Optical Coherence Tomography Angiography Biomarkers in Diabetic Nephropathy Patients in Thailand: A Diabetic Eye and Kidney Diseases (DEK-D) Study

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ลักษณะทางชีวภาพจากเครื่องตรวจภาพตัดขวางหลอดเลือดของจอประสาทตาในคนไข้โรค ไตจากเบาหวานในประเทศไทย

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นันทชัย สุรวัติเสถียร : ลักษณะทางชีวภาพจากเครื่องตรวจภาพตัดขวางหลอดเลือดของจอประสาทตาในคนไข้โรค ไตจากเบาหวานในประเทศไทย. (Optical Coherence Tomography Angiography Biomarkers in Diabetic Nephropathy Patients in Thailand: A Diabetic Eye and Kidney Diseases (DEK-D) Study) อ.ที่ปรึกษาหลัก : รศ. ดร.นพ.ณัฐชัย ศรีสวัสดิ์, อ.ที่ปรึกษาร่วม : ผศ. พญ.แพร์ พงศาเจริญนนท์ เฟอร์เรร่าห์

โรคเบาหวานสามารถทำให้เกิดภาวะแทรกซ้อนของหลอดเลือดขนาดเล็กได้ในหลายอวัยวะในร่างกาย รวมไปถึงภาวะเบาหวานขึ้นจอตา (diabetic retinopathy – DR) และภาวะไตเสื่อมจากโรคเบาหวาน (diabetic nephropathy – DN) มีการประมาณการว่ากว่า 1 ใน 3 ของผู้ป่วยเบาหวานนั้นสามารถตรวจพบภาวะเบาหวานขึ้นจอตา และกว่า 40% ของผู้ป่วยเบาหวานมีภาวะไตเสื่อมจากโรคเบาหวานร่วมด้วย โดยสองภาวะนี้มีพยาธิสภาพที่บริเวณหลอดเลือดฝอยคล้ายคลึงกันในจอประสาทตา และในไต เครื่องตรวจภาพตัดขวางหลอดเลือดของจอประสาทตา (optical coherence tomography angiography – OCTA) นั้นถูกพัฒนาขึ้นเพื่อทำให้การตรวจหลอดเลือดฝอยบริเวณจอประสาทได้อย่างละเอียด โดยไม่ต้องฉีดสีเข้าหลอดเลือด การศึกษานี้จึงมีขึ้นเพื่อศึกษาลักษณะทางชีวภาพของหลอดเลือดจอประสาทตาจากเครื่อง OCTA ที่มีความสัมพันธ์กับปริมาณโปรตีนที่รั่วในปัสสาวะ 24 ชั่วโมงจากภาวะไตเสื่อมจากโรคเบาหวาน การศึกษานี้เป็นการศึกษาเชิงสังเกตแบบตัดขวาง โดยศึกษาลักษณะต่างๆที่ได้จากการถ่ายภาพจอประสาทตาด้วยเครื่อง OCTA ในตา 186 ดวงจากอาสาสมัคร 93 คน โดยแบ่งเป็น 3 กลุ่มตามปริมาณโปรตีนรั่วในปัสสาวะ 24 ชั่วโมง เป็น กลุ่มไม่มีภาวะไตเสื่อมจากโรคเบาหวาน (no DN) กลุ่มภาวะไตเสื่อมจากโรคเบาหวานระยะต้น (early DN) และกลุ่มภาวะไตเสื่อมจากโรคเบาหวานระยะท้าย (late DN) โดยลักษณะที่ศึกษาประกอบด้วย ความหนาแน่นของเส้นเลือด (vessel density – VD) มิติเศษส่วนของเส้นเลือด (fractal dimension – FD) พื้นที่ปราศจากหลอดเลือดบริเวณจุดรับภาพชัด (foveal avascular zone – FAZ) พื้นที่ระหว่างหลอดเลือดฝอย (intercapillary area) ความหนาของจอประสาทตาบริเวณจุดรับภาพชัด (central retinal thickness – CRT) และความหนาของชั้นคอรอยด์ใต้จุดรับภาพชัด (subfoveal choroidal thickness – subfoveal CT) จากผลการศึกษาพบว่า ค่า VD ของเส้นเลือดฝอยในจอประสาทตาชั้นตื้น (superficial capillary plexus – SCP) เส้นเลือดฝอยในจอประสาทตาชั้นลึก (deep capillary plexus – DCP) และเส้นเลือดฝอยจอประสาทตาทั้งหมด (whole retina) ในกลุ่ม early DN มีค่าต่ำกว่ากลุ่ม no DN อย่างมีนัยสำคัญทางสถิติ (adjusted p-value 0.007, 0.003 และ 0.003 ตามลำดับ) และค่า VD ของ DCP และ whole retina ในกลุ่ม late DN มีค่าต่ำกว่ากลุ่ม no DN อย่างมีนัยสำคัญทางสถิติเช่นเดียวกัน (adjusted p-value 0.032 และ 0.021 ตามลำดับ) ส่วนค่า FD ค่า intercapillary area พื้นที่ FAZ ค่า CRT และค่า subfoveal CT ไม่พบความแตกต่างกันอย่างมีนัยสำคัญทางสถิติระหว่างอาสาสมัคร 3 กลุ่ม จากการศึกษานี้สรุปได้ว่าค่า VD จากเครื่อง OCTA นั้นอาจมีประโยชน์ในการตรวจคัดกรองภาวะไตเสื่อมจากโรคเบาหวาน การศึกษาเพิ่มเติมในประชากรกลุ่มใหญ่และหลากหลายขึ้น อาจมีประโยชน์ในการพัฒนาเครื่องมือการตรวจคัดกรองต่อไป

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KEYWORD: diabetic nephropathy, diabetic retinopathy, vessel density, foveal avascular zone, fractal dimension, urine albumin, optical coherence tomography angiography, diabetes mellitus

Nuntachai Surawatsatien : Optical Coherence Tomography Angiography Biomarkers in Diabetic Nephropathy Patients in Thailand: A Diabetic Eye and Kidney Diseases (DEK-D) Study. Advisor: Assoc. Prof. NATTACHAI SRISAWAT, M.D., Ph.D. Co-advisor: Asst. Prof. PEAR PONGSACHAREONNONT FERREIRA, M.D.

Diabetes mellitus (DM) can cause many microvascular complications including diabetic retinopathy (DR) and diabetic nephropathy (DN). It is estamated that about one-third of diabetic patients have DR and about 40% have DN. DR and DN share a common pathology in microvasculature. With the development of optical coherence tomography angiography (OCTA), the examination of retinal capillary is more convenient and less invasive. This is a cross-sectional observational study aimed to identify OCTA parameters as biomarkers that predict the diabetic nephropathy and association with 24-hour urine albumin level in diabetic patients. 186 eyes from 93 individuals were divided into 3 groups according to 24-hour urine albumin level: no DN, early DN, and late DN. Vessel density (VD), fractal dimension (FD), foveal avascular zone (FAZ) area, intercapillary area, central retinal thickness (CRT), subfoveal choroidal thickness (CT) were measured from OCTA images to determine the association between DN status. VD values of superficial capillary plexus (SCP), deep capillary plexus (DCP), and whole retina were significantly lower in early DN group compared to no DN group (adjusted p-value 0.007, 0.003, and 0.003, respectively). VD values of DCP and whole retina were significantly decreased in late DN group compared to no DN group (adjusted p-value 0.032 and 0.021, respectively). Mean FD, intercapillary area, FAZ area, CRT, and subfoveal CT were not statistically different between 3 groups. VD may be a useful tool for non-invasive screening of DN. Further studies in larger population are needed to establish a cutoff value for detection.

Field of Study:	Clinical Sciences	Student's Signature .....
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Nuntachai Surawatsatien

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CHAPTER I Introduction

Background and rationale

Diabetes mellitus (DM) is one of the non-communicable diseases (NCDs) which has many end-organ damages. It is estimated that there were 563 million adults living with diabetes and expected to reach 783 million by 2045.[1] Approximately one-third of diabetic patients have diabetic retinopathy which is diagnosed by dilated fundus examination.[1] Moreover, about 40% of people with diabetes will develop chronic kidney disease (CKD) which is diagnosed with urinalysis or blood chemistry as a result of functional change of kidney.[1]

In Thailand, there were 1,360,955 chronic kidney disease patients receiving hospital care in 2019.[2] Furthermore, 151,343 of these patients required renal replacement therapy which includes hemodialysis, peritoneal dialysis and renal transplantation. Diabetic nephropathy was the leading cause of renal replacement therapy which was accounted for 44% of patients.[3] Overall cost of care for chronic kidney disease patients in Thailand was 9.4 billion baht in 2020 and increased to 9.7 billion baht in 2021.[4]

Both diabetic retinopathy (DR) and diabetic nephropathy (DN) share the common pathology, that is a microvasculopathy. The retinal and renal circulations share some similar anatomical, physiological, and pathological features.[5] Hence, retinal microvascular abnormalities may be associated with renal vascular pathology and the development of CKD. Furthermore, anatomical changes can be detected earlier than functional changes and might offer the opportunity to prevent further damage to the kidney[6]. But there are a few gaps in current knowledge. The evidence of retinal lesions that associated with deteriorating kidney function is remain controversy.[7]

With the recent development of optical coherence tomography angiography (OCTA), retinal capillaries can be examined thoroughly without the need of an invasive injection of fluorescein or indocyanine green dye. OCTA also offers numerous possibilities for disease screening and detection. Retinal and choroidal vascular pathology detected by OCTA might be an early sign of anatomical changes

in diabetic nephropathy. These changes may precede functional changes detected by blood or urine chemistry, such as blood urea nitrogen (BUN), serum creatinine (SCr) and urine albumin creatinine ratio (ACR).[7] Earlier detection of diabetic nephropathy can lead to earlier treatment and prevention of the progression of the disease and therefore saves lives, decreases the burden of the disease for both patients and relatives and eventually reduces the costs of the healthcare system.

Research questions

Primary research question

- Which retinal vascular pathology detected by OCTA are associated with diabetic nephropathy?

