

Correlation between age and thickness of Descemet's membrane in Chinese people

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Foundation items: The National Natural Science Foundation of China (No. 81200661; 81470606); The Nature Science Foundation of Hubei Province (No. 2014CFB973; 2014CFB442)

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Received: 2015-12-27 Accepted: 2016-06-23

中国人角膜后弹力层厚度与年龄的相关性研究

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基金项目:国家自然科学基金(No. 81200661;81470606);湖北省自然科学基金(No. 2014CFB973;2014CFB442)

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摘要

目的:测量中国人的角膜后弹力层厚度并分析其与年龄的关系。

方法:收集27例角膜标本,年龄范围从0.2岁到78岁,所有标本经HE染色后行组织切片并通过400倍光学显微镜行显微照相,于每张相片中选取四个测量点并人工标记后弹力层的边界,通过软件Motic Images Plus 2.0自动计算其厚度并取平均值。采用线性回归方法分析角膜后弹力层厚度与年龄的关系。

结果:所有标本的角膜后弹力层厚度为1.78 μm 到9.30 μm ,平均4.63 \pm 2.00 μm 。角膜后弹力层厚度与年龄具有高正相关性($r=0.776$, $P=0.000$),线性回归方程可描述为:角膜后弹力层厚度(μm)=2.010+0.063年龄(岁)。

结论:中国人的角膜后弹力层厚度与年龄之间具有显著正相关性。

关键词:角膜;后弹力层;厚度;年龄

引用:王玮,徐玲娟,罗班,张宪,李贵刚. 中国人角膜后弹力层厚度与年龄的相关性研究. 国际眼科杂志 2016;16(9):1599-1602

Abstract

• **AIM:** To examination the thickness of Chinese Descemet's membrane and investigate the correlation with age.

• **METHODS:** Twenty-seven normal corneas with ages ranging from 0.2 to 78y old were collected. All specimens were stained with Hematoxylin and Eosin, and viewed and photographed at $\times 400$ through a calibrated Motic light photomicroscope. Four measurement sites on each specimen micrograph were chosen and the boundary of Descemet's membrane was designated manually, then the Descemet's membrane thickness was measured by the software of Motic Images Plus 2.0 automatically. The relationship between Descemet's membrane thickness and age was analyzed by using linear regression methods.

• **RESULTS:** The thickness of Chinese Descemet's membrane various from 1.78 to 9.30 μm , averaged 4.63 \pm 2.00 μm . There is a highly significant positive correlation between age and thickness of Descemet's membrane ($r=0.776$, $P=0.000$). The estimated equation that best describes the relationship of thickness with age can be expressed as: Thickness (μm)= 2.010+0.063y.

• **CONCLUSION:** There is a significant positive association between age and the thickness of Descemet's membrane in Chinese people.

• **KEYWORDS:** cornea; Descemet's membrane; thickness; age

DOI:10.3980/j.issn.1672-5123.2016.9.01

Citation: Wang W, Xu LJ, Luo B, Zhang X, Li GG. Correlation between age and thickness of Descemet's membrane in Chinese people. *Guoji Yanke Zazhi (Int Eye Sci)* 2016;16(9):1599-1602

INTRODUCTION

Descemet's membrane is the basement membrane of the corneal endothelium^[1]. This posterior limiting membrane fulfills important structural and physiologic functions in the cornea^[2].

Descemet's membrane is composed of anterior banded zone and posterior non-banded zone when viewed through scanning electron microscope^[3-4]. The anterior banded zone is first secreted in utero by the endothelial cells at about four months' gestation and acquires recognizable banding by eight months' gestation. The posterior non-banded zone is secreted by the endothelial cells after birth. Observations in mammals suggest that only the posterior non-banded zone is synthesized continuously throughout adult life^[3-6].

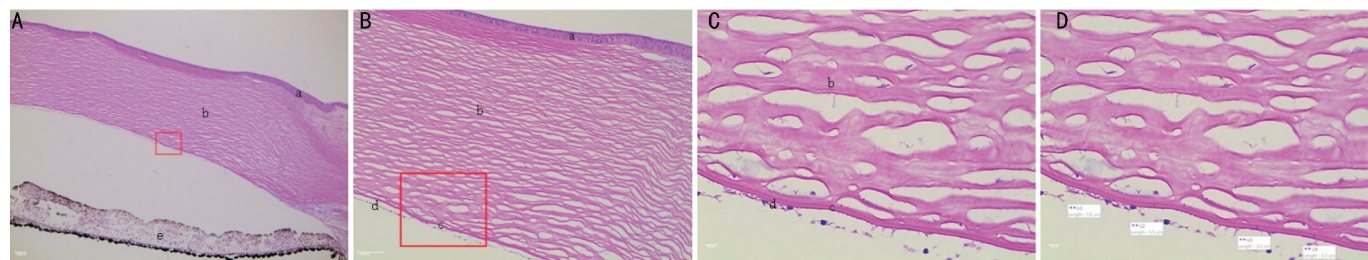


Figure 1 Histopathologic appearance of the cornea with hematoxylin and eosin staining (A $\times 40$, B $\times 100$, C $\times 400$, D is same to C but shows the Measurement sites): a is the epithelium cell layer, b is the stroma, c is the Descemet's membrane, d is the endothelial cell layer, e is the iris.

