EVALUATION OF RETINAL VASCULAR CALIBER IN MALAY CHILDREN

BY

DR EVELYN TAI LI MIN
MD (USM)

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DISCLAIMER

I hereby certify that the work in this dissertation is my own except for quotations and summaries which have been duly acknowledged.

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Dr. Evelyn Tai Li Min

P-UM 0029/12

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ABSTRAK (BAHASA MALAYSIA)

Latar belakang:

Fotografi digital salur darah retina merupakan satu kaedah yang sedang berkembang dan selamat digunakan untuk menganalisa dan menilai salur darah pada lapisan retina manusia. Kanak-kanak merupakan subjek yang sesuai digunakan kerana secara amnya mereka tidak dibatasi oleh faktor-faktor kesihatan. Walaubagaimanapun, setakat ini kajian mengenai diameter salur darah retina kanak-kanak, terutamanya di kalangan etnik Melayu yang merupakan etnik majoriti di Asia Tenggara, masih lagi terhad. Kajian ini bertujuan untuk menganalisa pengaruh faktor sistemik dan okular pada diameter salur darah retina dikalangan kanak-kanak Melayu.

Kaedah:

Ini merupakan kajian keratan rentas yang melibatkan seramai 86 kanak-kanak Melayu berumur 6 hingga 12 tahun. Refraksi, biometrik mata dan fotografi retina dilakukan mengikut protokol yang telah ditetapkan. Di samping itu, tekanan darah juga diukur. Diameter salur darah retina diterjemahkan sebagai “central retinal ateriolar equivalent” (CRAE) dan central retinal venular equivalent (CRVE) melalui kaedah berasakan komputer yang telah ditetapkan. Kaedah multiple linear regression digunakan untuk menentukan kesan faktor sistemik dan okular pada diameter salur darah retina.

Keputusan:

Setelah mengambil kira faktor pembesaran okular, ukuran purata CRAE dan CRVE masing-masing ialah 171.40 um dan 248.02 um. Didapati tekanan darah dan panjang bola mata adalah faktor-faktor yang mempengaruhi CRAE. Selepas pengubahsuaian data “multivariable”, didapati bagi setiap unit (1 mm Hg) kenaikan dalam purata tekanan darah dikaitkan dengan 0.451 um penurunan dalam CRAE (p=0.013). Manakala, bagi setiap kenaikan (1 mm) dalam panjang bola mata dikaitkan dengan 4.582 um penurunan dalam CRAE (p=0.024). Walaubagaimanapun, semua faktor yang telah dikaji tidak menunjukkan sebarang kesan statistik yang signifikan pada CRVE.

Kesimpulan:

Ukuran purata CRAE dan CRVE masing-masing ialah 171.40 um dan 248.02 um. Kajian ini mendapati bahawa bagi golongan kanak-kanak yang normal, tekanan darah dan panjang bola mata yang lebih tinggi berkait dengan diameter salur darah arteri retina yang lebih sempit. Ini menunjukkan bahawa penyempitan salur darah yang berlaku dalam penyakit seperti tekanan darah tinggi dan miopia patologikal mungkin merupakan kesan tambahan daripada perubahan fisiologi yang normal.

ABSTRACT (ENGLISH)

Background:

Digital retinal vessel imaging is evolving as a non-invasive method of evaluating the microvasculature. In children, the general absence of disease-related confounding factors makes them an ideal study population. However, childhood studies in this field are limited, especially among Malays, the main ethnicity in South East Asia. The present study aims to evaluate the influence of ocular and systemic factors on retinal vessel caliber in a childhood Malay population.

Methods:

This was a cross-sectional study involving 86 Malay children aged 6 to 12 years old in Hospital Universiti Sains Malaysia, Malaysia. Ocular examination, refraction, retinal photography and axial length were performed by standardized protocols. Anthropometric measurements including blood pressure were likewise obtained. Retinal vessel diameters were summarized as the central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE) using validated computer-based methods. Multiple linear regression was used to determine the effect of systemic and ocular biometry on CRAE and CRVE.

Results:

After correcting for ocular magnification, the mean CRAE and CRVE were 171.40 um and 248.02 um respectively. Blood pressure (SBP, DBP, MAP) and axial length were the main factors affecting the CRAE. After multivariable adjustment, each unit (1 mm Hg) increase in mean arterial pressure was associated with a 0.451 um reduction in CRAE (p=0.013). Each 1 mm increase in axial length was associated with a 4.582 um narrowing of CRAE (p=0.024). All variables analyzed in our study had no statistically significant effect on the CRVE.

