·Investigation·

Corneal endothelial cell density and morphology and central corneal thickness in Guangxi Maonan and Han adolescent students of China

Hao Liang[1], Hui-Yi Zuo[1], Jin-Mao Chen[1], Jie Cai[1], Yu-Zhua Qin[2], Yu-Ping Huang[2], Ying-Ying Chen[1], Dong-Yong Tang[1], Shao-Jian Tan[1]

[1]Department of Ophthalmology, the First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China

[2]People's Hospital of Huanjiang Maonan Autonomous County, Huanjiang 547100, Guangxi Zhuang Autonomous Region, China

Co-first authors: Hao Liang and Hui-Yi Zuo

Correspondence to: Shao-Jian Tan. Department of Ophthalmology, the First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China. shaojiantan@163.com

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Abstract

• AIM: To investigate the corneal endothelial cell density and morphology and central corneal thickness in the Guangxi Maonan and Han adolescent students of China.

• METHODS: Noncontact specular microscope (Topcon SP3000P, Tokyo, Japan) was performed in 133 adolescent students of Maonan nationality (M:F 54:79) and 105 adolescent students of Han nationality (M:F 50:55), 5 to 20y of age, who were randomly selected from 3 schools in Huanjiang Maonan Autonomous County of Guangxi Zhuang Autonomous Region of China. Parameters studied included endothelial cell density, mean cell area, coefficient of variation in cell size, percentage hexagonality and central corneal thickness.

• RESULTS: Endothelial cell density, mean cell area, coefficient of variation in cell size, percentage hexagonality and central corneal thickness in the study population were (2969.50±253.93) cells/mm^2, (339.23±29.44) μm^2, (29.96±4.07) %, (64.58±9.41) % and (523.71±32.82) μm in Maonan and (2998.26±262.65) cells/mm^2, (336.11±30.07) μm^2, (29.89±5.03) %, (64.91±11.64) % and (524.39±33.15) μm in Han, respectively. No significant differences were observed in endothelial cell density, mean cell area, coefficient of variation in cell size, percentage hexagonality and central corneal thickness between Maonan and Han (P=0.615, 0.659, 0.528, 0.551, 0.999). In Maonan and Han, we found age was negatively correlated with endothelial cell density and percentage hexagonality and positively correlated with mean cell area and coefficient of variation in cell size. Negative correlation was also found between central corneal thickness and age in Han, whereas no correlation was found in Maonan.

• CONCLUSION: There were no differences between Maonan and Han in corneal endothelial cell density and morphology and central corneal thickness. In these two nationalities, there were statistically significant decrease in endothelial cell density and percentage hexagonality with increasing age and statistically significant increase in cell area and coefficient of variation in cell size with increasing age. Central corneal thinned with increasing age in Han, whereas difference did not attain statistical significance in Maonan.

• KEYWORDS: endothelial cell density; morphology; central corneal thickness; Maonan

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INTRODUCTION

A layer of hexagonal flat cells composes the endothelium. Close connection between the corneal endothelial cells can stop aqueous humor into the extracellular space, which maintains corneal relative state of dehydration through the "pump" and barrier function of the endothelium-a prerequisite for corneal optical transparency[1]. It is well known that the endothelial repair is limited to enlargement and sliding of existing cells, with little capacity for cell division [2]. Several authors in their studies have confirmed that there were decrease in endothelial cell density and percentage hexagonality with increasing age and increase in cell area with increasing age[3,4].

There are wide variations in endothelial morphology between individuals at any age. These variations are partly due to extrinsic factors, such as genetic factors, ocular trauma, intraocular surgery, and ultraviolet radiation[5]. Due to corneal

endothelial cell density is necessary to maintain normal state of dehydration and transparent cornea, polymegathism and polymorphism can better reflect the integrity and function of cornea than endothelial cell density alone [6]. Despite these variations can influence endothelial cell chatacteristics, it may be essential to determine normal endothelial cell densities at each age level.

China is a multi-ethnic country, including the Han and 55 minority nationalities. Maonan nationality is one of the 55 minorities with population of 107 200 according to the sixth national census statistics of China in 2010. Forty percent of them live in the Huanjiang Maonan Autonomous County of Guangxi Zhuang Autonomous Region of China. Some studies have showed that statistically significant differences of corneal endothelial cell densities and morphologies among races and ethnic groups [7,8]. Although a cross-sectional population-based studies has investigated the distribution of corneal endothelial cell density and morphology in the healthy Chinese population[9], no such study has been done in the different ethnic groups of China. This study described the corneal endothelial cell density and morphology and central corneal thickness in the Guangxi Maonan and Han adolescent students in relation to age and gender.

