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Original Article

SPECULAR MICROSCOPIC EVALUATION OF CORNEAL ENDOTHELIUM IN NORMAL EGYPTIAN EYES AND ITS RELATION TO AGE, GENDER, AND REFRACTION

ElSebaity, D.[(*)], Aly, T., Mohamed, Kh., Aly, M. & Fathalla. A.
Ophthalmology dept., Faculty of Medicine, Assuit Univ. Hospital, Assuit, Egypt.

[*]*E-mail: drdalia@aun.edu.eg*

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Abstract
***Aim**: Describe the parameters of corneal endothelial cell of normal Egyptians and to determine its correlation with sex, age, and refractive eye state. **Methods**: Non-contact specular microscopy was performed in 390 eyes of healthy Egyptian. Age, sex, refractive state, Endothelial cell density (ECD), the standard deviation of hexagonal cells HEX, coefficient of variation (CV) were analyzed. The system recorded the mean cell density MCD (cell/mm2), mean cell area MCA (µm2), central corneal thickness CCT, and HEX percentage. **Results**: The population's mean age was 48.90±15.77. There were 158 (40.5%) males and 232 (59.5%) females. The MCD was 2762.27±327.72. The mean CV in the cell size was 30.91±5.50, and the HEX percentage was 65.62±6.39. The mean CCT was 542.48±37.57. The ECD decreased from 2822.32 in the 20-30-year age group to 2475.64 in the 71–80-year age group, with no substantial correlation between ECD and age. There were no substantial differences in HEX percentage, CV, and ECD between females and males. However, there was a statistical difference in CCT between females and males, with a P-value of 0.007. There was a marked difference in ECD between emmetropes, hypermetropic, and myopes, with a P-value of 0.001. Moreover, there was a substantial difference in CCT between emmetropes, hypermetropic, and myopes. Nonetheless, there were no statistically significant differences in HEX percentage and CV among emmetropes, hypermetropic, and myopes. **Conclusion**: Our study provides normative data for the corneal endothelium in healthy Egyptian eyes, adding to the expanding body of information about CEC parameters in healthy Egyptian eyes.*

Keywords: *Corneal endothelium, Cell density, Specular microscopy*.

1. Introduction
The critical role of a healthy cornea in retaining clear vision cannot be ignored. The two most significant criteria in the morphological and functional evaluation of the cornea for diagnostic purposes and prior to any intraocular surgical procedure are corneal endothelial cell (CEC) morphology as well as central corneal thickness (CCT) [1]. Descemet's membrane has a single layer of CECs on its back surface. Endothelial cell dimensions are uniform in a normal cornea and are metabolically active, maintaining a normal dehydrated condition of 70% water in the corneal stroma [2]. The average normal corneal endothelial cell density (ECD) is greatest at birth (3,000

cells/mm2) and gradually decreases with age with a rate of 0.3-0.6% per year, approximating a value of 2000-3000 cells/mm^2 in a normal adult eye. ECD declines with age, diabetes mellitus, corneal dystrophy, glaucoma, intraocular surgery, refractive surgery, and trauma [1-3]. A lower ECD of 400-500 cells/mm2 is necessary to maintain endothelial pumping activity, and values below this range have been related to bullous keratopathy [4]. CECs are characterized by a limited repair capability. Damage to CECs is compensated by cell spread as well as cell expansion, resulting in a progressive decrease in ECD and hexagonally with increased cellular pleomorphism and cell size [5]. CCT is another essential factor for corneal well-being because intraocular pressure (IOP) is influenced by corneal thickness, and CCT must be considered when assessing patients' glaucoma or suspects. Several studies revealed that ECD, morphology, and CCT differ depending on ethnicity, race, sex, and age [1,6,7]. The progression of refractive error is strongly associated with the overall visual stimulation influencing the corneal parameters, and sometimes even slight changes in the development of refractive error, such as myopia, can result in irreversible steeper cornea and axial expansion when compared to emmetropic individuals. Flat corneal curvature (CC) has a reciprocal effect on hyperopic individuals. Furthermore, astigmatism is another component where CC varies across various meridians [8]. Today's specular microscope makes it feasible to study endothelium. In order to quantify the functional states of endothelial cells, quantitative specular microscopic examination assigns endothelial cell values. It allows for the evaluation of cell size variations (polymegathism), the mean cell area (MCA), MCD, and mean cell shape (polymorphism). Normative data for endothelium parameters have been established and compared between ethnic groups and gender using the specular microscope [2]. Since endothelium parameters differ between populations, studying normative data from each population, it is essential to set a reference range for various populations to aid predictability and identify abnormal values, particularly when considering the recent rise in intraocular procedures. A study of ECD in the Indian, American, and Japanese populations [9,10] demonstrated that the Japanese population had a much higher value while the Indians had a significantly lower value. There is limited availability of normative data on corneal morphological parameters in the Egyptian population, which might be different compared to other races. This study aimed to characterize normal Egyptians' CEC parameters and to investigate the correlation between sex, age, and refractive eye state with ECD.