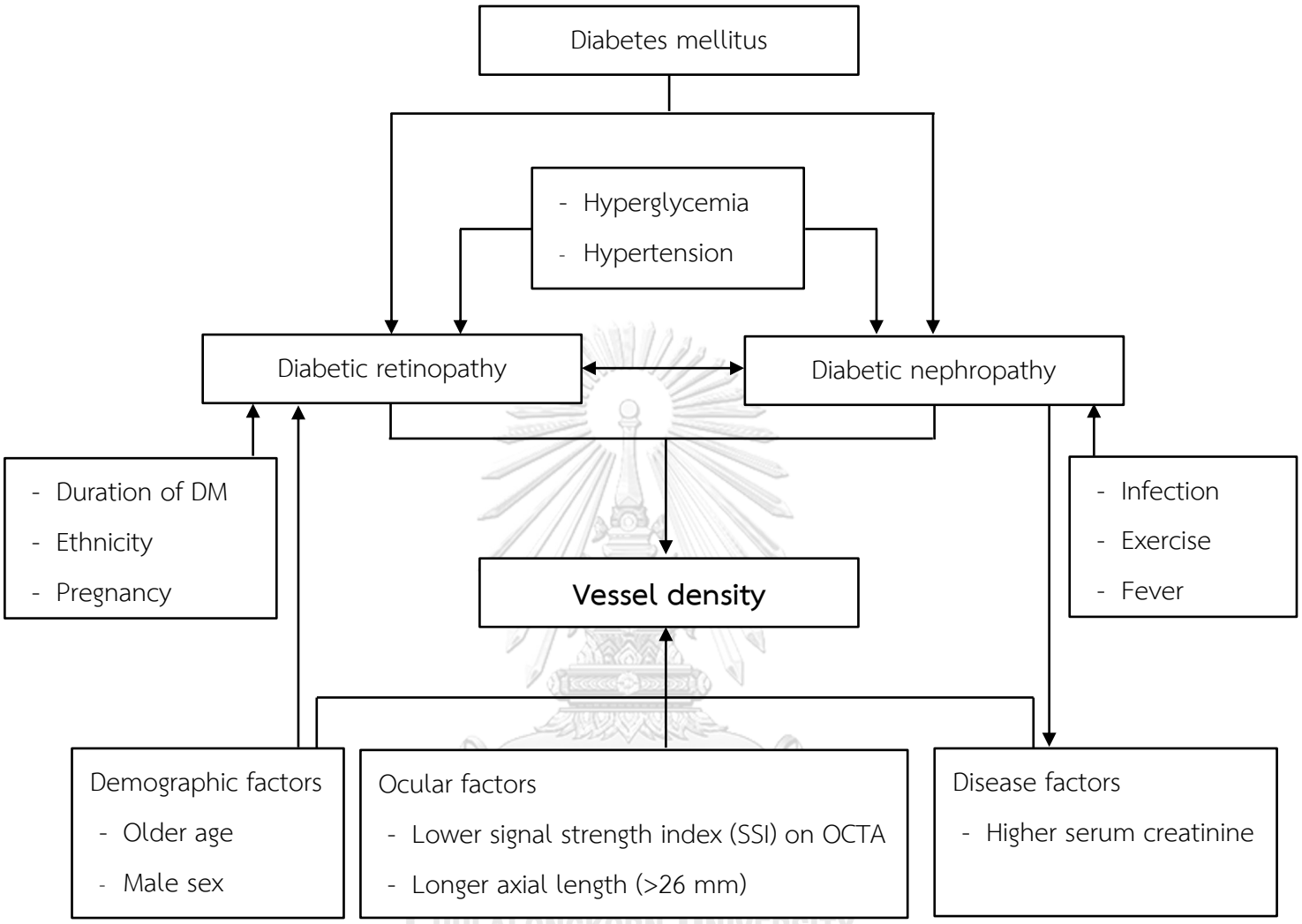
Secondary research question

- Does the degree of visual function changes (visual acuity (VA)) and retinal anatomical changes, (DR severity, and central retinal thickness (CRT)) correlate with the degree of renal function changes?

Objectives

1. To identify OCTA characteristics associated with diabetic nephropathy
2. To find correlation between renal function (urine albumin level) and VA, DR severity, and CRT

Conceptual framework



Keywords

diabetic retinopathy, diabetic nephropathy, optical coherence tomography angiography, OCTA, microalbuminuria, macroalbuminuria, vessel density

Research design

Cross-sectional observational study

Expected benefits and applications

The expected benefit of this study can be divided into participants' benefit and the healthcare system's benefit. The former includes special screening for diabetic retinopathy and other retinal vascular diseases by the OCTA machine. If any risks or conditions were detected, participants will be offered a treatment and follow-up by an ophthalmologist. Moreover, the benefit to the healthcare system is the potential biomarker for quick, non-invasive diabetic nephropathy screening program which could be used in telemedicine. In this artificial intelligence era, the result and data from this study could be a part of biomarker or learning database for machine learning algorithm. Lastly, a prediction model for diabetic nephropathy based on visual function, DR severity and OCTA characteristics could be originated from this study.

Chapter II Literature Review

Diabetic retinopathy (DR)

Diabetic retinopathy (DR) is one of the leading causes of preventable blindness worldwide. It is estimated that about 30-40% of diabetic patients develop DR, making it the most common diabetic complication. And about one-third of DR patients have vision threatening retinopathy. There are several risk factors for developing DR such as ethnicity, genetic, puberty, pregnancy, hyperglycemia, hypertension, dyslipidemia, and cataract surgery. But the most important risk factor is duration of diabetes.[1, 8]

Originally, Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale was developed based on modified Airlie House classification. It is a gold standard in DR severity grading based on fundus photograph. Due to detailed scoring system and impracticality, it is mainly used in research settings. Until in 2002, the Global Diabetic Retinopathy Group proposed a new simplified and practical grading system called "International Clinical Diabetic Retinopathy and Diabetic Macular Edema Disease Severity Scales" which is still mainly used in clinical settings worldwide until present. The DR stages comprised of no DR, mild non-proliferative diabetic retinopathy (mild NPDR), moderate non-proliferative diabetic retinopathy (moderate NPDR), severe non-proliferative diabetic retinopathy (severe NPDR), and proliferative diabetic retinopathy (PDR).[9] The detail and findings of each stage will be discussed later in the method section. The DR staging system is used for diagnosis, investigation, initiation of treatment, and selecting the duration of follow-up.[8]

Diabetic nephropathy (DN)

Chronic kidney disease (CKD) occurs in about 40% in diabetic patients. It is defined by persistent albuminuria (>30 mg/g creatinine), low estimated glomerular filtration rate (eGFR <60 ml/min/1.73 m^2), or evidence of kidney damage either from radiological or pathological evidence. It often develops in the late stage of DM. But due to the natural history of type II DM, it can be presented at the time of diagnosis.[10] DN is a leading cause of renal replacement therapy and renal

transplantation which are major burdens to both patients and the healthcare system. The American Diabetes Association (ADA) recommends annual screening for albuminuria using spot urine albumin-to-creatine ratio (UACR). Although 24-hour urine albumin is the gold standard for diagnosis, it is more burdensome and adds little value to accuracy. However, patients are required to have two of three abnormal UACR tests within 3-6 months to be diagnosed with diagnosed with albuminuria.[10]

Albuminuria is classified into 3 stages; normal to mildly increased, moderately increased, and severely increased albuminuria Firstly, normal to mildly increased albuminuria and formerly known as normoalbuminuria is defined as UACR less than 30 mg/g creatinine or 24-hour urine albumin less than 30 mg. Secondly, moderately increased albuminuria or microalbuminuria is defined as UACR 30-299 mg/g creatinine. Lastly, if UACR 300 mg/g creatinine and above, patients will have severely increased albuminuria or macroalbuminuria. Diagnosis of DN is based on the presence of albuminuria and/or decreased eGFR without any signs of kidney damage from other causes.[10]

There are some factors that can interfere with urine albumin result such as infection, exercise, heart failure, fever, marked hyperglycemia, hypertension, and menstruation.[10]

Clinical relationship between diabetic retinopathy and diabetic nephropathy

In a Multiethnic Asian Population Study by Sabanayagam et al. analyzed the data from 2,964 participants between the ages of 40 and 80 years with the median follow-up time of 8.8 years. The study revealed that 29.9% of diabetic participants had DR, and 20.7% had DN. Over 28.3% of DR participants had DN which is also associated with hypertension, history of cardiovascular diseases, and longer duration of diabetes. Moreover, risks of mortality were higher in those with DR or DN and increased significantly with the increasing stages of both diabetic complications. However, the study did not find the interactions between DR and DN on the mortality rate.[11]

A similar study that used the data from Singapore Prospective Study Program and The Singapore Malay Eye study was conducted by Yip et al. In this study, the

patients with DR had significantly lower estimated glomerular filtration rate (eGFR) and DR was found to be significantly associated with the prevalence of end-stage renal disease (ESRD). After adjusting for other factors such as hypertension, diabetes, and eGFR, the risk of ESRD was persisted.[12]

Another population-based study in Spain by Pedro et al. showed that among 8,675 diabetic patients aged 7-87 years, prevalence of DR was 36.47% in type I DM and 26.11% in type II DM. Microalbuminuria which is defined as abnormal urine albumin excretion (30-300 mg of albumin/24 hours), was found in 25.61% of patients with type I DM and 17.78% in type II DM, while overt nephropathy (urine albumin excretion exceed 300 mg/24 hours) was presented in 8.6% and 6.74% in patients with type I and type II DM respectively. Overt nephropathy was also a significant risk factor for diabetic retinopathy (OR 3.66 for type I DM and 2.87 for type II DM) and diabetic macular edema (OR 6.63 for type I DM and 3.94 for type II DM). On the other hand, proliferative diabetic retinopathy (PDR) was a significant risk factor for diabetic microalbuminuria (OR 3.14 for type I DM and 2.46 for type II DM) and diabetic overt nephropathy (OR 4.89 for type I DM and 5.88 for type II DM).[13]

Cao et al. conducted a retrospective study in a Chinese population and compared between DN patients with and without DR. 48.8% of patients with DN had DR. The DN without DR group had significantly lower serum creatinine levels and higher serum albumin levels than the DN with DR group. Prevalence of other complications of diabetes such as diabetic neuropathy and diabetic vascular diseases were also significantly higher in the DN with DR group.[14]

A large registry-based study consisting of 54,670 type II DM patients in Saudi Arabia by Al-Rubeaan et al. reported that prevalence of DN was 10.8%. Prevalence of DR in patients with DN was 48.8% which significantly higher than patients without DN. When exploring in each stage of DN, prevalence of DR in microalbuminuria group (albumin excretion 30-300 μg/mg creatinine) was 23.8% while in macroalbuminuria group (albumin excretion >300 μg/mg creatinine) the prevalence was increased to 60.1%. Furthermore, 41% of patients with ESRD, which was defined as glomerular filtration rate (GFR) <30 ml/min/1.73 m^2 body surface area, had DR. DR is also found

to be the second significant risk factor for developing DN. Patients with DR had the chance of developing DN 3.95 times more than those without retinopathy.[15]

A prospective study by Saini et al. also confirmed the association and correlation between these two microvascular complications. The study showed statistically significant association between the severity of DR and DN, both GFR staging and urine protein staging. The authors explained that the pathogenesis of microvascular complications from hyperglycemia is similar, and the onset and progression of both complications are closely related. The authors also purposed that DR can be an important predictor for the progression of kidney complications of diabetes.[16]

A meta-analysis by Li et al., which included 26 articles and involved 60,136 participants. DR was statistically significantly related to DN and vice versa. The difference in risk of DN between any DR and proliferative DR was also significant. But there was no significant difference in correlation with DR between any DN and overt DN. The study also revealed that hypertension was a correlation factor between DN and DR.[17]

Another meta-analysis by Jiang et al. explored the diagnostic performance of DR in the detection of DN. 45 studies were included in the analysis with a total number of 4,561 participants. The study showed that DR had a pooled sensitivity of 67% and a specificity of 78% in diagnosing DN. When applying to clinical utility, using only DR as an only test for confirming or excluding DN was not appropriate. However, the presence of DR was still useful in differentiating between DN and non-diabetic kidney diseases.[18]

Li et al. conducted a meta-analysis that also focused on the predictive value of DR on DN. The systematic review analyzed 10 prospective cohort studies. The pooled sensitivity and specificity were 64% and 77% respectively. When considering positive and negative likelihood ratio, DR showed only moderate accuracy in diagnosing or excluding DN. After performing subgroup analysis, both sensitivity and positive likelihood ratio of DR in younger patients were significantly higher than in older patients. However, as previous studies suggested, DR was still an important risk of DN in type II DM patients. The limitation of these meta-analyses was the

heterogeneity between studies which occurred from the different definitions of the diagnosis of DR and DN.[19]

There is also conflicting evidence about correlation between the severity of DR and DN. The Renin-Angiotensin System Study by Klein et al. found no significant relationship between severity of DR and serum creatinine nor GFR.[20] However, it is worth mentioning that this study included only patients with type I DM. And this same study found significant association between DR and renal anatomical end points which will be mentioned in the next section.

In conclusion, based on available evidence, there is a strong clinical association between DR and DN. But the correlation between these two diabetic complications is still in debate. The definitions of DN between studies are also vary which may affect the result of meta-analyses and systematic reviews.