Conclusion:

The mean retinal CRAE and CRVE in Malay children were 171.40 um and 248.02 um. Retinal arteriolar calibers are narrower in healthy children with higher blood pressure and longer axial lengths. These associations suggest that the pathological changes in the microvasculature seen hypertension and pathological myopia may be an amplification of the normal physiological response of blood vessels to various systemic and ocular dynamics.

Chapter 1

Introduction

INTRODUCTION

The human microvasculature, which previously could be evaluated only by relatively invasive methods, is now gradually becoming an open book to us via the retinal circulation. Direct visualization of the retinal vasculature has been simplified by the advent of digital fundus photography, which is rapidly evolving as an integral part of the diagnosis and monitoring of a range of conditions.

In adults, retinal vascular calibers have been associated not only with ocular problems like glaucoma and diabetic retinopathy, but also with various systemic diseases, including coronary heart disease, stroke, obesity and mental health. Although studies in children are comparatively few, similar associations have been demonstrated, in which ocular biometry like axial length, as well as systemic biometry, such as blood pressure influence the retinal vessel parameters.

A significant proportion of the population living in South East Asia fall under the category of 'Malay', or 'Austronesian people'. This indigenous group of people have been estimated to number approximately 300 million in South East Asia. Malaysia is a nation with among the highest proportion of Malays, who comprise almost 70% of the population. Although Malays comprise the main ethnic group in South East Asia, the data on retinal vessel parameters in Malays, whom the anthropologist Blumenbach described as "tawny-coloured, with black hair", is particularly limited, especially in Malay children.

Ethnicity and the degree of iris and retinal pigmentation have been shown to influence the measurements of retinal vascular caliber in children. However, to the best of our knowledge, apart from a multi-ethnic study by Cheung et al in 2007, no other studies of retinal vessel calibers in children have included this large ethnic group. In children, the general absence of confounding factors related to systemic and ocular disease makes them an ideal study population not only for acquiring normative data, but also in analyzing the relationship between anatomico-physiological factors and retinal vessel calibers. The present study aims to mitigate the aforementioned dearth of data in Malay children by analyzing the factors affecting retinal vessel caliber in healthy Malay children.

Chapter 2

Objectives

2.1 GENERAL OBJECTIVE

To study the retinal vascular caliber in Malay children

2.2 SPECIFIC OBJECTIVES

i. To determine the mean retinal arteriolar caliber among Malay children

ii. To determine the mean retinal venular caliber among Malay children

iii. To determine ocular and systemic factors affecting retinal venular caliber in Malay children

iv. To determine ocular and systemic factors affecting retinal venular caliber in Malay children

Chapter 3

Manuscript

3.1 TITLE PAGE

Evaluation of Retinal Vascular Calibers in Malay Children

Evelyn-Tai LM[1,2], Wan-Hazabbah Wan Hitam[1,2], Tien Yin Wong[3,4,5], *Shatriah Ismail[1,2]

[1]Department of Ophthalmology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

[2]Hospital Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

[3] Singapore Eye Research Institute, Singapore National Eye Centre, Singapore

[4] DUKE-NUS Medical School, Singapore

[5] Centre for Eye Research Australia, University of Melbourne, Australia

* Corresponding author email: shatriah@usm.my (SI)

Author roles:

ETLM designed, carried out the study and wrote the manuscript. WHWH and SI co-designed the study. TYW contributed retinal vessel analysis via the Singapore I Vessel Analysis (SIVA) 4.0 cloud system, as part of a collaborative research project between Universiti Sains Malaysia and Singapore Eye Research Institute.

3.2 ABSTRACT

Background:

Digital retinal vessel imaging is evolving as a non-invasive method of evaluating the microvasculature. In children, the general absence of disease-related confounding factors makes them an ideal study population. However, childhood studies in this field are limited, especially among Malays, the main ethnicity in South East Asia. The present study aims to evaluate the influence of ocular and systemic factors on retinal vessel caliber in a childhood Malay population.