2. Subjects

2.1. *Study design*

The research was conducted at Alforasn Eye Center, Assuit, Egypt, between Nov. 2021 and Aug. 2022. The study included 390 eyes of healthy volunteers among the center's visitors, outpatients, and staff, ages ranging between 18 and 80 years of both sexes, were included in the study.

2.2. *Exclusion criteria*

The individual under the age of 18, history of ocular trauma, an ocular surface disorder, intraocular surgery or endothelial dysfunction, uveitis, glaucoma, corneal opacity, and diabetics were all excluded.

3. Methods

All subjects underwent routine ophthalmic examination as well as specular microscopy utilizing a non-contact specular microscope NIDEK Specular Microscope, Model CEM-530 (Hiroshi-Cho, Gama Gori, Aichi 443-0038, Japan). A single examiner (Dr. Momen Ahmad Aly) carried out all measurements. Specular microscopy procedures were as follows: A slit of light was focused on the surface of the corneal endothelium, with secularly (mirror-like) reflecting light rays focused onto the film plane for viewing one real-time monitor. The same objective lens collected the reflected light and focused it onto a film plane for examination.

3.1. *Data collection*

Age, sex, refractive state, average cell area and cell density, percentage, the standard deviation of hexagonal cells HEX, maximum cell area, coefficient of variation (CV), as well as lowest cell area, were all collected and analyzed. The system recorded the MCD (cell/mm2), MCA (μm2), CCT, HEX percentage, and CV in cell size (standard deviation divided by the MCA), which was utilized as an index of variation degree in the cell area (polymegathism). HEX percentage in the analyzed area was utilized as an index of variation in the shape of cells (polymorphism). Data were analyzed utilizing the 26^{th} version of SPSS software. Quantitative data normality was tested by the Shapiro-Wilk test data and expressed as mean ± SD, whereas qualitative data were expressed as percentages and frequencies. One-way ANOVA and the independent Sample T-test were utilized for comparing the mean difference between two independent groups and more than two groups, respectively, to identify changes occurring in CECs based on sex and different age groups. Additionally, the Pearson correlation was utilized to identify the association between CECs and age. The significance level was determined at a P-value < 0.05.

3.2. *Ethics*

The study conformed to the Helsinki Declaration. Assiut University Hospital's Faculty of Medicine, Assuit, Egypt Ethical Committee granted ethical approval. All study subjects signed informed consent, and all their data was anonymized.