Anatomical relationship between diabetic retinopathy (DR) and diabetic nephropathy (DN)

Numerous anatomical evidence also supports the relationship between retinal and renal microvasculature. Histopathological studies revealed that early glomerular lesions in DN are thickening of glomerular basement membrane. Glomeruli which are a type of fenestrated capillaries play an important role in the pathophysiology of DN. Other major histological changes in DN are mesangial expansion and glomerular sclerosis. Glomerulosclerosis is believed to be associated with hyaline deposits in the glomerular arterioles that cause by plasma proteins accumulation.[20] Thickening of retinal capillary basement membrane which is shown as hyalinization of capillaries via light microscopy is also found in the early stage of DR. This pathological feature found in many organs of diabetic patients and is a hallmark of microvasculopathy of DM. One possible explanation is that the pathology is the result of chronic hyperglycemia and accumulation of advanced glycation end products which cause the activation of various cytokines and oxidative stress.[21, 22]

Another common pathology is the loss of pericytes in retina and podocytes which are specialized forms of pericytes in glomeruli that is contributed to basement

membrane abnormality. Loss of both pericytes and podocytes can disrupt the barrier function of the retinal and renal vessels and cause plasma protein leakage into tissues. Other associated features include microaneurysm, arteriolar hyalinosis and exudative lesions.[22, 23]

A study by Klein et al. which is mentioned in previous section is one of few studies that obtained percutaneous renal biopsies from participant and assessed with renal function and retinal examination. The univariate analysis showed that the severity of DR was significantly related to glomerular basement membrane (GBM) width, mesangial fractional volume per glomerulus, mesangial matrix fraction volume per glomerulus, peripheral GBM surface density per glomerulus, and glomerulopathy index. However, in multivariate analysis, when controlling duration of diabetes and HbA1c level, only GBM width and glomerulopathy index were found to be significantly associated with DR severity. The authors suggested that DR severity could be used to assess the renal risk in patients with type I DM without clinical nephropathy.[20]

The previously mentioned study by Yip et al. failed to find the association between renal function and other retinal pathological characteristics such as retinal arteriolar caliber, retinal venular caliber, and retinal vascular fractal dimension. Only DR severity was significantly related to the incident of ESRD in diabetic patients.[12]

The Atherosclerosis Risk in Communities Study, a longitudinal community-based study by Wong et al. explored the retinal pathological characteristics on fundus photographs compared with six-year change in serum creatinine. The authors reported that microaneurysm, retinal hemorrhage, soft exudates, and arteriovenous nicking were significantly associated with larger change in serum creatinine and high odds of developing nephropathy in a multivariate analysis. Whereas lower arteriole-to-venule ratio which reflects narrowed arteriolar and venular diameters, was only associated with larger change in serum creatinine but not risk of developing renal dysfunction. These findings confirmed the established concepts about systemic microvasculopathy in patients with DM.[24]

Data from The Multi-Ethnic Study of Atherosclerosis suggested that narrower central retinal artery caliber was significantly associated with increased odds of

developing albuminuria and lower GFR after adjusting for age, sex, and race. But central retinal vein caliber failed to show any association with albuminuria. Both arterial and venous caliber were not associated with the incidence of CKD in overall population. However, in a subgroup analysis, narrower retinal arterial caliber was associated with higher incidence of CKD in white population. The authors suggested that due to the distribution of prevalence of albuminuria, other factors including hypertension and coronary artery disease might play a role in arteriolar narrowing.[5, 25]

Sabanayagam et al. also found similar result from The Singapore Malay Eye Study. Reduced central retinal artery diameter was found to be significantly associated with reduced GFR and micro/macroalbuminuria. The association persisted after adjusted for venous diameter, HbA1c level, and blood pressure. However, such association was not found with central retinal vein diameter.[26]

Another article using the cross-sectional data from The Singapore Malay Eye Study by Lim et al. found the significant association between GFR and retinal arteriolar caliber, retinal vascular fractal dimension, arteriovenous nicking, and arteriovenous opacification after adjusting for other variables. But none of retinal pathology were associated with chronic kidney disease. Albuminuria is another factor that reported to be associated with fractal dimension, focal arteriolar narrowing, arteriovenous nicking, and arteriovenous opacification.[6]

The Cardiovascular Health Study is a large multicenter prospective study by Edwards et al. that followed participants' renal function for 4 years. Apart from the significant association between retinopathy and increased serum creatinine, the authors also discovered that specific retinal pathology including retinal exudates and hemorrhages were associated with worse renal function. But microaneurysms, retinal arteriolar abnormality, focal arteriolar narrowing, arteriovenous nicking, and arteriovenous ratio were not found to be associated with renal function.[27]

A novel anatomical marker for retinal pathology is fractal dimension which represent the complexity of retinal vascular network. Sng et al. used the data from the Singapore Prospective Study Program and proposed that lowest and highest quintiles of retinal vascular fractal dimension were associated with increased

incidence of CKD. The authors suggested that suboptimal microvasculature, both above and below optimal value, can cause hemodynamic change and leads to end-organ pathology.[28]

To our knowledge, retinal pathology can be signs of diffuse systemic microvasculopathy which leads to the breakdown of blood-tissue barrier. But the causes of end-organ microvascular changes can be from other diseases such as hypertension. Many studies suggested that some retinal characteristics can be predictors for a renal dysfunction.

Optical coherence tomography angiography (OCTA) and diabetic nephropathy (DN)

Apart from direct visualization from fundus examination or fundus photography, retinal vascular pathology can be examined using optical coherence tomography angiography (OCTA). With the development of OCTA, retinal blood flow can be investigated in a non-invasive and faster than traditional fluorescein angiography (FA) which required an injection of fluorescein dye into patient's body. Using the principle of swept-source optical coherence tomography (SS-OCT), OCTA can provide detailed images of various depth of retina including superficial, intermediate, deep capillary plexus, peripapillary capillary network, and outer retinal choriocapillaris layer. Research on OCTA is rapidly expanding because of its utility and convenience.[29]

The first commercial OCTA machine uses laser at the wavelength of 1,050 nm and can produces 100,000 A-scans per second. With continuous development, the OCTA machines now can produce up to 400,000 A-scans per second which can produce better image resolution and less motion artifact. The A-scan data are then combined into cross-sectional B-scan images. Multiple B-scan images can create a volumetric data which enables 3D visualization of the area of the retina. En-face images or C-scans are created from this volumetric data. En-face images are used to visualize multiple retinal surfaces and vessels of various depth.[30]

However, OCTA still has some limitations. Due to the principle of OCTA, it cannot detect dynamic change such as vascular leakage or alteration of blood flow.

Although the scanning speed is very fast, patients are still required to stay very still for a period of time and the field of view is narrower than FA. And because it is a relatively new technology, algorithm and imaging protocol including automated segmentation protocol differ between manufacturers. Lastly, there are many artifacts of various causes such as low signal strength, localized loss of signal strength, motion artifact, projection artifact, segmentation artifact, and false positive decorrelation. But manufacturers are developing automated software to detect and remove those artifacts.[29]

There are 2 main circulations that supply blood to the eye; central retinal artery, and posterior ciliary arteries, both of which are branches of ophthalmic artery that originates from internal carotid artery. The posterior ciliary arteries supply the choroid and outer one third of the retina layers. The central retinal artery branches into small retinal arterioles which supply the inner two third of the retina. The retinal arterioles then divide and form into four retinal vascular networks. The first and most superficial one is the radial peripapillary capillary plexus which is in the nerve fiber layer (NFL). The second plexus that locates in retinal ganglion cell layer (GCL) is caller superficial capillary plexus (SCP). The innermost ones are intermediate capillary plexus (ICP) and deep capillary plexus (DCP) which locate on both sides of inner nuclear layer (INL). ICP and DCP are formed by anastomoses of vessels originate from SCP. Both plexuses are not clearly demarcated thus they are sometimes grouped together as DCP.[29]

There are many OCTA features that can be detected in patients with DR, such as capillary dropout, enlarged foveal avascular zone (FAZ), microaneurysms, decreased vessel density (VD), presence of intraretinal microvascular abnormalities (IRMA), and development of neovascularization. These changes can be visualized very early by OCTA in patients without clinically detected DR.[29]

Vessel density is one of the most studied OCTA parameters. It is defined as a proportion of vascular area with blood flow detected with OCTA over the total scan area. There are some factors that can affect VD values including older age, male sex, worse best-corrected visual acuity, longer axial length, thinner inner retinal thickness,

higher HbA1c level, lower signal strength index, worse DR severity, and higher serum creatinine.[29, 30]

Cheung et al. studied various OCTA parameters including FAZ area, FAZ circularity, intercapillary area, VD, fractal dimension (FD), and vessel diameter index. Only intercapillary area was found to be significantly associated with reduced GFR. The authors proposed that larger intercapillary area in retina may reflect reduction in peritubular capillary flow, renal ischemia, and blood-renal barrier disruption.[31] These findings conceded with prior histopathological studies which observed pericyte and endothelium changes and capillary dropout in retinal capillaries in diabetic eyes.[32, 33]

A cross-sectional study by Cankurtaran et al. focused on the relationship between vessel density and albuminuria. The authors reported that there was statistically significant reduction in vessel density of the superficial capillary plexus (SCP), the whole disc, and the peripapillary area in participants with microalbuminuria compared to normoaluminuria and participants without diabetes. The vessel density of the deep capillary plexus (DCP) in participants with microalbuminuria were also significantly reduced compared to participants without diabetes. But the mean retinal thickness between 3 groups were not found to be significantly different. However, the study included only participants without retinopathy. The result demonstrated that alteration in retinal microcirculation may occurred before retinal thinning.[7]

A prospective case-control study conducted in Iran by Ahmadzadeh Amir et al. included 78 eyes from 46 participants with and without overt nephropathy. Participants with overt nephropathy were found to have significantly larger FAZ area that those without nephropathy. The authors suggested the DN was a significant risk of diabetic macular ischemia.[34] The result of this study concurs with a prior study of Shukla et al. which reported significantly enlarged FAZ area from fluorescein angiography in patients with diabetic nephropathy.[35]

Choroidal thickness can be detected by a conventional optical coherence tomography angiography (OCT) with special technique called enhanced depth imaging (EDI-OCT) or using SS-OCT which is the principle of OCTA machines. Choroidal vessels are another site that believed to be affected by hyperglycemia. Kocasarac et al. compared the choroidal thickness of 35 eyes of DN patients with 35 eyes of

diabetic patients without DN and 34 eyes of healthy controls. DN patients had statistically significant thinner choroid than non-DN patients and controls. But when comparing between normal participants and non-DN patients, no significant difference was found. In DN groups, there were a significant negative correlation between CT and proteinuria, and a positive correlation between CT and GFR.[36]

OCTA is a relatively new technology and there are limited publications regarding its characteristics in DN patients. Moreover, as with other studies in DN, there is heterogeneity between studies which is cause by different diagnostic criteria for DN, different imaging protocol, and participants' characteristics. And to our knowledge, there is no such study in Thai population, so we aim to study there OCTA biomarkers in Thai diabetic patients. All the relevant studies are summarized in Table 1.