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Conclusion:

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3.3 INTRODUCTION

The human microvasculature, which previously could be evaluated only by relatively invasive methods, is now gradually becoming an open book to us via the retinal circulation [1, 2]. Direct visualization of the retinal vasculature has been simplified by the advent of digital fundus photography, which is rapidly evolving as an integral part of the diagnosis and monitoring of a range of conditions [3]. In adults, retinal vascular calibers have been associated not only with ocular problems like glaucoma [4] and diabetic retinopathy [5], but also with various systemic diseases, including coronary heart disease [6], stroke [7], obesity [8] and mental health [9]. Studies in children are comparatively few, and the available data on this parameter in children has focused mainly on Caucasian and East Asian subjects [10] in Sydney, with the contribution from our region being mainly Singaporean Chinese children [11].

A significant proportion of the population living in South East Asia fall under the category of 'Malay', or 'Austronesian people'[12]. This indigenous group of people have been estimated to number approximately 300 million in South East Asia[13]. However, to the best of our knowledge, apart from a multi-ethnic study by Cheung et al in 2007, no other studies of retinal vessel calibers in children have included this large ethnic group [11, 14]. In children, the general absence of confounding factors related to systemic and ocular disease makes them an ideal study population not only for acquiring normative data, but also in analyzing the relationship between anatomico-physiological factors and retinal vessel calibers [11]. The present study aims to mitigate the aforementioned dearth of data in Malay children by analyzing the factors affecting retinal vessel caliber in healthy Malay children.

3.4 MATERIALS AND METHODS

This was a hospital-based, cross-sectional prospective study involving 86 Malay girls aged 6 to 12 years old, conducted in the Eye Clinic of Hospital Universiti Sains Malaysia between January 2015 and March 2016. The study was approved by the Human Research Ethics Committee USM (HREC] and the conduct of the study followed the tenets of the declaration of Helsinki.

Children who fulfilled the inclusion and exclusion criteria were invited to participate. The children were included into the study if they were aged between 6 and 12 years old at the time of examination, had best corrected visual acuity better than 6/12, had normal anterior and posterior segment findings and could claim to be at least a third generation ethnic Malay. Informed written consent was obtained from at least one parent, as well as verbal assent from the child. After initial recruitment, 86 children were included in this study after exclusion of strabismus, amblyopia, optic nerve abnormalities, high refractive errors (based on spherical equivalent of ±4.0 diopters], history of ocular trauma, ocular pathology, systemic illnesses or developmental delay.

Distance visual acuity measurement was measured monocularly using a Snellen chart for distance (Reichert, NY, USA) at 6 meters. A comprehensive eye examination, including a cover test, assessment of extraocular motility, pupillary examination and slit lamp biomicroscopy was also performed to rule out any associated ocular conditions (eg. strabismus, ocular trauma, previous ocular surgery, glaucoma) which would have precluded participation in the study.

Ocular biometry was performed by a trained technician in the eye clinic. Autorefraction was performed using an autokeratorefractometer (model RK5; Canon, Inc). Three consecutive readings of sphere and cylinder were obtained; a difference between the lowest and highest readings of up to 0.25 diopters was considered acceptable. Spherical equivalent was taken as the value of the sphere plus half of the value of the cylinder. A non-contact partial coherence interferometer (IOL Master, Carl Zeiss Meditec, Inc, Germany) was used to measure the axial length of the right eye. The mean axial length was derived from an average of five consecutive readings, with a signal-to-noise ratio of more than 2 mm, and a difference of up to 0.05 mm between the lowest and highest reading was considered acceptable. Corneal curvature and anterior chamber depth were likewise derived from a mean of five consecutive readings.

After dilation with topical phenylephrine 2.5% and tropicamide 1%, 45 ° digital retinal photographs were taken of the optic disc and macula bilaterally using a digital fundus camera (Model VX-10/KOWA/Japan). Retinal images were then analyzed by a single grader, masked to participant identity and characteristics, using the Singapore I Vessel Algorithm (SIVA 4.0 cloud system) software, which is a partially-automated computed software, in which all retinal vessels greater than 25 um in diameter located between one-half to two disc diameters from the optic disc margin are outlined, their edges marked using a pixel density histogram, and the retinal arteriolar or venular calibers calculated using the Knudtson-Parr-Hubbard formula [15-17]. This formula summarizes the diameters of the six largest arterioles and venules to generate a central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE) respectively. Right eye fundus photographs were

analyzed. In cases where these were ungradable, the images from the left eye were used. The Bengtsson formula was used to correct for ocular magnification on retinal vascular caliber measurement [18, 19].

