (26.9)	17 (65.4)	2 (7.7)	0	0	
D+7						
RL	22 (84.7)	3 (11.5)	1 (3.8)	0	0	0.525
BSS	24 (92.3)	2 (7.7)	0	0	0	
Flare	0	+1	+2	+3	+4	*P*
D+2						
RL	6 (23.1)	16 (61.5)	4 (15.4)	0	0	0.369
BSS	7 (26.9)	18 (69.2)	1 (7.7)	0	0	
D+7						
RL	24 (92.3)	2 (7.7)	0	0	0	0.552
BSS	25 (96.1)	1 (3.9)	0	0	0	

RL: Ringer lactate; BSS: Balanced salt solution.

the BSS Plus solution was 20.2%±4.0%, this increase was statistically insignificant. Central corneal thickness significantly increases on day one, 8, 15, and 60 in both groups, yet this increase was not statistically significant[13].

Another study done by Vasavada *et al*[14] revealed that the percentage of endothelial cell loss at 3mo after phacoemulsification in the BSS group was 5% and 8% for the RL group. Average change in corneal endothelial coefficient of variance pre-surgery in the BSS group was 3 and 5 in the RL group, and also the two results did not show a statically significant difference. Futhermore, a different study by Nayak and Shukla[15] revealed postoperative percentage of endothelial cell loss was not significantly different between the 2 groups at 1wk (P=0.582), 1mo (P=0.668), or 6mo (5.03% and 8.35% in Group A and Group B, respectively) (P=0.483). The postoperative percentage change in pachymetry was not significantly different between the 2 groups at 1wk (P=0.179) or 1mo (P=0.170) but was significant at 6mo (P<0.001; -1.59% and 0.54% in Group A and Group B, respectively).

Previous study related to the correlation between endothelial cell loss and ultrasound parameter. Crema *et al*[16] reports that there was no significant correlation between the time of ultrasound and the decrease of endothelial cell percentage, however some researches have contradicting results. A research by Lee *et al*[17], Baradaran-Rafii *et al*[18], and Mahdi *et al*[19] reports that there was a relationship between ultrasound energy and endothelial cell loss. Other factor affecting corneal endothelial loss was surgical technique, Elnaby *et al*[20] described that mean effective ultrasound time intraoperatively and percentage endothelial loss postoperatively were significantly lower when the phaco prechop technique was used compared to divide-and-conquer technique.

In this study, the corneal endothelial cell count was reported that was lower with BSS when compared to RL. It might related with the level of homogeneity of BSS within the humor aqueous. BSS and RL both have aqueous humor compositions such as sodium phosphate, sodium bicarbonate, dextrose and glutathione. The superiority of BSS to RL lies in the fact that BSS comprises of different compositions from RL, including magnesium, which was responsible for pumping endothelial Mg-ATPase, an addition of dextrose as a pure source of energy for the endothelial cells, sodium phosphate and bicarbonate that are physiological buffers contained within the aqueous humor, and glutathione that was an important peptide that functions as an antioxidant and to maintain intercellular links. Furthermore, the osmolality and pH of BSS plus was similar to that of aqueous humor, while RL was more hypotonic and acidic than BSS plus[21].

In addition, it was revealed that inflammation signs were equal between the two groups. Similarly, Vasavada *et al*[14] reported that at grade 0 flare (no flare) 24 subjects were from the BSS group and only 4 from the RL group, this result was statistically significant (P<0.05). A similar result with grade 1 flare, 53 subjects were from the BSS group and only 29 from the RL group. On the contrary, for grade 3 flare, there were more subjects from the RL group (50 subjects) than the BSS group (18 subjects). For the average cell count, half of the subjects from the BSS group had 0 (5 subjects) and 1 degree cell count (47 subject), whereas the RL group had a 0 (0 subject) and 1 degree cell count (36 subjects). The result for the comparison of the cell count degree was not statistically significant (P>0.05). The researcher believes a statistically significant result in the amount of subjects from the RL group with flare grade 2 and 3 in first postoperative day may be due to slightly acidic fluctuating pH and lack of buffer system in RL, which could affect the blood aqueous barrier stability. On the other hand, BSS has an acetate citrate buffer system and the pH, which might slightly alkaline and was known stable.

In conclusion, it was revealed from this study that BSS and RL were similar in their capability of maintaining endothelial cell loss and endothelial cell morphologic change after phacoemulsification in senile cataract. The limitation of this study was objective examination of inflammation, which is flare and cells on the anterior chamber used a biomicroscope slit lamp. Further multicenter study is needed to strengthen the result of this study.

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