SUBJECTS AND METHODS

Subjects One hundred and thirty-three adolescent students of Maonan nationality (M:F 54:79) and 105 adolescent students of Han nationality (M:F 50:55) were randomly selected from 3 schools in Huanjiang Maonan Autonomous County of Guangxi Zhuang Autonomous Region of China. They were randomly selected from our previous stratified randomized cluster samples. Students ranged in age from 5 to 20, with a mean of (12.91±3.18)y in Maonan and (12.11±4.44)y in Han, and no significant difference was observed (P=0.125). The participants were excluded if they had evidence of ocular trauma, history of intraocular surgery, corneal disease, endothelial dystrophy, history of wearing contact lens and a family history of hereditary corneal disorders. We also requested informed consent from the school authorities and all students before the examination. The experimental design was approved by the Institutional Review Board of the First Affiliated Hospital of Guangxi Medical University.

Methods Routine ocular examination was performed, corneal endothelial cell was measured using a noncontact specular microscope (Topcon SP3000P, Tokyo, Japan). A single examiner performed all of the measurements. Three images from the central cornea were taken, with the sharply focused image saved, and the three measuring results were averaged. Parameters studied included endothelial cell density, mean cell area, coefficient of variation in cell size, percentage hexagonality and central corneal thickness.

Statistical Analysis Statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The right eye of all subjects was analyzed. Differences in corneal endothelial cell density and morphology and central corneal thickness in different ethnic groups and all indexes between males and females within ethnic groups were assessed using the covariance analysis. Spearman correlation was used to determine the variation characteristics of endothelial cells and central corneal thickness with age. A P value of <0.05 was considered statistically significant.

RESULTS

Endothelial cell density, mean cell area, coefficient of variation in cell size, percentage hexagonality and central corneal thickness in the study population were (2969.50±253.93) cells/mm^2, (339.23±29.44) μm^2, (29.96±4.07) %, (64.58±9.41) % and (523.71±32.82) μm in Maonan and (2998.26±262.65) cells/mm^2, (336.11±30.07) μm^2, (29.89±5.03) %, (64.91±11.64) % and (524.39±33.15) μm in Han, respectively. Since age and gender can impact indicators, covariance analysis can further improve the accuracy and sensitivity of statistical test. No significant differences were observed in endothelial cell density, mean cell area, coefficient of variation in cell size, percentage hexagonality and central corneal thickness between Maonan and Han (P=0.615, 0.659, 0.528, 0.551, 0.999). In different age groups, there were no significant differences between Maonan and Han in corneal endothelial cell density and morphology and central corneal thickness (Table 1). No significant differences between males and females within ethnic groups in corneal endothelial cell density and morphology and central corneal thickness (Table 2).

In Maonan, there was a statistically significant decrease in endothelial cell density with increase in age (P=0.009, correlation -0.227). Regression analysis indicated a cell loss rate of 0.6% per year of age. A significant increase in mean cell area (P=0.009, correlation 0.227), and coefficient of variation in cell size (P<0.001 correlation 0.339) with age was noted. The percentage of hexagonal cells in the endothelium showed a statistically significant reduction with increasing age (P<0.001, correlation -0.373). The difference did not attain significance in the corneal thickness with age (P=0.481, correlation 0.062). In Han, there was a statistically significant decrease in endothelial cell density with increase in age (P<0.001, correlation -0.341). Regression analysis indicated a cell loss rate of 0.5% per year of age. A significant increase in mean cell area (P<0.001, correlation 0.341), and coefficient of variation in cell size (P<0.001 correlation 0.437) with age was noted. The percentage of hexagonal cells in the endothelium showed a statistically significant reduction with increasing age (P<0.001, correlation -0.526). A decrease in corneal thickness with age was noted (P=0.046, correlation -0.195).

Table 1 Corneal endothelial cell density and morphology and central corneal thickness in different age groups $\bar{x} \pm s$ x

Parameters	≤10 years old			11-15 years old			≥16 years old		
	Maonan	Han	*P*	Maonan	Han	*P*	Maonan	Han	*P*
Central corneal thickness (μm)	519.32±4.27	530.64±3.66	0.052	529.08±4.71	511.60±8.47	0.076	519.23±6.51	521.44±6.18	0.816
Mean cell area (μm^2)	326.30±4.20	325.86±3.60	0.939	342.93±3.55	340.05±6.38	0.695	347.01±6.12	349.59±5.82	0.772
Variation coefficient (%)	27.69±0.74	27.89±0.64	0.839	30.56±0.42	30.02±0.76	0.536	31.44±0.88	32.85±0.84	0.272
Cell density (cells/mm^2)	3084.68±40.67	3086.15±34.87	0.979	2935.07±29.15	2960.20±52.39	0.677	2903.69±50.04	2884.88±47.56	0.796
Hexagonality (%)	70.52±1.69	70.03±1.45	0.831	63.57±1.04	62.91±1.87	0.760	59.73±1.87	58.10±1.78	0.548

Age and gender as covariate.