The normal thickness of human Descemet's membrane is about 2–3 μm at birth, increasing to approximately 5–6 μm in children and 8–14 μm in adults^[3-4]. To our knowledge, there is no morphometric data available that describing the relationship between Descemet's membrane thickness and age in Chinese people. The purpose of our investigation is to provide a comprehensive Chinese standard derived from measurements of 27 specimens from 0.2 to 78y old.

SUBJECTS AND METHODS

Human corneas were obtained from the Wuhan Red Cross Eye Bank. Informed consent was obtained prior to the study from patients or relatives according to usual procedures, the corneas were managed in accordance with the declaration of Helsinki. Twenty-seven ostensibly normal corneas which ages ranging from 0.2 to 78y old were collected (Table 1). The central parts of 12 corneas were used for transplantation and peripheral parts were used for this study, the other corneas were not suitable for transplantation for HBs positive or cancer disease. Corneas from both male and female patients were included, and only one eye per patient was studied.

Standard methods were used for fixation and tissue processing of the cornea. Sectioning of the paraffin-embedded tissue was in a meridional plane perpendicular to the corneal surface. Transverse sections (3.0 μm thick) of Descemet's membrane and adjacent corneal tissue were stained with hematoxylin and eosin(HE), then viewed and photographed at $\times 400$ through a calibrated Motic light photomicroscope (Figure 1). The boundary of Descemet's membrane was designated manually on the micrographs which allowed for an easy identification of the borders, and the thickness from the anterior boundary adjacent to the stroma to the posterior boundary adjacent to the endothelial cell layer was measured by the software of Motic Images Plus 2.0. This software can measure the thickness automatically by comparing the scale length when the measurement sites were chosen. And the measurement sites choice were not random, as the boundary domelike extension and tangentially sectioned foci were avoided. We took one $\times 400$ micrograph of each cornea specimen, chose four measurement sites on each micrograph, and calculated the average thickness (Figure 2, 3). The relationship between Descemet's membrane thickness and age was analyzed by using linear regression methods.

Table 1 General information for the corneas

No.	Age (a)	Sex	Thickness of Descemet's membrane (μm)
1	0.2	M	1.78
2	0.5	M	2.18
3	2	F	2.68
4	6	M	1.83
5	10	M	2.85
6	16	M	2.83
7	16	M	3.40
8	30	M	2.88
9	35	M	3.80
10	39	M	6.13
11	39	M	6.23
12	40	M	5.03
13	40	M	7.15
14	41	M	4.20
15	42	M	2.33
16	44	M	4.93
17	56	F	4.45
18	56	M	2.30
19	60	M	4.85
20	60	M	6.68
21	62	M	5.15
22	63	M	5.38
23	67	M	6.90
24	67	M	5.88
25	76	M	7.30
26	78	F	6.58
27	78	F	9.30

RESULTS

The thickness of Chinese Descemet's membrane varies from 1.78 to 9.30 μm , averaged $4.63 \pm 2.00 \mu\text{m}$ ($n=27$). There is a highly significant positive correlation between age and thickness of Descemet's membrane ($r=0.776$, $P=0.000$) (Figure 4). The estimated equation that best describes the relationship of thickness with age can be expressed as: thickness (μm) = $2.010 + 0.063y$. But sufficient variation in thickness of Descemet's membrane between individuals, it's inaccurate to predict the exact thickness of any people from the age.

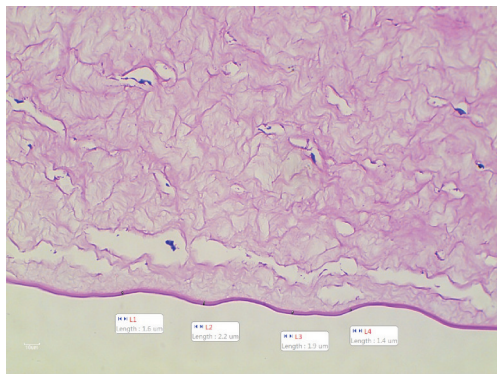


Figure 2 The micrograph of 0.2-year-old infant's Descemet's membrane with HE staining ($\times 400$), the average of 4 measurements of thickness is $1.78 \mu\text{m}$. This is the youngest specimen which has the thinnest thickness.



Figure 3 The micrograph of 78-year-old woman's Descemet's membrane with HE staining ($\times 400$), the average of 4 measurements of thickness is $9.30 \mu\text{m}$. This is the oldest specimen which has the thickest thickness.