Table 1 Overview of the reviewed literature

Authors	Year	Design	N	Retina imaging	DN diagnosis	Retinal parameters	Renal parameters	Results
Wong TY[24]	2004	Cohort (cross sectional fundus) (2 years)	10056 (687)	Fundus photograph	SCr increase >0.4	DR, MA, retinal hemorrhage, soft exudates, AV nicking, AV ratio	SCr	Any retinopathy, MA, retinal hemorrhages, soft exudates, and AV nicking associated with larger change in SCr and higher odds of renal dysfunction ($p < 0.05$)
Shukla D[35]	2004	Cross sectional	102	Fundus photograph and FFA	Urine dipstick, SCr	ETDRS severity scale, FAZ size	Urine albumin, SCr	Increased FAZ area and irregularity associated with albuminuria ($p < 0.05$)
Edwards MS[27]	2005	Cohort (4 years)	1394	Fundus photograph	SCr increase >0.3	Retinopathy, retinal arteriolar abnormalities, AV ratio	SCr, eGFR	Retinopathy, exudates, and hemorrhage associated with higher SCr ($p < 0.05$)

Klein R[20]	2005	Cross sectional	252	Fundus photograph	Renal biopsy	DR severity (Modified Airlie House)	BP, AER, SCr, GFR, GBM width, mesangial fractional volume/glomerulus, mesangial matrix fractional volume/glomerulus, Vv, peripheral GBM surface density/glomerulus, glomerulopathy index	DR severity associated with GBM width, mesangial fractional volume/glomerulus, mesangial matrix fractional volume/glomerulus, and glomerulopathy index ($p < 0.0001$)
Sabanayagam C[11]	2009	Cross sectional	3280	Fundus photograph	eGFR <60 or micro/macroalbuminuria	Retinal arteriolar and venular diameter (CRAE, CRVE)	eGFR, micro/macroalbuminuria	Reduced CRAE and retinopathy associated with reduced eGFR and increased micro/macroalbuminuria ($p < 0.05$)
Sng CC[28]	2010	Cross sectional	261	Fundus photograph	eGFR <60	Fractal dimension retinal vasculature	eGFR <60	Fractal dimension in extreme quintiles associated with increased odds of CKD ($p < 0.05$)

Awua-Larbi S[25]	2011	Cross sectional	675	Fundus photograph	Urine albumin/creatinine ratio	CRAE, CRVE	Urine albumin/creatinine ratio	Narrower and wider CRAE associated with increased odds of albuminuria (p < 0.01)
Yau JW[5]	2011	Cohort (cross sectional fundus) (5 years)	4594	Fundus photograph	eGFR <60	Retinal microvascular caliber – CRAE and CRVE, Retinopathy	eGFR <60	Narrower CRAE associated with CKD in whites (p = 0.04)
Lim LS[6]	2013	Cross sectional	3280	Fundus photograph	CKD - eGFR <30	Retinal vascular caliber, retinal vascular fractal dimension, retinal vascular tortuosity, retinal branching	Urine albumin, SCr, eGFR	Reduced vascular caliber, reduced fractal dimension, and presence of AV nicking associated with reduced eGFR and increased urine albumin (p < 0.05)
Lee WJ[37]	2014	Cross sectional	971	Fundus photograph	Albuminuria	Presence of DR (ETDRS severity scale)	Albuminuria, albumin/creatinine ratio	DR and PDR associated with CKD in overall and microalbuminuria group (p < 0.05)

Yip W[12]	2015	Cross sectional	5763	Fundus photograph and FFA	ESRD – eGFR, SCr, treatment for CKD	Presence of DR, modified Airlie House, retinal vascular caliber, retinal vascular fraction dimension	eGFR, SCr	Presence of retinopathy and retinal arteriolar narrowing associated with risk of ESRD ($p < 0.05$)
Kocasarac C[36]	2018	Cross sectional	104	SD-OCT	N/A	Choroidal thickness	Proteinuria, GFR	Choroidal thickness significantly thinner in DN compared with no DN and normal control ($p < 0.05$)
Zhang J[38]	2018	Cross sectional and retrospective cohort	250	Fundus photograph	Renal biopsy	Presence of DR	Kidney biopsy, 24-h urine protein, SCr, eGFR	DN and DR have significantly worse renal survival rate, higher SCr, higher proteinuria, and lower eGFR than DN alone ($p < 0.05$)
Cheung CY[31]	2018	Cross sectional	184	OCTA	eGFR <60 and >60	Intercapillary area, FAZ area, FAZ circularity, vessel density, fractal dimension, vessel diameter index	eGFR	Average of 10 and 20 largest intercapillary area associated with eGFR ($p = 0.006$ and 0.008)

Jiang S[18]	2019	Meta-analysis	4561	Fundus photograph and FFA	Renal biopsy	Presence of DR, DR severity	DN	DR showed sensitivity of 0.67 and specificity of 0.78 in diagnosis of DN
Cankurtaran V[7]	2019	Cross sectional	137	OCTA	UAE	Vessel densities in SCP, DCP, disc area	UAE rate (normoalbuminuria, microalbuminuria)	Significant reduction in VD of SCP in microalbuminuria compared to normoalbuminuria and control ($p < 0.05$) Significant reduction in VD of DCP in microalbuminuria compared to control ($p < 0.05$)
Ahmadzadeh Amiri A[34]	2020	Cross sectional	78	OCTA	Gross albuminuria (>300mg/L)	FAZ area, CSF thickness	Overt nephropathy (>300mg/L), SCr	Significant thicker CSF and larger FAZ area in overt nephropathy ($p < 0.05$)

Abbreviation: Scr – serum creatinine; DR – diabetic retinopathy; MA – microaneurysms; AV – arteriovenous; FFA – fundus fluorescein angiography; ETDRS – Early Treatment of Diabetic Retinopathy Study; FAZ – foveal avascular zone; eGFR – estimated glomerular filtration rate; BP – blood pressure; AER – albumin excretion rate; GFR – glomerular filtration rate; GBM – glomerular basement membrane; Vv – mesangial cell fractional volume/glomerulus; CRAE – central retinal artery equivalent; CRVE – central retinal vein equivalent; CKD – chronic kidney disease; ESRD – end stage renal disease; SD-OCT – spectral-domain optical coherence

tomography; OCTA – optical coherence tomography angiography; DN – diabetic nephropathy; UAE – urinary albumin excretion; SCP – superficial retinal capillary plexus; DCP – deep retinal capillary plexus; CSF – central foveal subfield

Chapter III Research Methodology

Target population

Patients who are diagnosed with diabetes mellitus.

Sample population

Diabetic patients in the internal medicine out-patient department of King Chulalongkorn Memorial Hospital with recent 24-hour urine albumin testing less than 3 months.

Inclusion criteria

1. Participants aged 18 and above with a diagnosis of type 2 diabetes mellitus according to diagnostic criteria by the American Diabetes Association[39]
2. History of 24-urine albumin and creatinine testing within 3 months prior the recruitment
3. For no DN group, participants with 24-hour urine albumin level <30 mg
4. For early DN group, participant with 24-hour urine albumin level 30-300 mg
5. For late DN group, participants with 24-hour urine albumin level >300 mg
6. Absence of retinal anomaly other than diabetic retinopathy

Exclusion criteria

1. History of ocular trauma or surgery other than uncomplicated cataract surgery
2. History corneal disorders, strabismus, nystagmus, uveitis, glaucoma, optic nerve abnormalities, neurological disorders or other abnormalities affecting the visual pathway
3. History of retinal laser treatment or intravitreal anti-vascular endothelial growth factor (anti-VEGF) treatment
4. Presence of diabetic macular edema or tractional retinal detachment
5. Media opacities or other pathology preventing reliable retinal imaging
6. Active urinary tract infection
7. Suspected of any non-diabetic renal diseases

8. End-stage kidney disease requiring dialysis or kidney transplantation
9. Participants who could not cooperate adequately for the OCTA examination

Sample size calculation

Using sample size formula for independent continuous outcomes as following,

$$N = \frac{2\left(Z_{1-\alpha/2} + Z_{1-\beta}\right)^2 \sigma^2}{MCD_E^2}$$

$$= \frac{2\,(2.41 + 0.84)^2 3.4^2}{2^2} = 61.05$$

With standard deviation of 3.4, calculated from standard deviation of vessel densities of superficial capillary plexuses of normoalbuminuria and microalbuminuria groups from previous study.[7] Expert opinions and result from previous study suggested that mean clinical difference of 2% is considered significant.[7] The sample size of 62 eyes in each group would be a sufficient population to attain power of 80% with an alpha value of 0.016 (from pairwise comparison using Bonferroni's method). Total of 186 eyes would be required to be enrolled into this study. Both eyes of participants will be included if they were eligible for the study.

Materials

1. Early Treatment of Diabetic Retinopathy Study (ETDRS) visual acuity chart

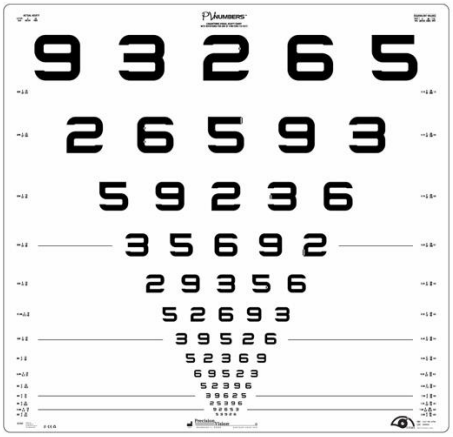


Figure 1 ETDRS visual acuity chart

(from https://www.precision-vision.com)

Participants were tested for visual acuity (VA) of each eye using ETDRS chart (PV Numbers ETDRS Series, Precision Vision, Illinois, USA) (Fig. 1) by standing at 4 meters from the chart. Then participants were asked to read the numbers on the chart starting from the top row using only one eye. Participants then read down the chart until the letters are incorrectly read at least 3 letters. The process was done again in the same eye but looking through a pinhole. After the first eye was done, the process was then repeated in another eye. And the result was reported in the greatest number of letters that participants can correctly identified.

2. Non-contact tonometer

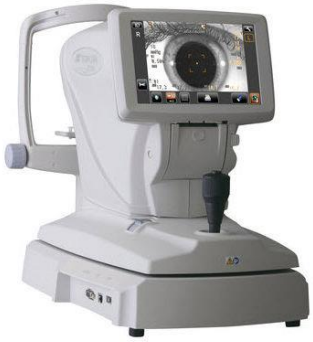


Figure 2 Topcon CT-800 non-contact tonometer
(from https://www.topconhealthcare.jp)

Non-contact tonometer (Topcon CT-800, Topcon Healthcare, Tokyo, Japan) (Fig. 2) was used for measuring participants' intraocular pressure in mmHg by blowing a column of air to flatten the cornea and the machine will detect the change in the reflection of laser beam by the flattened cornea.[40]

3. Slit lamp biomicroscopy

Figure 3 Topcon DC-4 slit lamp biomicroscopy
(from https://www.topconhealthcare.jp)

Slit lamp biomicroscopy (Topcon DC-4, Topcon Healthcare, Tokyo, Japan) (Fig. 3) was used for examining patients' eye including anterior segment and fundus examination at Ophthalmology Clinic, Department of Ophthalmology, King Chulalongkorn Memorial Hospital.