Table 2 Corneal endothelial cell density and morphology and central corneal thickness between males and females within enthic goups $\bar{x} \pm s$ x

Parameters	Male	Female	*P*
Maonan			
Central corneal thickness (μm)	530.15±4.46	519.31±3.68	0.065
Mean cell area (μm^2)	344.42±3.89	335.69±3.21	0.087
Variation coefficient (%)	29.92±0.53	29.99±0.44	0.925
Cell density (cells/mm^2)	2921.43±33.39	3002.36±27.53	0.065
Hexagonality (%)	65.31±1.20	64.08±0.99	0.433
Han			
Central corneal thickness (μm)	522.69±4.64	525.94±4.42	0.614
Mean cell area (μm^2)	340.30±3.94	332.30±3.76	0.146
Variation coefficient (%)	29.27±0.64	30.45±0.61	0.183
Cell density (cells/mm^2)	2959.31±34.49	3033.66±32.88	0.122
Hexagonality (%)	65.47±1.43	64.41±1.36	0.595

Age as covariate.

DISCUSSION

Many studies have been published on the relationship of endothelial cell density and morphology to age, gender, and ethnicity [5-7,9,10]. Decisions regarding endothelial health and function in an individual should be based on normative data derived from the underlying population. Although different investigators in their studies found that the relationship of age and gender to corneal endothelial cells, it was clear that significant differences in corneal endothelial density and morphology among races and ethnic groups [7,8]. However, it was unknown whether corneal endothelial cells characteristics between different ethnic groups in China had significant differences. The present study provided data on endothelial cell characteristics in the normal Maonan adolescent students and Han adolescent students living in Huanjiang Maonan Autonomous County of Guangxi Zhuang Autonomous Region of China. The results showed that endothelial cell density, mean cell area, coefficient of variation in cell size and percentage hexagonality of two ethnic groups was very approximate and no significant differences were observed. Corneal endothelial cell density could be used as indirect evaluation index to evaluate the function of corneal endothelium, whereas corneal thickness was related with corneal edema and the function of corneal endothelium. It was described that central corneal thickness among different races and ethnic groups was difference [11]. Significant difference was not noted in central corneal thickness between Maonan and Han.

Regarding the effect of sex on corneal endothelial cell densities and morphology and central corneal thickness, some studies showed statistically significant differences of corneal endothelial cell densities between genders [6,8], but others did not find statistically significant differences of corneal endothelial cell densities between genders [5,10,12]. Previous studies reported that males have thicker central corneas than females [13-15], other studies did not find such a difference[16-18]. The results revealed that significant differences were not noted in corneal endothelial cell density and morphology and central corneal thickness between males and females within ethnic groups.

The corneal endothelial cells in adolescent students, increased with age while showing some variation, mainly the number of cells and the morphological changes, the number of cells can be reflected by changes in cell density, and morphological indicators such as the mean cell area, coefficient of variation in cell size and percentage hexagonality reflects changes in cells morphology. Corneal endothelial cells from birth until death can not be regenerated, so the increase of the surface area of the cornea can only rely on the expansion of the cells to extend coverage. This causes the cell area expansion and lower density, while also changing morphology. The cell loss rate with age (0.6% per year) in Maonan and (0.5% per year) in Han are similar to that described in other cross-sectional studies (0.3%-0.6% per year)[5,9,10,19,20]. We estimated it from our data in Maonan a decrease in cell density of 3.1% between ages 5 and 10y, a decrease of 2.8% between ages 11 and 15y and a decrease of 2.0% between ages 16 and 20y, in Han a decrease in cell density of 3.7% between ages 5 and 10y, a decrease of 2.4% between ages 11 and 15y and a decrease of 1.6% between ages 16 and 20y. As the age grows, the cell loss rate went down. A posterior corneal surface growth in the absence of endothelial mitoses and the prepubertal horomonal change may contribute part to this finding [21]. Spearman correlation showed that in Maonan and Han, age was negatively correlated with endothelial cell density and percentage hexagonality and positively correlated with mean cell area and coefficient of variation in cell size, which was similar to the results of many previous investigations [5,9,10,22,23]. It was further revealed that the

characteristics of corneal endothelial cell density and morphology changed with increasing age. In our study, negative correlation was also found between central corneal thickness and age in Han, whereas no correlation was found in Mulao. These data confirmed that in Han central corneal thinned with increasing age, as was reported earlier [13,24], but in Maonan the difference did not attain statistical significance, as was reported by Rao *et al* [5]. Possible explanations for the thinning of the central cornea with aging include a decline in the density of keratocytes and a breakdown of corneal collagen fibers with age, as well as longer exposure to the environment and the effect of nutrition on central corneal thickness [13]. Our findings indicated that a trend toward decreased endothelial cell density and percentage hexagonality, increased means cell area and coefficient of variation in cell size with increasing age in Maonan and Han, central corneal thinned with increasing age in Han, whereas the difference did not attain in Maonan.

In conclusion, there were no differences between Maonan and Han in corneal endothelial cell density and morphology and central corneal thickness. In Maonan and Han, there were statistically significant decrease in endothelial cell density and percentage hexagonality with increasing age and statistically significant increase in cell area and coefficient of variation in cell size with increasing age. Central corneal thinned with increasing age in Han, whereas difference did not attain statistical significance in Maonan.

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