4. Results

The endothelial cell properties of 390 eyes of 390 participants (the right eye for each one) with healthy eyes were studied. There were 158 (40.5%) males and 232 (59.5%) females. The population's mean age was 48.90±15.77. The MCD was 2762.27±327.72. The mean CV in the cell size was 30.91±5.50, and the HEX percentage was 65.62±6.39. The mean CCT was 542.48±37.57, as shown in tab. (1). The studied population's mean age was 48.90±15.77 and ranged from 18 to 79 years old. 4.1 % were less than 20 years, and 12.8% were aged 20 to 39. 15.4% aged 31 to 40 years, 15.9% aged 41 to 50 years, 25.1% aged 51-60 years, 21 % aged 61 to 70 years, and 5.6% from 71 to 80 years old, as demonstrated in fig. (1). Regarding the spherical equivalent of the study participants, 9.7% were emmetrope, 19 % were hypermetropia (9.7%, 8.2%, and 1% were low, moderate, and high hypermetropia, respectively), and 71.3% were myope (31.8%, 23.1%, and 16.4% were low, moderate, and high myopia respectively), as depicted in fig. (2).

Table (1) Characteristics of study participants.

		Number of eyes=390
Gender	▪ Male ▪ Female	158 (40.5%) 232 (59.5%)
Endothelial cell density (cells/mm^2) ECD	▪ Mean ± SD (range)	2762.27±327.30 (2024-3692)
Coefficient of variation (%) CV	▪ Mean ± SD (range)	30.91±5.50 (21-53)
Hexagonal cell (%) HEX	▪ Mean ± SD (range)	65.62±6.39 (38-81)
Central corneal thickness (CCT)	▪ Mean ± SD (range)	542.48±37.53 (428-646)

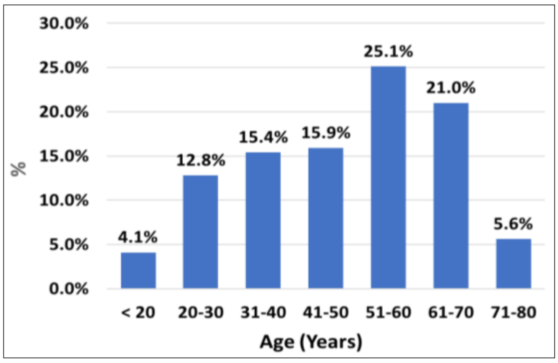

Figure (1) Age distribution of the study participants

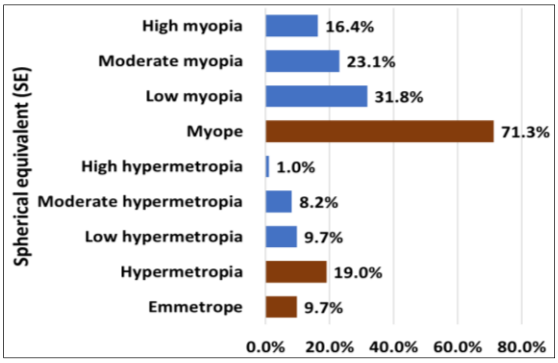

Figure (2) Distribution of study participants according to their spherical equivalent

4.1. *The relation of endothelial cell parameters with different age groups*

The ECD decreased from 2822.32 in the 20-30-year age group to 2475.64 in the 71-80-year age group, with no substantial correlation between ECD and age at a P-value of <0.002, as displayed in tab. (2). The CV increased from 25.38 in the age group less than 20 years to 32.00 in the age group more than 71. There was a marked correlation between CV and age at a P-value <0.001; the percentage of HEX did not change with age in our study. With a P-value of <0.001, there was a statistically significant difference between CCT and age. There was a statistically significant mild negative correlation between ECD and age (r= -0.301, P value <0.001) and a statistically significant mild positive correlation between the CV and age (r=0.204, P value =0.004), tab. (3). The scatter diagram shows a statistically significant mild negative correlation between age and ECD (r= -0.301, p value<0.001), fig. (3). The scatter diagram shows a statistically significant mild positive correlation between age and coefficient of variation (r= 0.204, p value=0.004), fig. (4). The scatter diagram shows a statistically significant mild positive correlation between age and CCT (r= 0.201, p value=0.011), fig. (5).