4. Optical coherence tomography (OCT) machine

Figure 4 Heidelberg Spectralis OCT machine (from https://www.heidelbergengineering.com)

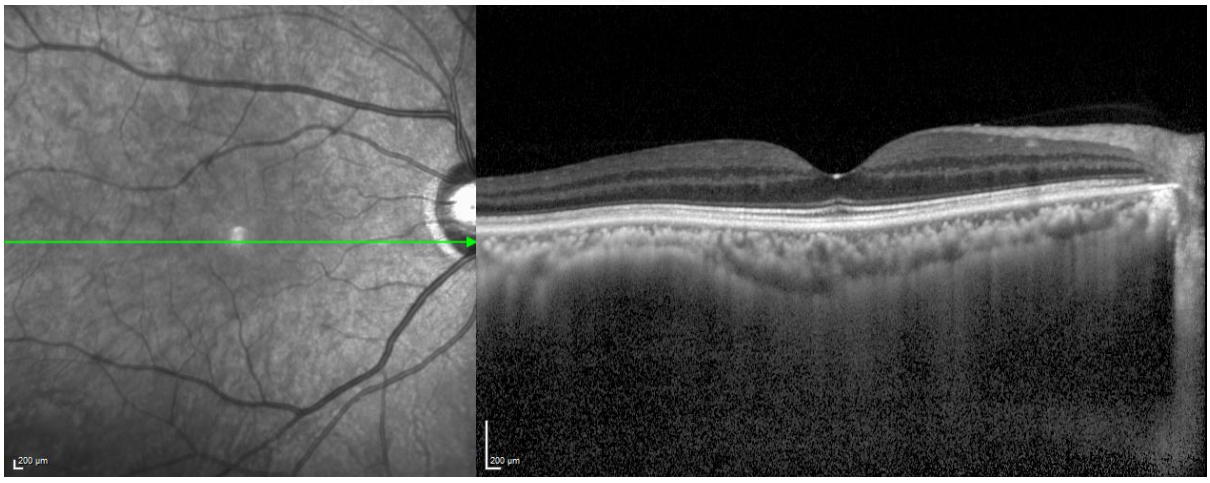


Figure 5 OCT image of fovea

Spectral domain OCT machine (Spectralis, Heidelberg Engineering, Heidelberg, Germany) (Fig. 4) with enhanced depth imaging (EDI) technology was used to visualize cross sectional images of retina (Fig. 5) and measure retinal layer thickness and choroidal thickness.

5. Optical coherence tomography angiography (OCTA) machine

Figure 6 Zeiss PLEX elite 9000 OCTA machine (from https://www.zeiss.com)

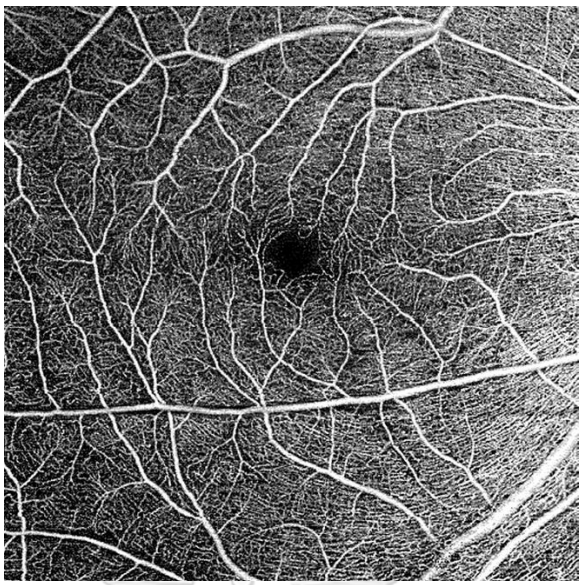


Figure 7 OCTA image of superficial capillary plexus of the 6x6 mm area around fovea

OCTA machine using swept source technology (PLEX Elite 9000 Swept-source OCT, Carl Zeiss Meditec AG, Jena, Germany) (Fig. 6) can produce very detailed images of retina and blood vessels at various depths (Fig. 7) by detecting the movement of red blood cells in blood vessels.[29]

6. Ultra-wide field fundus camera

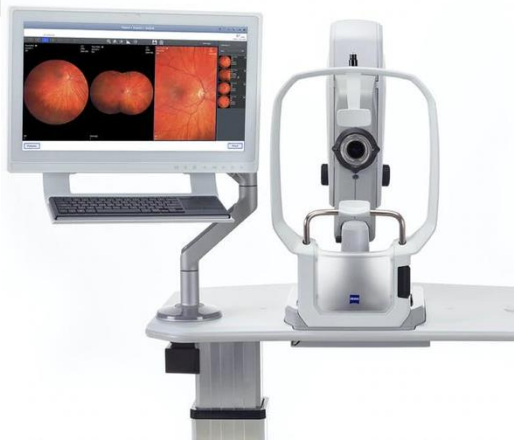


Figure 8 Zeiss CLARUS 700 Ultra-wide field fundus camera (from https://www.zeiss.com)

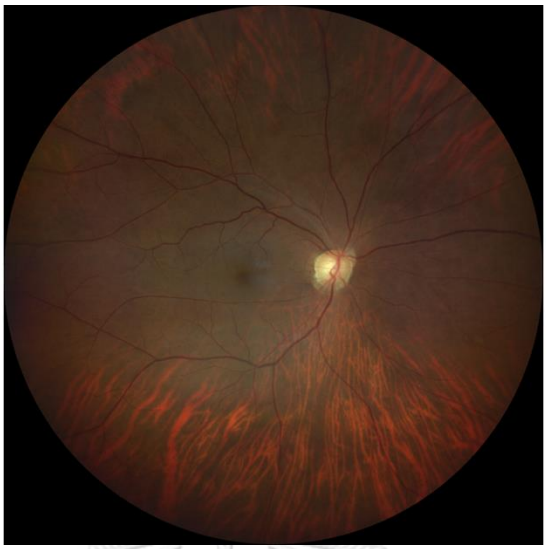


Figure 9 Wide field fundus photo

Ultra-wide field fundus camera (CLARUS 700, Carl Zeiss Meditec AG, Jena, Germany) (Fig. 8) was used for taking image of participants' fundus (Fig. 9) and sent for grading of diabetic retinopathy severity by independent investigators.

7. Optical biometer

Figure 10 Zeiss IOL Master 700 optical biometer (from https://www.zeiss.com)

Optical biometer (IOL Master 700, Carl Zeiss Meditec AG, Jena, Germany) (Fig. 10) was used to measure participants' eye axial length.

8. ImageJ Software

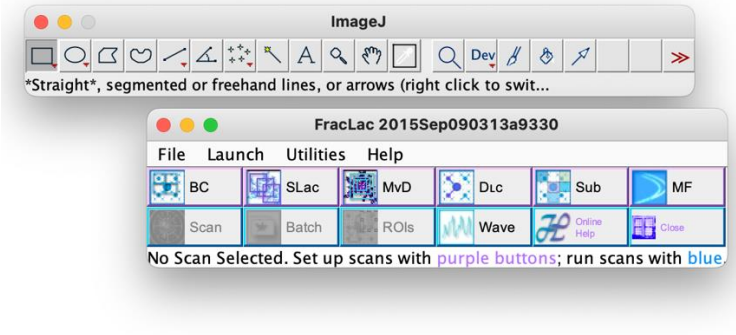


Figure 11 ImageJ and FracLac plugin user interface

ImageJ (National Institute of Health, Maryland, USA) (Fig. 11) is an image processing software commonly used in research. It has various image analysis feature including converting color images into binary images and calculating the area. FracLac (School of Community Health, Faculty of Science, Charles Sturt University, Australia) (Fig. 11) is a plug-in software for ImageJ. The plug-in was used to analyze fractal dimension of the images. Fractal dimension is a type of morphological analyses. It represents the complexity of the pattern in an image comparing to the scale of the image.

Methods

Participants who fulfilled the inclusion criteria were recruited from the internal medicine out-patient department in King Chulalongkorn Memorial Hospital, Bangkok, Thailand. After all information including risks and benefits of the study was discussed with participant and informed consent was obtained, blood and urine samples were collected and sent for blood urea nitrogen (BUN), serum creatinine (SCr) and serum glycated hemoglobin (HbA1c).

Participants were divided into 3 groups according to 24-hour urine albumin result in to 3 levels; (1) Participants with urine albumin less than 30 mg/24 hours will be in no DN group. (2) Participants with 24-hour urine albumin level of 30-300 mg was categorized as early DN, and (3) Participants with more than 300 mg were sorted into late DN group. The specimen for 24-hour urine test was obtained according to

the standard guideline of the hospital. The participant received an instruction to void and discard the urine at 8.00 am and then start a urine collection for 24 hours until 8.00 am of the next day. Participants were required to use a standard gallon for urine storage and transport to the hospital for laboratory analysis. Spectrophotometry method was used to quantify the amount of albumin in the specimen by an automated clinical chemistry machine (Alinity c; Abbott Core Laboratory, Illinois, USA). The laboratory was performed according to ISO 15189 standard and was accredited by The International Laboratory Accreditation Cooperation Mutual Recognition Arrangement (ILAC MRA) and Department of Medical Sciences (DMSc), Ministry of Public Health, Thailand (Accreditation No. 4006/47). The result was reported in milligram (mg) of total albumin in urine. Due to limitation of the laboratory, lower limit of detection of urine albumin is 6.8 mg. To guarantee the reliability of 24-hour urine collection, urine creatinine was also measured. Participants with urine creatinine less than standard 24-hour urine creatinine excretion rate were asked to repeat the collecting process.

Visual acuity was tested using an ETDRS chart (Precision Vision, Illinois, USA) and intraocular pressure was measured with a Topcon CT-800 non-contact tonometer (Topcon Healthcare, Tokyo, Japan). Participants were then received one drop of 0.5% tetracaine hydrochloride eyedrop (Alcon, Texas, USA) followed by one drop of 1% tropicamide eyedrop (Mydriacyl; Alcon, Texas, USA) every 15 minutes for 4 times in each eye. Anterior segment eye examination and dilated fundus examination were done by a single investigator using a Topcon DC-4 slit lamp biomicroscopy (Topcon Healthcare, Tokyo, Japan) and all the result was recorded in a case record form. Axial length was then measured using the IOL Master 700 optical biometer (Carl Zeiss Meditec AG, Jena, Germany).

Wide field fundus photograph was taken using a Clarus 700 ultra-wide field retinal camera (Carl Zeiss Meditec AG, Jena, Germany) by a masked technician and was randomly graded by 2 independent masked assessors (P.P. and W.K.) into no diabetic retinopathy (no DR), mild non-proliferative diabetic retinopathy (mild NPDR), moderate non-proliferative diabetic retinopathy (moderate NPDR), severe non-proliferative diabetic retinopathy (severe NPDR) and proliferative diabetic retinopathy

(PDR) according to the International Clinical Diabetic Retinopathy and Diabetic Macular Edema Disease Severity Scales (Table 2).[8] If there were disagreement between two graders, a decision was made by a third grader (N.S.).

Table 2 Diabetic Retinopathy Disease Severity Scale (adapted from Wilkinson et al.)[9]

Disease Severity	Findings on Dilated Ophthalmoscopy
No diabetic retinopathy	No abnormalities
Mild nonproliferative diabetic retinopathy	Microaneurysms only
Moderate nonproliferative diabetic retinopathy	More than just microaneurysm but less than severe nonproliferative diabetic retinopathy
Severe nonproliferative diabetic retinopathy	Any of the followings >20 intraretinal hemorrhages in each of 4 quadrants Venous beading in 2+ quadrants Intraretinal microvascular abnormalities in 1+ quadrant and no signs of proliferative retinopathy
Proliferative diabetic retinopathy	Neovascularization and/or vitreous/preretinal hemorrhage

OCT image was taken using Heidelbelg Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany) in EDI mode with 100 images by Automatic Real Time (ART) averaging function in 1 horizontal line scan at foveal center. Only images with signal strength of 20 or above were used. Central retinal thickness (CRT) was measured using automatic segmentation by the Spectralis Software. Automatic segmentation was also confirmed by the investigator. If there were automatic segmentation errors, manual segmentation and measurement were done instead. Subfoveal choroidal thickness (CT) was manually measured by a single masked assessor using the

Spectralis Software measurement function. Subfoveal choroidal thickness is defined as the perpendicular distance at the foveal center from outer portion of hyperreflective line corresponding to the retinal pigment epithelium and Bruch membrane complex to the hyperreflective line at the posterior choroid corresponding to the chorioscleral junction (Fig. 12).

