RETINAL VESSEL DENSITY MEASUREMENT AS A TOOL FOR MICROVASCULAR RESEARCH

BY

FADILAH BINTI AB HAMID

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Kulliyyah of Allied Health Sciences
International Islamic University Malaysia

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ABSTRACT

This study aimed to evaluate the vessel density parameter as another measurement to study the morphological changes of retinal vessel. This study assessed the reliability of the vessel density parameter; provided the empirical evidence of fractal dimension (D_F) as an indirect measure of the vessel density; investigated the effect of aging on the parameters and conducted a preliminary analysis of the ability of the parameters to distinguish between normal and diseased retinal states. A total of 200 coloured right eye fundus images were analyzed to obtain two quantitative parameters which were vessel density and D_F. The coloured fundus images were transformed into black and white using MyVessel software. A circular area of approximately 2.6 optic disc radii surrounding the center of the optic disc was cropped using GNU Image Manipulation Program (GIMP) version 2.6.12 and the non-vessel fragments were deleted using the same software. The values of retinal vessel density and D_F were obtained from the FracLac software version 2.5. The findings showed that the intragrader reliability of vessel density and D_F as assessed using intraclass correlation coefficient (ICC) were 0.9975 (95% CI = 0.9954 - 0.9987) and 0.9645 (95% CI = 0.9340 - 0.9811), respectively. For intergrader reliability, the ICC were 0.9910 (95% CI = 0.9831 - 0.9952) and 0.9725 (95% CI = 0.9484 - 0.9854) for vessel density and D_F, respectively. A strong correlation between vessel density and D_F parameters was detected with Pearson's correlation coefficient, R of 0.942 ($p<0.001$). In response to increase in age, both vessel density and D_F parameters showed declining trend, suggesting rarefaction of the retinal vessel. Preliminary analysis on the sensitivity of vessel density and D_F parameters to differentiate between normal and diseased retinal states was obtained from the area under the receiver operating characteristic curve (AUC). The AUC of vessel density and D_F were 0.91 ($p<0.001$) and 0.89 ($p<0.001$), respectively. This study demonstrated the ability of the vessel density parameter to describe the difference between younger and older age groups. The vessel density parameter also showed high association with currently used geometrical parameter of retinal vessel, D_F. Therefore, vessel density parameter may be considered as another parameter for quantitative analysis of retinal vessel.

خلاصة البحث

تهدف هذه الدراسة إلى تقييم مؤشرات كثافةِ الأوعيةِ كمقياسٍ آخرَ لدراسة التغيرات المورفولوجية في أوعية شبكيات العيون. قيمت هذه الدراسة صلاحيةَ مؤشرِ كثافةِ الأوعية؛ مع مراعاة الأدلةِ التجريبية للبعد الكُسُوري (D_F) كمقياسٍ غير مباشرٍ لكثافةِ الأوعية؛ وحققت في تأثير التشيُّخ على المؤشرات، وأجرت تحليلات أوليةً لمعرفة قدرة المؤشرات على التمييز بين الشبكيات السليمة والمصابة. تم تحليلُ 200 صورةٍ مختلفةٍ لقاعة عين اليمنى للحصول على مؤشراتٍ كميةٍ لكثافة الأوعية والبعد الكُسُوري. تم تحويل الصورِ الملونة لقيعانِ العيون الملونةِ إلى الأبيضِ والأسود باستخدام برنامج MyVessel. تم اقتصاصُ منطقةٍ دائريةٍ وسعها حوالي 2.6 نصف قطرٍ بصري حول مركز القرص البصري باستخدام برنامج معالجة الصور (GNU Image Manipulator Program - GIMP) نسخة 2.6.12 ، وتم حذف الشظايا الدخيلة باستخدام نفس البرنامج. تم الحصول على قِيمِ كثافةِ الأوعية الشبكية والبعد الكُسُوري من برنامج FracLac نسخة 2.5. أظهرت النتائج أن صلاحية ما هو داخلَ المُقَيّمات (intergrader) لكثافة الأوعية والبعد الكُسُوري، كما قُيِّمَت باستخدام مُعامِل ارتباط ما هو داخل الطبقات (interclass correlation coefficient, ICC)، وكانت النتائج كما يلي: 0.9975 (95% CI =0.9954 – 0.9987) لكثافة الأوعية و 0.9645 (95% CI = 0.9340 – 0.9811) للبعد الكُسُوري. أما لصلاحية ما هو داخل المُقَيّمات (intergrader) فكان الICC. 0.9910 (95% CI 0.9831 – 0.9952) لكثافة الأوعية و 0.9725 (95% CI 0.9484 – 0.9854) للبعد الكُسُوري. تم الكشف عن علاقةٍ قوية بين مؤشراتِ كثافة الأوعية و البعد الكُسُوري مع معامل ارتباط بيرسون، R من 0.942 (p<0.001). استجابةً لزيادةِ العمر، أظهرت كلٌّ من مؤشراتِ كثافة الأوعية والبعد الكُسُوري نمطاً تراجعياً، مما أشار إلى خلخلةِ الأوعية في شبكية العين. تم الحصول على التحليل الأولي لحساسية مؤشرات كثافة الأوعية والبعد الكُسُوري للتمييزِ بين الشبكيات السليمة والمصابة من المنطقة التى تحتَ منحنى التشغيلِ المُميِّزِ المستقبِل (AUC). كان AUC لكثافةِ الأوعية حوالي 0.91 (p<0.001) و 0.89 (p<0.001) للبعد الكُسُوري. أظهرت هذه الدراسة قدرةَ مؤشرِ كثافة الأوعية في وصف الفرقِ بين الفئات العمرية الأصغر والأكبر سناً. أظهرَ مؤشر كثافة الأوعية أيضاً ارتباطاً ملحوظاً مع المؤشِّر الهندسي المستخدَم حالياً مع أوعية الشبكية والبعد الكُسُوري. بناءً على ذلك، يمكن اعتبارُ مؤشرِ كثافة الأوعية كمؤشرٍ آخرَ للتحليل الكمي لأوعية شبكية العين.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences (Optometry).