Table (2) Endothelial cell parameters' characteristics in different age groups

Age (Years)	Eyes No.	ECD *(cells/mm^2; mean ± SD)*	CV *(%; mean ± SD)*	HEX *(%; mean ± SD)*	CCT *(mean ± SD)*
<20	16	2983.13±175.78	25.38±3.42	68.75±4.31	535.25±38.41
20-30	50	2822.32±339.11	29.88±4.94	65.52±4.43	537.48±42.11
31-40	60	2898.10±275.07	29.60±4.44	65.80±8.01	527.57±33.98
41-50	62	2748.45±370.03	31.65±5.58	65.23±5.73	556.26±35.63
51-60	98	2696.96±330.54	32.18±6.39	65.86±6.54	537.06±39.76
61-70	82	2748.56±283.92	31.22±4.66	64.46±6.62	555.54±30.62
71-80	22	2475.64±249.81	32.00±5.82	67.45±6.10	536.45±33.11
P-Value*		**<0.001**	**<0.001**	0.186	**<0.001**

***One Way ANOVA*

Table (3) Correlation between endothelial cell parameters and age

	Age	
	r	*P- value**
ECD (cells/mm^{2})	-0.301	<0.001
CV (%)	0.204	0.004
HEX (%)	-0.052	0.303
CCT	0.201	0.011

r** (correlation coefficient) / ***Pearson correlation

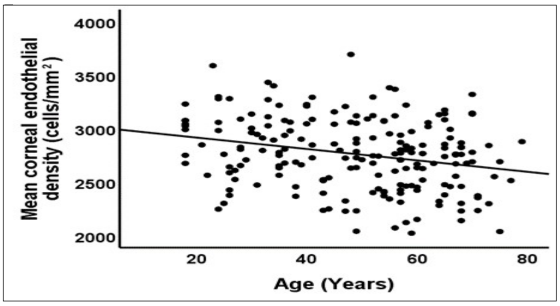

Figure (3) Scatter diagram for correlation between corneal cell density and age

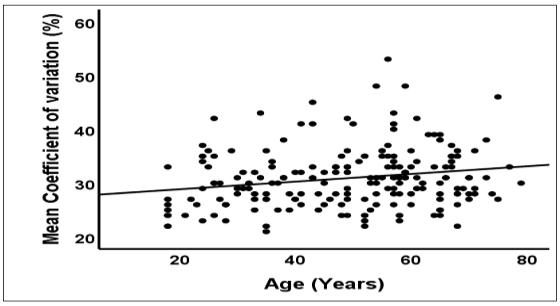

Figure (4) Scatter diagram for correlation between the coefficient of variation and age

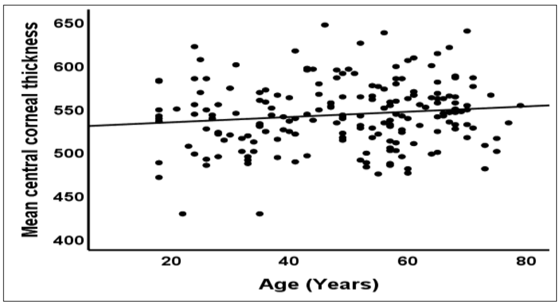

Figure (3) Scatter diagram for correlation between the central corneal thickness and age

4.2. *The relation of endothelial cell parameters with gender*

There were no substantial differences in HEX percentage, CV, and ECD between females and males. However, there was a statistical difference in CCT between females and males, with a P-value of 0.007, tab. (4)**.** Additionally, there was no substantial difference in ECD, tab. (5), CV, tab. (6), or HEX percentage, tab. (7) between females and males in different age groups. There was no statistical difference in HEX between females and males except in the age group 71-80 years, with a P-value of 0.006, tab. (7)**.** There was a statistical difference in CCT between females and males, with a P-value of 0.007, tab. (4), and a substantial difference in CCT between females and males in participants below the age of 20 years, with a P-value of 0.002; however other age groups demonstrated non-significant difference, tab. (8)**.**

Table (4) Endothelial cell parameters in females and males

	Male	Female	P-Value*
ECD (cells/mm²)	2780.20±320.50	2750.05±331.98	0.372
CV (%)	31.15±5.98	30.75±5.15	0.479
HEX (%)	64.78±5.46	66.19±6.90	0.055
CCT	536.25±36.55	546.72±37.66	**0.007**