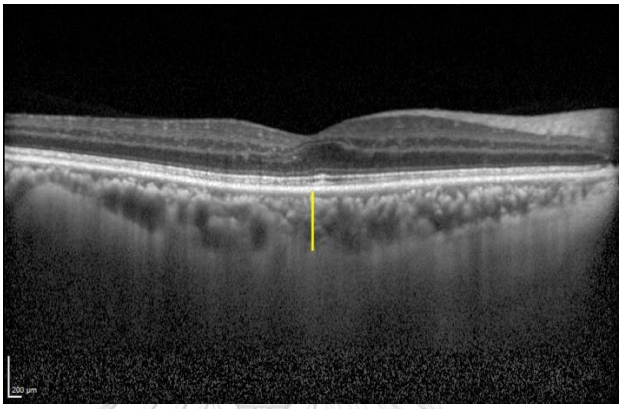


Figure 12 Subfoveal choroidal thickness measurement

OCTA images were obtained using PLEX Elite 9000 Swept-source OCT (Carl Zeiss Meditec AG, Jena, Germany) with 6x6 mm field of view in 4 seconds of total OCT scan time by a masked technician (Fig. 7). The en-face OCT image was segmented with an inner boundary at 3 μm beneath the internal limiting membrane (ILM) and the outer boundary was set at 15 μm beneath the inner plexiform layer (IPL) to obtain images of the superficial capillary plexus (SCP). The en-face image was segmented with an inner boundary of 15 μm beneath the IPL and the outer boundary was set at 70 μm beneath the outer plexiform layer (OPL) to obtain images of the deep capillary plexus (DCP). To ensure quality consistency, only images with signal strength of 6 and above were used and the investigator also examined the images again for any artifacts or missing portions. If the quality were unsatisfied, the participants would undergo image acquisition again. Participants were excluded from the study if images quality fail to meet the standard after the second image acquisition.

Image analysis

To calculate vessel density, OCT images of SCP, DCP, and whole retina were used. Images were converted into binary images using Otsu's auto-threshold algorithm[41] in the ImageJ Software (National Institute of Health, Maryland, USA) (Fig. 13). Vessel density (VD) is defined by all the white area in the image which represents blood vessels divided by total image area. Intercapillary area is defined by all the black area in the image divided by total image area. Using measurement function in the software, VD and intercapillary area were automatically calculated from the binary images.[42]

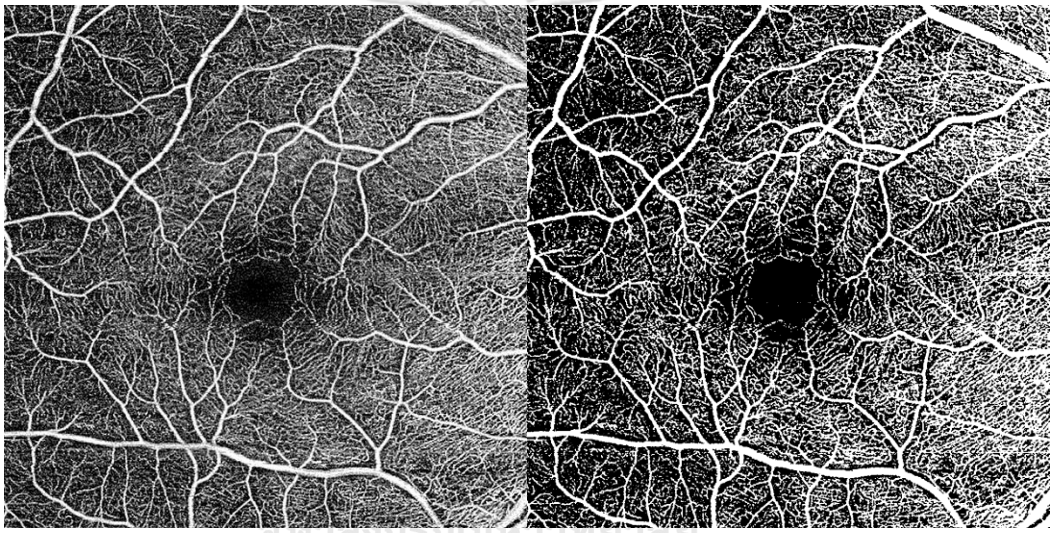


Figure 13 6x6 mm OCTA image of SCP (left) and binarized image by ImageJ (right)

Binarized OCTA images of SCP were then skeletonized using the same software (Fig. 14). Fractal dimension was automatically calculated by box counting method from skeletonized images using FracLac plug-in (School of Community Health, Faculty of Science, Charles Sturt University, Australia) and the result was reported in mean fractal dimension (Db).

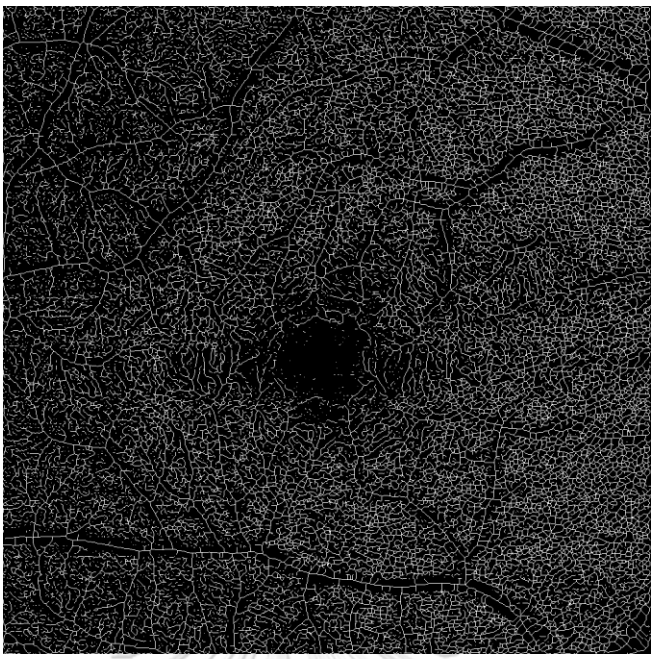


Figure 14 Skeletonized OCTA image of SCP

Foveal avascular zone (FAZ) area was calculated using automated macro function by Ishii et al.[43] By using repeated dilate and erode functions in ImageJ, central capillary ring was highlighted and FAZ area could be extracted and calculated from the center black area (Fig. 15) and reported in square millimeter (mm^2) with the reference area of the whole image is 36 mm^2 (6x6 mm).

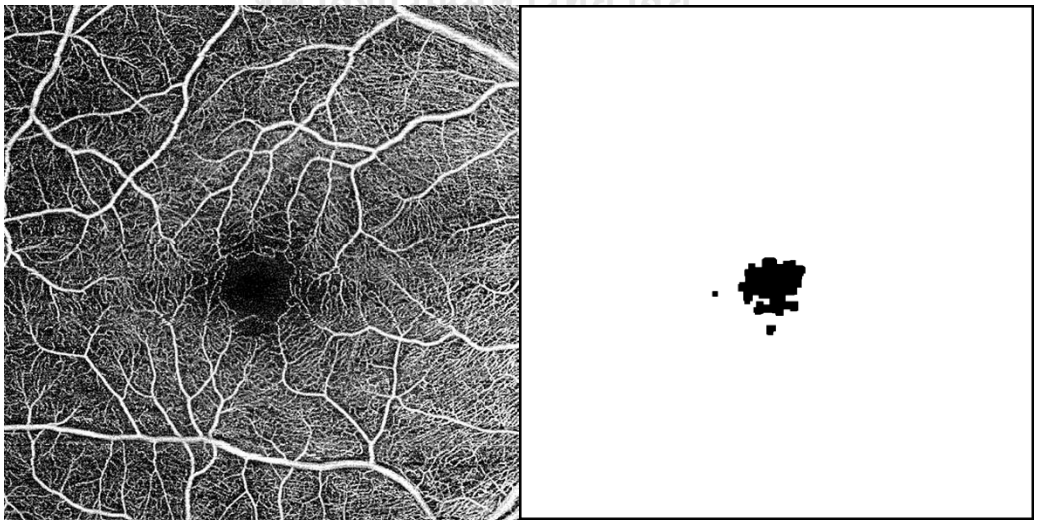


Figure 15 6x6 mm OCTA image before (left) and after (right) FAZ area is extracted

Data collection

Baseline characteristic including sex, age, weight, height, ethnicity, underlying diseases, current medication, systolic and diastolic blood pressure (SBP and DBP), side, pinhole-corrected visual acuity (VA), intraocular pressure (IOP), lens status, DR severity, axial length, serum creatinine (SCr), serum glycated hemoglobin (HbA1c), blood urea nitrogen (BUN), 24-hour urine albumin and 24-hour urine creatinine within 3 months prior the recruitment were collected. Data collecting methods are presented in Table 3. Data from swept source OCTA and automated software including VD, intercapillary area, FAZ area, FD and subfoveal choroidal thickness were collected and compared between no DN and early DN group, no DN and late DN group, and early DN and late DN group. Intraocular pressure and OCT images were obtained between 8.00 am – 10.00 am to reduce diurnal variations. All de-identified data was collected in a case record form shown in Appendix A and stored using Microsoft Excel software (Microsoft Corporation, New Mexico, USA).

Table 3 Data collecting methods for baseline characteristics

Baseline characteristics	**Unit**	**Data Collecting Methods**
Sex	-	Electronic medical record
Age	Years	Electronic medical record
Weight	kg	Weight scale on the day of observation
Height	cm	Height scale on the day of observation
Underlying diseases	-	Electronic medical record
Systolic and diastolic blood pressure	mmHg	Measured by an automatic sphygmomanometer on the day of observation
Visual acuity	Letter score	Tested on ETDRS Visual Acuity chart by a masked technician on the day of observation
Intraocular pressure	mmHg	Measured by a non-contact tonometer tested by a masked technician on the day of observation

Lens status	-	Examination by an investigator on the day of observation
DR severity	-	Graded by 2 masked investigators using wide-field fundus photographs
Axial length	mm	Measured by an optical biometer on the day of observation
Serum creatinine	mg/dL	Electronic medical record within 3 months prior the day of observation
Blood urea nitrogen	mg/dL	Electronic medical record within 3 months prior the day of observation
Serum glycated hemoglobin (HbA1c)	%	Electronic medical record within 3 months prior the day of observation
24-urine albumin	mg	Electronic medical record within 3 months prior the day of observation
24-urine creatinine	g	Electronic medical record within 3 months prior the day of observation

Data analysis

Statistical analysis was performed using STATA software version 14.0 (STATACorp LLC, Texas, USA). Data is presented as means ± standard deviations, frequencies, or percentages where appropriate. Shapiro-Wilk W test was used to test for normality. Baseline characteristic data was compared using chi-square test for both parametric and non-parametric categorical variables (sex, hypertension, insulin use, lens status, and DR severity). Continuous parametric variables (SBP, DBP) were compared between 3 groups (no DN, early DN, and late DN group) using one-way analysis of variance (ANOVA), while non-parametric variables (age, weight, height, SCr, BUN, HbA1c, 24-hour urine albumin, 24-hour urine creatinine, VA, and axial length) were tested using Kruskal-Wallis test. Due to small number of participants had diabetic retinopathy, participants with mild NPDR, moderate NPDR, severe NPDR, and PDR were categorized into DR group. Multilevel linear mixed model analysis with

post hoc pairwise comparison using Bonferroni's method was used to compare vessel density, intercapillary area, foveal avascular zone area, fractal dimension and subfoveal choroidal thickness between 3 groups with adjusted for DR status, age, sex, HbA1c level, SBP, DBP and axial length. 2-tailed *p* values were calculated using the mixed model approach regarding the eye as a unit of analysis and participant ID as the random effect. Global $p < 0.05$ is considered significant. For correlation analysis between OCTA characteristics, visual acuity, central retinal thickness and DR severity and urine albumin, average data from both eyes was used to evaluate the association and Pearson's correlation and Spearman's rank correlation were used to analyze the data and presented as Pearson's correlation coefficient (r) or Spearman's rank correlation coefficient (r_s) where appropriate.