…………………………………..
Mohd. Zulfaezal Bin Che Azemin
Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences (Optometry).

…………………………………..
Firdaus Bin Yusof @ Alias
Internal Examiner

…………………………………..
Lili Asma Binti Ismail
External Examiner

This thesis was submitted to the Department of Optometry and Visual Science and is accepted as a fulfilment of the requirement for the degree of Master of Health Sciences (Optometry).

…………………………………..
Noor Ezailina Binti Badarudin
Head, Department of Optometry and Visual Science

This thesis was submitted to the Kulliyyah of Allied Health Sciences and is accepted as a fulfilment of the requirement for the degree of Master of Health Sciences (Optometry).

…………………………………..
Wan Azdie Bin Mohd. Abu Bakar
Dean, Kulliyyah of Allied Health Sciences

DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Fadilah Binti Ab Hamid

Signature .... Date ....

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Thank you Allah, for His Blessings and Mercies on me for granting the completion of this thesis.

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LIST OF ABBREVIATIONS

a.	Artery
AMD	Age-related macular degeneration
ARIC	Atherosclerosis Risk in Communities
AUC	Area Under the ROC Curve
AV	Arteriovenous
AVR	Arteriovenous ratio
BMES	Blue Mountain Eye Study
BDES	Beaver Dam Eye Study
BH4	Tetrahydrobiopterin
BMI	Body mass index
CI	Confidence interval
CRAE	Central retinal artery equivalent
CRVE	Central retinal vein equivalent
d.f.	Degree of freedom
D	Diopter
D_F	Fractal dimension
DRIVE	Digital Retinal Images for Vessel Extraction
e.g.	(*exempli gratia*); for example
et al.	(*et alia*); and others
eNOS	Endothelial nitric oxide synthase
ETDRS	Early Treatment Diabetic Retinopathy Study
FAZ	Foveal avascular zone
g	Gram
GIMP	Gnu Image Manipulation Program
HR	Hazard ratio
i.e.	(*id est*); that is
ICC	Intraclass Correlation Coefficient
ID	Identification
IIUM	International Islamic University Malaysia
IREC	IIUM Research Ethics Committee
IRMA	Intraretinal microvascular abnormalities
L	Side length
LDR	Length to diameter ratio
mm	Milimeter
MABP	Mean arterial blood pressure
MESA	Multi-Ethnic Study of Atherosclerosis
N/A	Not applicable
NIH	National Institutes of Health
NO	Nitric oxide
NTG	Normotensive glaucoma
OR	Odds ratio
PDGF	Platelet-derived growth factor
PIA	Perifoveal intercapillary area
Post.	Posterior
ROC	Receiver Operating Characteristic

ROI	Region of interest
SD	Standard deviation
SIVA	Singapore Eye Vessel Assessment
SLM	Shape Language Modeling
SPSS	Statistical Package for the Social Science
STARE	STructured Analysis of the Retina
TGF-β1	Transforming growth factor-β1
TV	Television
v.	Vein
vs.	Versus
VEGF	Vascular endothelial growth factor
W_a	Calibre of the smaller branch vessel
W_b	Calibre of the larger branch vessel
Wc	Calibre of the trunk vessel
w_1	Width of the narrower branch
w_2	Width of the wider branch
W	Width of the parent trunk
$\hat{W}$	Arteriole or venule parent trunk estimation
WESDR	Wisconsin Epidemiologic Study of Diabetic Retinopathy