Independent sample T-test

Table (5) Endothelial cell density between males and females according to age groups

Age (Years)	Endothelial cell density (cells/mm²; mean ± SD)		P-Value*
	Male	*Female*	
<20	2971.83±203.31	3017.00±30.02	0.672
20-30	2859.73±372.40	2792.93±314.27	0.495
31-40	2883.64±224.18	2906.47±303.21	0.760
41-50	2786.90±452.02	2730.14±328.58	0.577
51-60	2687.39±277.51	2705.42±373.76	0.789
61-70	2800.92±257.15	2726.90±293.65	0.286
71-80	2556.33±303.36	2378.80±118.43	0.098

Independent sample T-test

Table (6) Coefficient of variation between males and females according to age groups

Age (Years)	Coefficient of variation (%; mean ± SD)		P-Value*
	Male	*Female*	
<20	25.50±3.94	25.00±1.15	0.810
20-30	29.82±5.50	29.93±4.56	0.938
31-40	29.09±3.42	29.89±4.96	0.504
41-50	31.90±4.84	31.52±5.95	0.806
51-60	33.35±7.45	31.15±5.15	0.090
61-70	30.92±3.87	31.34±4.97	0.708
71-80	33.83±6.36	29.80±4.44	0.107

******Independent sample T-test*

Table (7) Hexagonal cell between males and females according to age groups

Age (Years)	Hexagonal cell (%; mean ± SD)		P-Value*
	Male	*Female*	
<20	68.17±4.80	70.50±1.73	0.367
20-30	64.36±4.56	66.43±4.19	0.103
31-40	64.82±6.23	66.37±9.03	0.475
41-50	65.10±5.47	65.29±5.91	0.906
51-60	64.26±5.88	67.27±6.81	0.061
61-70	64.58±5.99	64.41±6.92	0.917
71-80	64.00±2.25	71.60±6.72	**0.006**

******Independent sample T-test*

Table (8) Central corneal thickness (CCT) between females and males according to age groups

Age (Years)	Central corneal thickness (mean ± SD)		P-Value*
	Male	*Female*	
<20	519.83±31.22	581.50±0.57	**0.002**
20-30	527.18±38.04	545.57±44.01	0.127
31-40	525.64±39.65	528.68±28.97	0.733
41-50	559.90±42.76	554.52±32.13	0.583
51-60	532.74±30.57	540.88±46.38	0.314
61-70	554.67±27.99	555.90±31.87	0.870
71-80	526.00±33.28	549.00±29.61	0.105

******Independent sample T-test*

4.3. *The relation of endothelial cell parameters with refractive eye state*

Table (9) shows a marked difference in ECD between emmetropes, hypermetropic, and myopes, with a P-value of 0.001. Moreover, there was a substantial difference in CCT between emmetropes, hypermetropic, and myopes, with a P-value of 0.001. Nonetheless, there were no statistically significant differences in HEX percentage and CV among emmetropes, hypermetropic, and myopes**.** There were no substantial differences between grades of myopia in ECD, CV, and HEX, as depicted in tab. (10). There were no substantial differences between grades of hypermetropia in ECD, with a P-value of 0.001, but no statistically significant difference in CV, HEX, tab. (11). There was a statistically significant low negative correlation between ECD and CV (r= -0.223, P value <0.001) and a statistically significant low negative correlation between CV and HEX percentage (r=-0.453, P-value <0.001) and a statistically significant low negative correlation between ECD and percentage of HEX (r=-0.297, P-value <0.001), tab. (12).