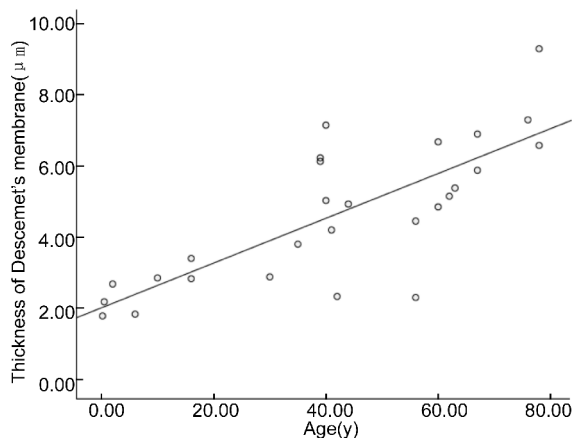


Figure 4 Thickness of Descemet's membrane as a function of age ($n=27$ specimens, ages from 0.2 to 78y). The estimated equation that best describes the relationship of thickness with age can be expressed as: thickness (μm) = $2.010+0.063y$.

The specimens which ages ranging from 35 to 44y have an average thickness of $4.98 \mu\text{m}$ with a standard deviation of $1.54 \mu\text{m}$ ($n=8$). And the specimens which ages ranging from 56 to 67y have an average thickness of $5.20 \mu\text{m}$ with a standard deviation of $1.45 \mu\text{m}$ ($n=8$).

DISCUSSION

All we know that the thickness of Descemet's membrane increases with age^[7-8], but this study is the first time to describe the relationship between Descemet's membrane thickness and age in Chinese people by morphometric data. Descemet's membrane has a complex structure which consists of collagen components, such as I, III, IV, V, VI, and VIII, and noncollagenous components, such as laminin, heparin sulphate, fibronectin, nidogen, tenascin, and P component, but a complete understanding of the composition of Descemet's membrane has not yet been achieved^[9-11]. Descemet's membrane is a basement membrane that lies in-between the stroma and the endothelial layer of the cornea, and eosin is a fluorescent red dye which can be used to distinguish Descemet's membrane from the stroma and the endothelial layer. But the boundary of Descemet's membrane viewed through light microscope is not clearer than electron microscope, the thickness measurement on light microscope graphs may cause more inaccuracy. And we found if the Descemet's membrane is thicker, the measurement inaccuracy is bigger. To reduce this inaccuracy, we abandoned some pathologic specimens which tissue is out of normal shape, we chose the measurementsites which the anterior boundary of Descemet's membrane parallel to the posterior boundary, and we avoided choosing the measurementsites that the anterior or posterior boundary was not clear.

Descemet's membrane is composed of anterior banded zone and posterior non-banded zone when viewed through scanning electron microscope, and only the posterior non-banded layer thicken with age. But there is no distinction between anterior banded layer and posterior non-banded layer when viewed through light microscope with HE staining. In this paper we analysis the relationship between the entire Descemet's membrane thickness and age in Chinese people. And the result is similar to previously published, we found a significant positive association between age and the thickness of Descemet's membrane.

Using transmission electron microscope, Murphy *et al*^[4] measured the average thickness of Descemet's membrane to be about $10.07 \pm 0.99 \mu\text{m}$ in the ages ranging from 32 to 44y ($n=6$) and $11.61 \pm 2.32 \mu\text{m}$ in the ages ranging from 55 to 68y ($n=10$), Johnson *et al*^[3] measured the average thickness of Descemet's membrane to be about $10.88 \pm 2.49 \mu\text{m}$ in the ages ranging from 52 to 68y ($n=6$). But our specimens which ages ranging from 35 to 44y have an average thickness of $4.98 \pm 1.54 \mu\text{m}$ ($n=8$), and specimens which ages ranging from 56 to 67y have an average thickness of $5.20 \pm 1.45 \mu\text{m}$ ($n=8$). Compared with the Westerners' data based on electron microscope, the Chinese data based on light microscope are significantly thinner. There are some elements should be consider which cause this difference in thickness. The Descemet's membrane boundary lack of precision which we have discussed may cause the variability. Tissue swelling or shrinkage may occur during tissue processing and fixation, and different methods of tissue processing and fixation between

electron microscopy and light microscopy may lead to artifacts. Compared Murphy's data which published based on the average thickness of whole membrane, our study chose only one $\times 400$ micrograph of each cornea specimen to measure the thickness which can't reflect the average thickness of whole membrane. Because the peripheral part of Descemet's membrane is thicker than the central part^[12-13]. Additionally, the thickness of Descemet's membrane in Chinese people may be thinner than Westerners, but this point need more specimens to support based on transmission electron microscope.

The most important result of our study is that we have been able to estimate the correlations between the thickness of Descemet's membrane and age in Chinese people for the first time. Although the thickness of Descemet's membrane increases with age, the range of thicknesses between individuals is large enough that age can't be accurately predicted from the thickness in any particular case.

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