Ethical consideration

The data collected in case record form is de-identified data. Participants will be identified as Case ID. The database contained participants' name and Case ID is kept in a secure place and can be accessed only by the principal investigator. The study was registered with Thai Clinical Trial Registry No. TCTR20210308001 and was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (COA No. 249/2021). The certificate is shown in Appendix B. The study was conducted following the tenets of the Declaration of Helsinki. Participants will receive treatment or be referred for treatment if required. Investigators will be responsible for any harm directly related to the study occurred during the period of the study and the participant will receive standard medical care. If participants refuse to participate in the study, their standard of treatment will not be affected.

CHAPTER IV Results

Baseline characteristics

A total of 188 eyes from 94 participants were enrolled into the study, but 2 eyes from 1 participant in early DN group were excluded due to media opacities which resulted in inadequate image quality (Fig. 16). All baseline characteristics are shown in Table 4.

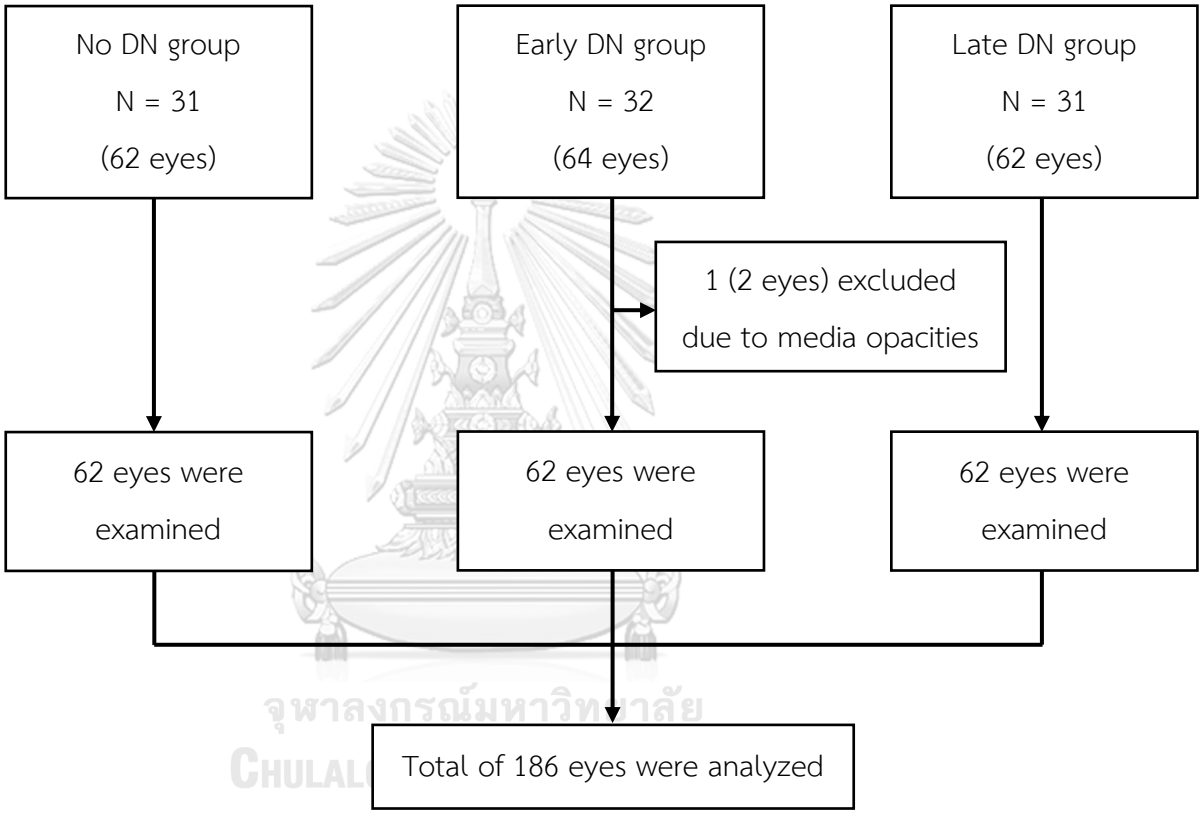


Figure 16 CONSORT diagram of study participants

Mean ages of the participants were similar among 3 groups, 65.1, 65.52, and 67.32 years in no DN, early DN, and late DN groups, respectively. There were more male participants in no DN and late DN groups (54.84% and 61.29%) but female participants were higher in early DN group (58.06%). All participants were Thais. Systolic and diastolic blood pressures (SBP and DBP) were similar among all groups.

There were no statistically significant differences in age, sex, ethnicity, SBP, and DBP between 3 groups.

Serum creatinine level was highest in late DN group (2.0 mg/dL), followed by early DN group (1.41 mg/dL) and no DN group (1.03 mg/dL). SCr was found to be significantly correlated with DN severity ($r_s = 0.62$, $p < 0.001$) (Fig. 17). As SCr levels, the BUN levels were also significantly different between 3 groups. BUN levels were also significantly correlated with SCr levels ($r_s = 0.84$, $p < 0.001$) (Fig. 18). Moreover, serum HbA1c levels were significantly different between 3 groups.

All participants in no DN group had 24-hour urine albumin levels of 6.8 mg due to lower limit of detection by the laboratory. In early and late DN groups, the mean 24-hour urine albumin level was 191.8 mg (range 53.68-285.9 mg) and 3236.84 mg (range 368.6-10506.5 mg), respectively. Numbers of participants who need insulin injection were similar in early and late DN groups (32.26% and 35.48%) which were higher than no DN group (19.35%) but there were no statistically significant differences in proportion between 3 groups.

There were no statistically differences in visual acuity between 3 groups and majority of participants in all 3 groups were phakic. In no DN group, 85.48% had no DR, 4.84% had mild NPDR, 8.07% had moderate NPDR, and 1.61% had severe NPDR. In early DN group, 90.32% had no DR, 1.61% had mild NPDR, 4.84% had moderate NPDR, and 3.23% had severe NPDR. None of the participants in no DN and early DN groups had PDR. For late DN group, the proportions were 54.84%, 4.84%, 24.19%, 4.84%, and 11.20% for no DR, mild NPDR, moderate NPDR, severe NPDR, and PDR, respectively (Fig. 19). Moreover, there were statistically differences in proportion of DR severity between the groups. The agreement between 2 fundus graders was excellent (ICC = 0.87 95% CI 0.82 – 0.9).

Table 4 Baseline characteristics of participants

Characteristics	**No DN (N=62)**	**Early DN (N=62)**	**Late DN (N=62)**	***p* value**
Age, years (range)	65.1 (39-84)	65.52 (30-84)	67.32 (45-89)	0.382[a]
Sex				0.089[b]
Male, N (%)	34 (54.84)	26 (41.94)	38 (61.29)	
Female, N (%)	28 (45.16)	36 (58.06)	24 (38.71)	
Ethnicity				
Thai, N (%)	62 (100)	62 (100)	62 (100)	
SBP, mmHg (SD)	132.94 (13.62)	130.9 (15.18)	135.42 (17.86)	0.276[c]
DBP, mmHg (SD)	72.77 (8.52)	72.26 (10.47)	71.55 (11.03)	0.793[c]
Weight, Kg (SD)	65.69 (12.06)	75.87 (15.64)	70.67 (11.64)	**0.002[a]**
Height, cm (SD)	161.61 (8.95)	161.37 (8.22)	162.76 (7.4)	0.538[a]
Hypertension, N (%)	44 (70.97)	58 (93.55)	50 (80.65)	**0.005[b]**
SCr, mg/dL (SD)	1.03 (0.42)	1.41 (0.87)	2.9 (1.51)	**<0.001[a]**
BUN, mg/dL (SD)	16.77 (7.3)	24.87 (14.4)	38.35 (16.63)	**<0.001[a]**
HbA1c, mg% (SD)	6.82 (1.16)	6.68 (0.81)	6.38 (0.87)	**0.049[a]**
24h urine albumin, mg (SD)	<6.8 (0)*	191.8 (54.82)	3236.84 (2701.72)	**<0.001[a]**
24h urine creatinine, g (SD)	1.14 (0.32)	1.84 (4.09)	1.06 (0.33)	0.289[a]
Insulin use, N (%)	12 (19.35)	20 (32.26)	22 (35.48)	0.112[b]
Visual acuity, letters (SD)	79.13 (8.58)	79.1 (7.06)	75.73 (11.87)	**0.048[a]**
Lens status				0.163[b]
Phakic, N (%)	48 (77.42)	40 (64.52)	39 (62.9)	
Pseudophakic, N (%)	14 (22.58)	22 (35.48)	23 (37.1)	
Axial length, mm (SD)	23.72 (1.14)	23.45 (0.99)	23.41 (0.85)	0.289[a]
DR severity				**<0.001[b]**
No DR, N (%)	53 (85.48)	56 (90.32)	34 (54.84)	
Any DR, N (%)	9 (14.52)	6 (9.68)	28 (45.16)	

Using eye as a unit of analysis. All data are presented in mean unless indicated.

*Lower limit of detection of urine protein is 6.8 mg.

[a] Kruskal-Wallis test. [b] Chi-square test. [c] Analysis of Variance (ANOVA). Bold values indicate $p < 0.05$.

Abbreviation: DN – diabetic nephropathy; SBP – systolic blood pressure; DBP – diastolic blood pressure, SCr – serum creatinine; BUN – blood urea nitrogen; DR – diabetic retinopathy; NPDR – nonproliferative diabetic retinopathy; PDR – proliferative diabetic retinopathy

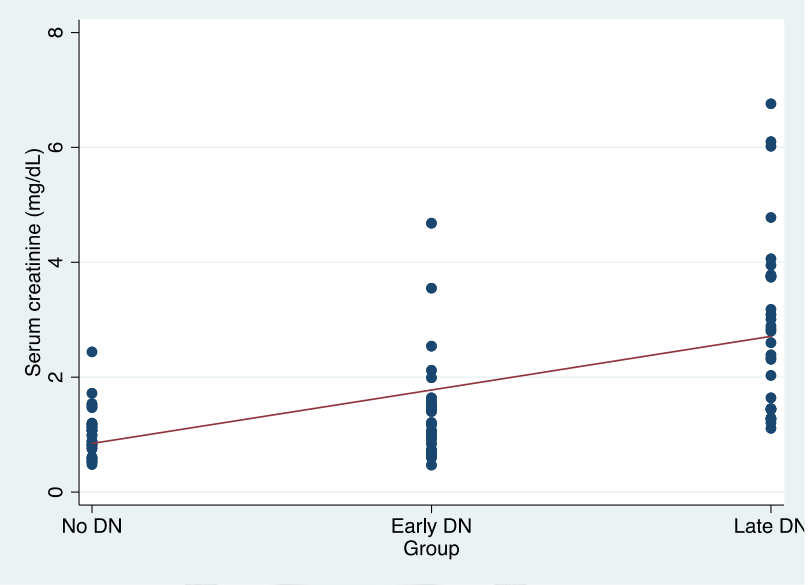


Figure 17 Scatter plot showing relationship between serum creatinine and diabetic nephropathy status