Table (9) Endothelial cell parameters according to spherical equivalent

Mean ± SD	Emmetrope	Hypermetropia	Myope	P-value*
ECD (cells/mm^2)	2952.21±211.30	2726.30±317.18	2745.88±335.17	0.001
P-Value**	Emmetrope Vs. Hyper <0.001	Hyper Vs. myope=0.642	Emmetrope Vs. myope <0.001	
CV (%)	28.95±3.99	31.46±5.76	31.04±5.56	0.058
HEX (%)	63.63±5.71	66.54±6.77	65.65±6.33	0.073
CCT	545.89±30.83	555.89±38.30	538.45±37.39	0.001
P-Value**	Emmetrope Vs. Hyper=0.176	Hyper Vs. myope <0.001	Emmetrope Vs. myope=0.245	

**One Way ANOVA, ** pairwise comparison between groups*

Table (10) Endothelial cell parameters according to grades of myopia (N=278)

Mean ± SD	Low myopia	Moderate myopia	High myopia	P-value*
ECD (cells/mm^2)	2737.60±361.10	2700.93±331.86	2825.13±272.89	0.071
CV (%)	30.74±5.33	31.60±6.11	30.81±5.27	0.505
HEX (%)	66.32±6.06	65.44±7.13	64.63±5.53	0.206
CCT	536.45±36.14	537.22±44.30	544.03±27.96	0.393

**One Way ANOVA*

Table (11) Endothelial cell parameters according to grades of Hypermetropia (N=74)

Mean ± SD	Low Hypermetropia	Moderate Hypermetropia	High Hypermetropia	P-value*
ECD (cells/mm^2)	2657.42±325.68	2739.00±254.79	3279.00±0.01	**0.001**
P-Value**	Low Vs. moderate=0.244	Moderate Vs. High=0.001	Low Vs. High <0.001	
CV (%)	30.95±5.01	31.63±6.79	35.00±0.01	0.404
HEX (%)	67.42±5.71	66.06±8.10	62.00±0.01	0.276
CCT	558.63±38.43	549.13±39.17	584.00±0.01	0.189

**One Way ANOVA, ** pairwise comparison between groups*

Table (12) Inter-correlation between endothelial cell parameters

		ECD	CV	HEX	CCT
ECD	r		-0.223**	-0.297**	0.124
	P-Value		<0.001	<0.001	0.014
CV	r	-0.223**		-0.453**	-0.005
	P-Value	<0.001		<0.001	0.390
HEX	r	-0.297**	-0.453**		0.001
	P-Value	<0.001	<0.001		0.986
CCT	r	0.124	-0.005	0.001	
	P-Value	0.014	0.390	0.986	