Height and weight were measured with the patient standing barefoot, according to a standard protocol of height and weight measuring scale (Seca model 220; Seca, Hamburg, Germany). Height was recorded to the nearest 1 mm while weight was recorded to the nearest 0.1 kg. Body mass index (BMI) was calculated as weight divided by the height squared (kg per meter squared).

Using an appropriate-sized cuff (bladder length approximately 80% and width 40% of the arm circumference), blood pressure and heart rate was measured in the sitting position after 5 minutes of rest, using a digital automated sphygmomanometer. Two separate readings were taken, after which the average systolic and diastolic blood pressure was calculated. A third attempt would be added if the difference between the first two readings were greater than 10 mm Hg in systolic blood pressure (SBP) and/or 5 mm Hg in diastolic blood pressure (DBP). The formula used to obtain mean arterial pressure (MAP) was one-third of the systolic blood pressure plus two-thirds of the diastolic blood pressure. A data collection sheet was used to document all relevant parameters.

Statistical analyses were performed using SPSS version 22.0. Simple and multiple linear regression were performed to determine the effect of ocular (eg. axial length, intraocular pressure) and systemic factors (eg. body mass index, blood pressure) on retinal arteriolar and venular calibers.

3.5 RESULTS

A total of 86 children were included in this study. The mean age was 9.44 years. Other systemic demographics are summarized in Table 1. The mean refractive error in our study sample was -0.47. After correcting for ocular magnification, the mean CRAE and CRVE were 171.40 um and 248.02 um respectively. Other ocular biometric parameters are listed in Table 2.

Among the variables affecting retinal arteriolar caliber, blood pressure (SBP, DBP, MAP) and axial length were the main factors affecting the CRAE, as shown in Table 3. After multivariable adjustment, each unit (1 mm Hg) increase in mean arterial pressure was associated with a 0.451 um reduction in CRAE (p=0.013), while each 1 mm increase in axial length was associated with a 4.582 um narrowing of retinal arteriolar caliber (p=0.024). With regard to retinal venular caliber, Table 4 shows that all variables analyzed had no statistically significant effect on the venular caliber.

3.6 DISCUSSION

Malaysia is a nation with among the highest proportion of Malays, who comprise almost 70% of the population [20]. Although Malays comprise the main ethnic group in South East Asia, the data on retinal vessel parameters in Malays, whom the anthropologist Blumenbach described as “tawny-coloured, with black hair”, is limited, especially in Malay children [21]. We present new data of retinal vessel calibers using SIVA cloud 4.0 in primary school children of Malay ethnicity.

The mean CRAE of 171.4 um in our study closely parallel the results of Rochtchina et al., who studied ethnic differences in retinal vessel caliber and observed that the greatest difference in CRAE was seen between Caucasian children, with a mean CRAE of 160.5 um, and East Asian children, with a mean of 171.5 um [10] (Table 5). Likewise, our mean CRVE, which is larger than that seen in studies of predominantly Caucasian and Chinese children[11, 22, 23], most closely mirrors the findings among the East Asian children in the study by Rochtchina [10]. Ethnicity and the degree of iris and retinal pigmentation have been shown to influence the measurements of retinal vascular caliber in children, with Cheung et al. demonstrating that Malays have wider retinal vessels than Chinese [10, 11]. It has to be noted, however, that the mean CRAE and CRVE among Malay children in that study are also narrower than our results; these differences may possibly be due to differences in ocular magnification which were not accounted.

We demonstrated that blood pressure was significantly associated with a reduction of CRAE, but not CRVE, in primary school children. Our results concur with those of Mitchell et al., who evaluated children aged 7 to 9 years old in the Singapore Cohort Study of the Risk Factors for Myopia (SCORM); Gopinath et al., who evaluated 12-year-old children in the Sydney Myopia Study; and Zheng et al., who studied children aged 7 to 19 years old in the Guangzhou Twin Eye Study [22, 24, 25]. Conversely, Gishti et al and Li et al demonstrated that increased blood pressure is associated with both narrower retinal arterioles and wider retinal venules in younger children [26, 27]. The significance of higher blood pressure being associated with greater narrowing of CRAE even in non-hypertensive children is that narrowed retinal arterioles are not only associated with atherosclerotic changes [28], but this narrowing has also been found to precede the development of clinical hypertension [29-32]. Whether this CRAE narrowing is a transient autoregulatory phenomenon in response to the elevated blood pressure is uncertain. However, it is likely that the pathological changes which occur in chronic hypertension may have their roots in the normal physiological response of retinal arterioles to blood pressure changes.