LIST OF SYMBOLS

β	Beta
κ	Kappa
n	Sample size
p	Probability
R	Pearson product moment correlation
R^2	Coefficient of determination
µm	Micrometer
°	Degree
%	Percent
-	To
>	More than
<	Less than
≥	Greater than or equal to
≤	Less than or equal to
±	Plus-minus
x	Times
=	Equal to
*	Statistical significance denotation

CHAPTER ONE

INTRODUCTION

Investigating retinal images using a computer analysis method is both of scientific and clinical importance as understanding the retinal vascular network would be useful in the detection of several systemic diseases and improving specific treatments of retinal disorders.

1.1 BACKGROUND OF THE STUDY

Image analysis has widely been used in medical field to aid in the diagnosis of medical images and improving clinician's confidence in the analysis of retinal images (Abràmoff, Garvin, & Sonka, 2010; Sharma, Sample, Zangwill, & Schuman, 2008; Patton, Aslam, Macgillivray, Deary, Dhillon, Eikelboom, Yogesan, & Constable, 2006). Recently, studies have shown a link between various retinal microvascular signs and both clinical and subclinical cerebrovascular (Kawasaki, Azemin, Kumar, Tan, Liew, Wong, Mitchell, & Wang, 2011; Ikram, de Jong, Bos, Vingerling, Hofman, Koudstaal, de Jong, & Breteler, 2006), cardiovascular (Wang, Liew, Wong, Smith, Klein, Leeder, & Mitchell, 2006; Witt, Wong, Hughes, Chaturvedi, Klein, Evans, McNamara, Thom, & Klein, 2006; Wong, Klein, Sharrett, Duncan, Couper, Tielsch, Klein, & Hubbard, 2002a) and metabolic outcomes (Grauslund, Hodgson, Kawasaki, Green, Sjølie, & Wong, 2009; Ikram, Janssen, Roos, Rietveld, Witteman, Breteler, Hofman, van Duijn, & de Jong, 2006; Witt et al., 2006).

Human retina has a unique property due to the direct accessibility of the circulatory system *in vivo*. Thus, offers an opportunity for researchers to study the structural features of the microcirculation. Advances in the retinal imaging technology enable the image to be photographed, visualized and accessed at any time, without any need for mydriatic agent. The process of capturing the retinal image is easy and requires short time. Despite limited viewing of retinal blood vessel through the subjective clinical assessment, the revolution of digital imaging systems enables the retinal photographs to be captured with a larger field of view. Furthermore, the vascular network in terms of its branching, tortuosity as well as the abnormalities can be easily documented.

Several review papers have reported that the alteration in the retinal microvasculature possibly reflects similar changes in the cerebral and the coronary microvasculature due to the similar anatomical and physiological characteristics shared by these microcirculation systems (MacGillivray, Trucco, Cameron, Dhillon, Houston, & Van Beek, 2014; Cheung, Ikram, Sabanayagam, & Wong, 2012b; Ikram, Cheung, Wong, & Chen, 2012; Patton, Aslam, Macgillivray, Pattie, Deary, & Dhillon, 2005). Therefore, the findings from retinal vessel analysis may provide additional insight into early microvascular abnormalities before the appearance of clinical disease. Retinal arteries and veins represent the complexity of the retinal vessel branching system. Various geometric parameters have been employed by researchers in microvessel analysis, including vascular calibre, arteriovenous ratio, bifurcation angle, tortuosity, and fractal dimension.

Cheung, Tay, Mitchell, Wang, Hsu, Lee, Lau, Zhu, Klein, Saw, & Wong (2011a) reported that the quantitative assessment of retinal vascular structure is a precise and reliable method in investigating the relationship between microcirculation and cardiovascular disease. Recent studies focused on the geometrical analysis of retinal vascular branching pattern known as fractal dimension (D_F).

1.2 RESEARCH OBJECTIVES

This study was aimed to achieve the following objectives:

1.2.1 General Objective

The goal of this study was to examine the vessel density parameter as another measurement to study the morphological changes of retinal vessel.

1.2.2 Specific Objectives

i. To assess the intragrader and intergrader reliability of computer-assisted measurement of retinal vessel, as derived from computed vessel density and D_F.

ii. To provide the empirical evidence of D_F as an indirect measure of retinal vasculature density.

iii. To investigate the mathematical model of vessel density and D_F, their trend and rate of changes in response to increase in age.

iv. To conduct a preliminary analysis on the ability of vessel density and D_F parameters to distinguish between normal and pathological retinal condition.