***r** (correlation coefficient), *Pearson correlation*

5. Discussion

Endothelial cell studies revealed significant clinical information on corneal viability and function. In order to obtain more information about the endothelial cell layer, which is essential for maintaining corneal transparency, ECD measurement is now a standard clinical and research technique. Clinical applications include the evaluation of the endothelium in donor corneas, the observation of various anterior segment surgical procedures, and the study of the long-term effects of intraocular surgeries such as cataract surgery or phakic intraocular lens implantation [2]. It was reported that endothelial cells diminish with age. This decrease is most likely due to necrosis or apoptosis caused by light-induced oxidative damage. ECD levels should be evaluated prior to nearly all intraocular procedures [11]. Numerous studies have discovered an association between ECD and shape concerning ethnicity, sex, and age. Corneal endothelium characteristics vary between races and ethnic groups [10, 12]. Consequently, populations of diverse ethnic and racial backgrounds must produce normative data in order to determine endothelial function. Evaluations of an individual's endothelium function and health should be according to the population's normative data. This work investigates the endothelial cell parameters of the normal Egyptian population. Numerous studies investigated the relationship between sex and endothelial cell characteristics. Some studies revealed differences between sexes [13], whereas others revealed substantial differences [14,15]. Our study revealed nonsignificant variations between females and males in ECD, CCT, or CV but a statistical difference in CCT between males and females in participants below the age of 20 years, as well as no statistically significant difference in other age groups. There was a nonsignificant difference in hexagonal cells between females and males except in the age group 71-80 years, in contrast to a study by Abdallah MM et al. [16], who reported a significant difference between Egyptian males and females in all age groups. According to the current study's findings, there was a general tendency toward diminished ECD, increased MCA, and elevated CV in cell size with increasing age. These results are in accordance with the results of several earlier studies [10,17]. Even though several studies have indicated a substantial negative association between ECD and age in healthy individuals [10], other studies demonstrated no marked correlation between ECD and age in those older than 40. Padilla et [13] demonstrated that, while there was a definite tendency toward reducing MCD and MCA with increasing age up to the sixth decade of life, there was a reversal tendency at 61 and above. These findings have been linked to a substantial elevation in polymegathism during the fourth decade [18]. This study showed that a decreased CCT with age might be due to some degenerative changes in corneal stroma or epithelium. Variation among CC of the various refractive error groups is one of the most studied topics, with numerous research studies revealing significant correlation values [19]. The progression of myopia or hyperopia induces the steepening or flattening of CC. Several studies have observed that the shift in refractive error, CC alterations, and axial length expansion have a significant relationship [20, 21]. CC may also have a significant effect on emmetropization, as it tends to slow the progression of refractive error [22]. The polymegathism value is a coefficient that describes the variance in the cell area. In the current research, CV demonstrated a substantial positive correlation with age, with no significant differences regarding sex. According to this study, increasing age is associated with a reduction in ECD, an increase in MCA, and an increase in the CV of cell size. These findings are compatible with several earlier studies [10,16, 17]. Our study revealed that the CEC layer with an elevated percentage of hexagonal appearance of the cells tends to have less CV in the cell size. Although there was a minor correlation between CV distribution and refractive error ($r = 0.05$, $p = 0.65$), the correlation between ECD and CV distribution was remarkable ($r = 0.34$, p 0.05). Previous research found a mixed response between ECD and CV among different refractive error groups, with some individuals with myopia having a lower ECD ratio [23]. The results of this study agreed with those of some previous investigations, which found that high myopes with an average age of 22.2 years had low corneal ECD [24]. However, it has been well-established that the variability in ECD among individuals with hyperopia and emmetropia stays within the normal range. The present study demonstrated a marked relationship between ECD and CCT, which aligns with Nieider et al. [25], who found a negative relationship between ECD and CCT, and inconsistent with another study that revealed an insignificant relationship between ECD and CCT [16]. This study demonstrated no correlation between ECD, CCT, and CC in contrast to the previous study by Muller et al. [23], who found a significant correlation between ECD, CCT, and CC. There was a statistically significant low negative correlation between ECD and CV, between CV and HEX percentage, as well as between ECD and HEX percentage. A previous study by Chang et al. [24] showed a correlation between longer axial length and low ECD. The possible explanation of these abnormalities in endothelial parameters in high myopia is that elongation of the eyeball leads to flattening endothelial cells to cover the inner surface of the cornea of elongated eyes, thereby decreasing ECD. Recent evidence suggests that extreme myopia is an inflammatory condition. Therefore, higher levels of inflammatory cytokines in the aqueous humor are related to ECD loss. Our study showed no statistical difference in endothelial cell parameters among grades of myopia in contrast to a study done by Delshadet al. [26], who stated that the mean ECD and HEX were lower in moderate myopia compared to low myopia in young participants with a mean age of 21.6 years without any history of contact lenses wear. However, our study revealed a statistically significant variation in ECD across hypermetropia grades. The study's strength was the proper sample size with presumably healthy corneas and the first time in the Egyptian population evaluation of multiple corneal parameters (hexagonality, Avg cell size, CV, and CCT), and one examiner performed all measurements. The study's limitations include the absence of multivariate analysis and the failure to account for contributing factors like corneal diameter, IOP, and smoking. This work contributes to our knowledge of corneal morphology in the general population, particularly in light of the significance of pre-operative examination prior to intraocular surgeries.

6. Conclusion

Our study provides normative data for the corneal endothelium in healthy Egyptian eyes, adding to the expanding body of information about CEC parameters in healthy Egyptian eyes. In addition, our results may serve as a baseline for comparisons between Egyptians and other populations, as well as for research into endothelial cell reserve, corneal transplantation capacity, and intraocular surgeries.

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