Our study found no statistically significant effect of body mass index on the retinal venular caliber. This finding parallels that of Xiao et al, who found that among children aged less than 12 years old, there was no significant association of body mass index and retinal vessel caliber [23]. Although we are aware that other research has shown a positive association between body mass index and retinal venule calibers [14, 33-37], ethnic-related differences in body fat composition may apply in our population. As body mass index is merely a proxy measure or obesity, body components like body fat mass are increasingly being used as quantitative measures

of obesity [38]. Body fat has been shown to differ between ethnicities, with Malays having a relatively high percentage of body fat despite a low body mass index [39]. A recent analysis of the association between body composition parameters and retinal vessel calibers in the Guangzhou Twin Eye Study found significant associations in adolescents, but not in children [23]. This may be related to the wide variation in fatness for a given body mass index value in children, which is attributed to variations in fat mass and fat free mass as the child grows [40].

We observed that axial length was significantly associated with retinal arteriolar caliber, but not venular caliber, after correction for ocular magnification. This is consistent with the results of Li et al, who likewise corrected for ocular magnification [41], and Gopinath et al, who evaluated children with no significant refractive error, in whom the impact of ocular magnification was deemed insignificant [42]. On the contrary, Cheung et al found that after adjusting for ocular magnification, the association between longer axial length and narrower retinal vessels disappeared [43]. As refractive errors may affect the measured dimensions of retinal vessels on fundus photography due to differences in ocular magnification, it is important to correct for this magnification difference. It has been postulated that a longer axial length is associated with a thinner ocular wall (possibly due to elongation during growth of eyeball] and likewise, stretched, attenuated retinal vessels [44]. The association of increased axial length with narrowed retinal arterioles may explain the reduced efficiency of the circulation in myopic eyes and indirectly, be related to the pathophysiologic changes seen in this condition [45-48].

The effect of intraocular pressure on retinal vessel calibers was not statistically significant. Our results concur with de Haseth et al's study involving 386 Chinese children in the Singapore Cohort Study of the Risk factors for Myopia [49]. No other studies have been conducted to evaluate the effect of intraocular pressure on retinal vessel calibers in children. In adults, dilation of retinal vessels has been noted in response to an acute rise in intraocular pressure; however, autoregulatory constriction of these vessels to baseline calibers occurs as the intraocular pressure normalizes, and has been shown to be accelerated by medical therapy [50]. Conversely, adult eyes with normal-tension glaucoma have been noted to have a narrower CRAE and CRVE than normal eyes, suggesting that the narrower retinal vessels seen in this condition may not be a direct consequence of intraocular pressure, but rather, be a result of the reduced perfusion drive in damaged retinas [51, 52].

Few limitations of our study must be acknowledged. Firstly, available methods of adjusting for ocular and camera magnification may still not encompass the full scope of the variation in measured vessel caliber secondary to magnification error. Secondly, retinal vessel calibers are not static; as they vary with the cardiac cycle and can thus be affected by changes in the autonomic nervous system, measurements obtained from a single retinal image may have errors due to this variability [53]. Finally, due to financial constraints, we were unable to analyze the role of genetics, which has been found to account for a large proportion of the variance in retinal arteriolar and venular calibers (70% and 83% respectively) [54-57].

We provide this data as a baseline for Malay children aged six to twelve years old. Our study in healthy primary school children suggests that the pathophysiologic changes underlying the retinal arteriolar narrowing characteristic of hypertensive patients may actually begin in early childhood. Measurement of retinal arteriolar caliber in children is a simple, non-invasive method of assessing the body's microvasculature, and may give early clues of future microcirculatory disease. However, vessel calibers are only part of the spectrum of retinal vascular research, and further studies are required to explore the other retinal vessel parameters of clinical relevance.

CONCLUSION

The mean retinal CRAE and CRVE in Malay children were 171.40 um and 248.02 um. Retinal arteriolar calibers are narrower in healthy children with higher blood pressure and longer axial lengths, independent of other factors. These associations suggest that the microcirculatory compromise occurring in pathological increases of these parameters may have its origin in the normal physiological response of blood vessels to various systemic and ocular dynamics.

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