1.3 RESEARCH QUESTIONS

i. How reliable are the results from the quantification of the retinal vessel density and D_F measurements using computer-assisted method?

ii. How does the vessel density measurement correlate with the D_F measurement of retinal vessel?

iii. Does the correlation between vessel density and D_F measurements will be affected when a different region of interest was used for retinal vessel analysis?

iv. How does the vessel density measurement of retinal vessel structure affected when tested for the increase in age?

v. How sensitive are the vessel density and D_F measurements to differentiate between normal and diseased retinal condition?

1.4 RATIONALE AND HYPOTHESES OF THE STUDY

Fractal dimension (D_F) estimation employed a box-counting method which incorporates four different grids orientation in the computation. The illustration of the different orientation is depicted in Figure 1.1.

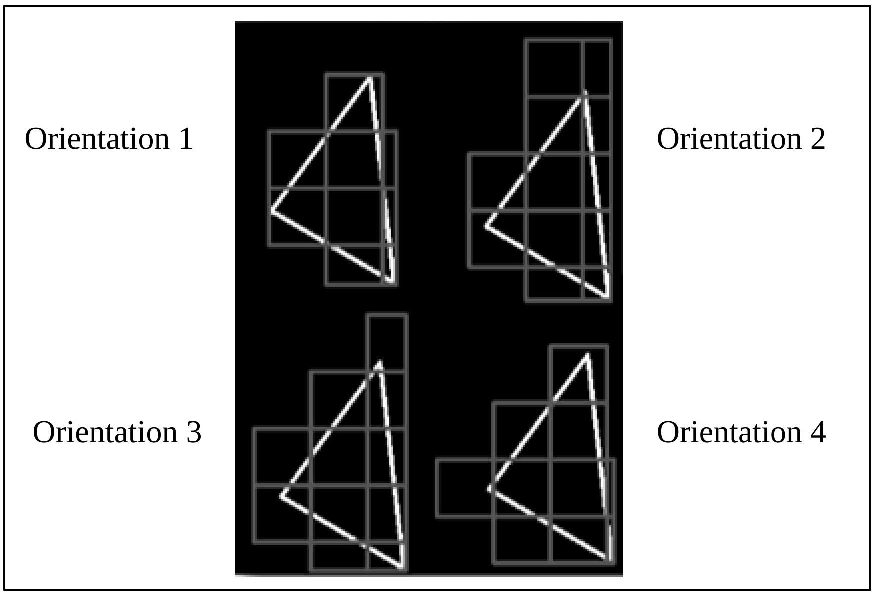

Figure 1.1 Different orientations of grid positions with respect to an image in a box-counting method. Source: Karperien (2013).

Many studies focused on the D_F to estimate the morphological changes in retinal vascular structure (Azemin, Kant, Wong, Jin, Mitchell, Kawasaki, & Wu, 2012; Li, Mitchell, Liew, Rochtchina, Kifley, Wong, Hsu, Lee, Zhang, & Wang, 2010; Liew, Wang, Cheung, Zhang, Hsu, Lee, Mitchell, Tikellis, Taylor, & Wong, 2008a; Masters, 2004; Landini, Misson, & Murray, 1993). These studies gave inconsistent results of D_F, possibly due to different methodologies used by different investigators. The D_F measurement is also dimensionless (Liew et al., 2008a) which makes it hard to do a comparative analysis on the subject. D_F measurement can take a value for instance of 1.4858. However, there is no direct physical meaning that can be interpreted by merely looking at one single D_F value. Therefore, the main goal of this study was to examine vessel density parameter which may potentially serve as a tool to study the morphology of human retinal vessel. The vessel density parameter is also dimensionless, however, the parameter is able to produce more explicit results when using a single datum as compared to the retinal vessel complexity measurement using D_F.

Previous studies on D_F were more on the elderly population aged 49 years old and older (Azemin et al., 2012; Li et al., 2010; Liew et al., 2008a). A study conducted by Landini et al. (1993) included data from a younger age group, ranging from 14 to 73 years old, however the number of subjects were very limited, only 23 subjects' data was used for the large age spectrum leading to low variations among samples. Moreover, Landini et al. (1993) hand-traced the retinal vessel and the method of image acquisition was invasive (Avakian, Kalina, Helene Sage, Rambhia, Elliot, Chuang, Clark, Hwang, & Parsons-Wingerter, 2002; Landini et al., 1993; Remky, Arend, & Hendricks, 2000). In the study with invasive approach, fluorescein dye was used.