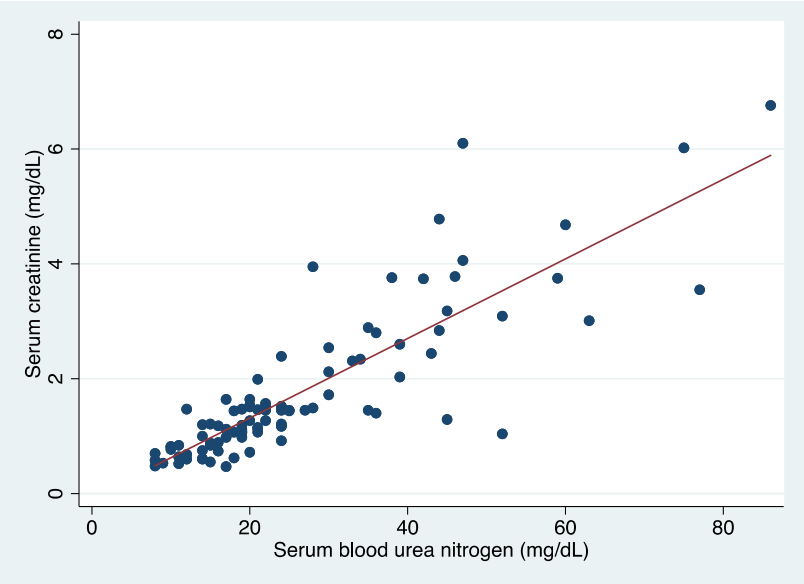


Figure 18 Scatter plot showing relationship between serum creatinine and serum blood urea nitrogen

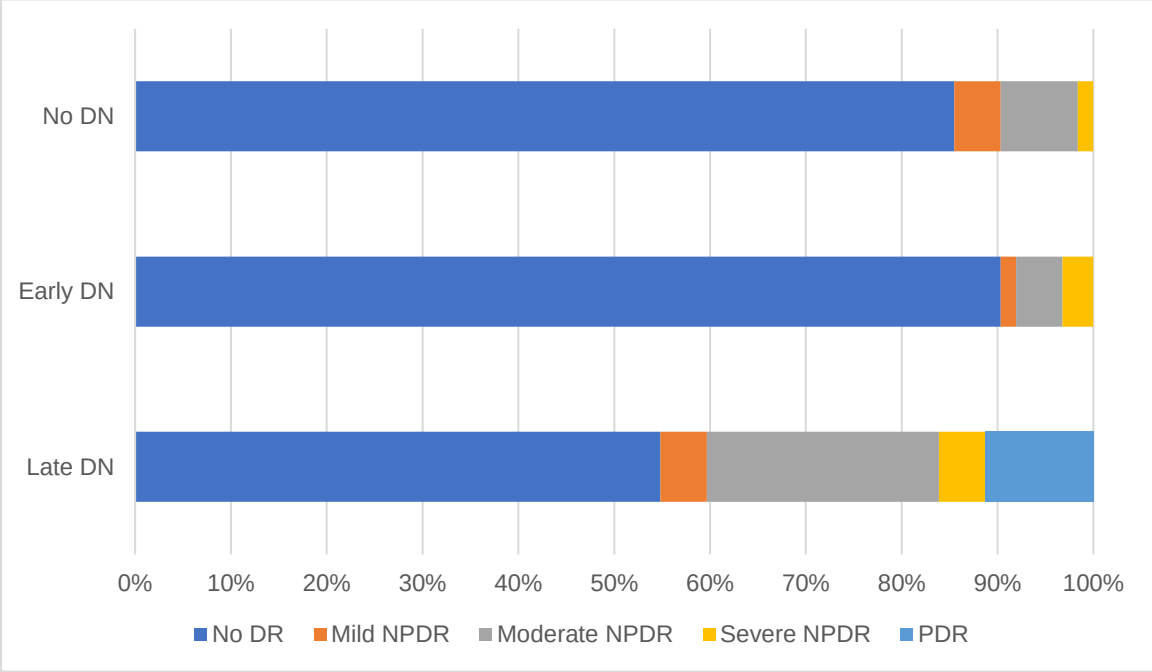


Figure 19 Stacked bar chart showing proportions of diabetic retinopathy severity among participants in each group

Vessel densities and diabetic nephropathy

The mean VD values of 3 groups are presented in Table 5. Mean VD values of SCP were significantly different between DR severity (p = 0.038) and were significantly lower in participants with any DR compared no DR (p = 0.045). Trends of reduction in mean VD of all layers were found with increasing 24-hour urine albumin. There were significant differences in mean VD of SCP, DCP and whole retina among participants in no DN groups compared to late DN groups (SCP p = 0.02; DCP p = 0.008; whole retina p = 0.023). But after adjusted for age, sex, HbA1c, SBP, DBP, DR status, axial length, and lens status, only VD values of DCP and whole retina were significantly different between groups (DCP p = 0.032; whole retina p = 0.021). Moreover, there were no significant differences in mean VD of SCP and DCP between no DN group and early DN group (SCP p = 0.061; DCP p =0.051). But after adjusted for aforementioned factors, there was significant reduction in both mean VD of SCP and DCP (SCP p = 0.007; DCP p = 0.003). Interestingly, significant reduction was also found in mean VD of whole retina between no DN group and early DN group (p = 0.032) and the significance persisted after adjusting for confounding factors (p = 0.003). Additionally, no significant differences were found in all the mean VD values compared between early DN group and late DN group.

Logistic regression analysis between VD values and risk of developing diabetic nephropathy was done. Result showed significantly increased risk of developing any degree of DN in participants with decreasing VD values of SCP (OR = 1.13, 95% CI 1.04 - 1.24, p = 0.005). Participants with decreased VD values of DCP and whole retina also have significantly increased risk of developing any degree of DN (DCP – OR = 1.16, 95% CI 1.06 – 1.28, p = 0.002; whole retina – OR 1.13, 95% CI 1.04 – 1.23, p = 0.004).

Spearman's correlation analysis was also done between 24-hour urine albumin level and average VD value of both eyes of participants. We found a significant negative correlation between 24-hour urine albumin level and average VD value of SCP but the correlation was weak (r_s = -0.26, p = 0.013). Significant correlations between 24-hour urine albumin level and average VD values of DCP and

whole retina were also weak (DCP – r_s = -0.30, p = 0.004; whole retina – r_s = -0.28, p = 0.007).

Table 5 Mean vessel density values of the groups

	No DN (N=62)	Early DN (N=62)	Late DN (N=62)	Adjusted p value[a]		
				No DN and Early DN	No DN and Late DN	Early DN and Late DN
SCP	48.35 (3.31)	46.8 (4.09)	46.44 (3.92)	**0.007**	0.135	0.307
DCP	47.18 (3.26)	45.74 (3.35)	45.21 (3.61)	**0.003**	**0.032**	0.558
Whole retina	44.88 (4.12)	42.91 (4.52)	42.8 (4.2)	**0.003**	**0.021**	0.613

Values are presented as mean (standard deviation). Statistical analysis using linear mixed-model analysis

[a] Adjusted for age, sex, hemoglobin A1c, SBP, DBP, diabetic retinopathy severity, axial length, and lens status. Bold values indicate $p < 0.05$.

Abbreviation: SCP – superficial capillary plexus; DCP – deep capillary plexus.

Other OCTA parameters and diabetic nephropathy

Mean fractal dimension, intercapillary area, FAZ area, CRT, and subfoveal CT are presented in Table 6. For fractal dimension, there is a tendency to decrease with increasing 24-hour urine albumin. However, the significant difference only found between no DN group and late DN group (p = 0.022) and the significance disappeared when adjusting for confounding factors (p = 0.056). After doing logistic regression analysis using mean FD in the first quartile as a cut point, participants with FD less than 1.8401 Db had significantly increased risk of developing macroalbuminuria (OR = 2.25, 95% CI 1.12 – 4.5, p = 0.022).

Similarly, there is a trend of increasing intercapillary area with increasing 24-hour urine albumin. Only significant increase was found between no DN group and late DN group (p = 0.02), but after adjusting for confounding factors, there were no significant differences (p = 0.139). Logistic regression analysis revealed that

participants with increased intercapillary area had significantly increased risk of developing any degree of DN (OR = 1.42, 95% CI 1.11 – 1.81, p = 0.005). No significant differences were found in FAZ area, CRT, and subfoveal CT between the groups.

In addition, no significant correlations were found between 24-urine albumin level and intercapillary area, FAZ area, FD, CRT, and subfoveal CT (p = 0.125, 0.642, 0.065, 0.946, and 0.724, respectively)

Table 6 Mean OCTA parameters values of the groups

	No DN (N=62)	Early DN (N=62)	Late DN (N=62)	Adjusted p value[a]		
				No DN and Early DN	No DN and Late DN	Early DN and Late DN
Fractal dimension (Db)	1.8413 (0.002)	1.8411 (0.0019)	1.8401 (0.0031)	0.668	0.056	0.111
Intercapillary area (mm^2)	18.59 (1.19)	19.15 (1.47)	19.28 (1.41)	0.071	0.139	0.506
FAZ area (mm^2)	0.279 (0.084)	0.279 (0.095)	0.266 (0.093)	0.822	0.858	0.975
Central retinal thickness (μm)	229.08 (53.63)	224.47 (44.54)	237.1 (57.29)	0.521	0.941	0.601
Subfoveal choroidal thickness (μm)	228.87 (90.14)	232.42 (93.34)	228.5 (79.82)	0.640	0.737	0.912

Values are presented as mean (standard deviation). Statistical analysis using linear mixed-model analysis

[a] Adjusted for age, sex, hemoglobin A1c, diabetic retinopathy severity, and axial length. Bold values indicate p <0.05.

Abbreviation: Db – fractal dimension by box counting method; FAZ – foveal avascular zone.

Correlation between visual function and anatomy and 24-hour urine albumin

There were no statistically significant correlations between VA and 24-hour urine albumin level (p = 0.566). DR severity was also not found to be significantly

correlated with 24-hour urine albumin level (p = 1.00). Lastly, no significant correlations between CRT and 24-hour urine albumin level were found (p = 0.946).

CHAPTER V Discussion

Diabetes mellitus and its complications are still a global burden for both patients and healthcare systems. Even though there are many modern treatments available, microvascular complications are still a major factor at affect patients' quality of life. Asymptomatic phase of type II diabetes can last from 4-7 years.[44] Patients with newly diagnosed type II DM may already have diabetic complications. Even in targeted screen procedure, the prevalence of microvascular complications was not different from symptomatic patients in clinical practice.[44] To reduce morbidity and mortality from diabetic complications, early screening, diagnosis, and treatment are essential.

Diabetic nephropathy is one of the major diabetic microvascular complications. An estimation of 40% of diabetic patients will develop kidney dysfunction.[1] DN is also a leading cause of renal replacement therapy worldwide.[1] A screening method for DN requires urine chemistry test which reflects kidney functional changes. However, anatomical changes that precede functional changes can only be directly demonstrated by an invasive renal biopsy.[6] Interestingly, from the result of our study, majority of participants in early DN group had no clinically detectable DR which was more than the result of previous studies.[15, 37]

As microvascular complications of DM are systemic and each affected organs tend to be associated. Although causal or chronological relationship might not be fully understood, many studies showed clinical correlations between DN and DR.[11-19] Histopathological studies confirmed this association.[21, 22] Therefore, anatomical pathologies observed in retinal microvasculature may reflect similar changes in renal vessels. Such observations can lead to earlier identification and diagnosis of those who are at risk of DN. Many retinal lesions and characteristic detected by fundus examination, fundus photography, or fluorescein angiography are proven to be associated with declined renal function.[5, 6, 11, 24-28]

With the development of OCTA which can be a replacement of the more invasive FA in many aspects. This new technology has been proven to be useful in many diseases including detecting pre-clinical DR.[29] But its use in detecting systemic

diseases is less explored. With these reasons, in this study, we aim to investigate microvascular characteristic detected in patients with DN. We found that there were significant reductions in mean VD of SCP, DCP, and whole retina in participants in late DN group compared to no DN group. But after adjusting for age, sex, HbA1c, DR severity, and axial length, significances were found only in mean VD of DCP and whole retina. Additionally, we also found statistically significant decrease in mean VD of SCP, DCP, and whole retina in early DN group compared to no DN group after adjusting for confounding factors. The reason that DCP showed significant difference between groups might be due to the inclusion of major vessels in the SCP images. While the study by Cankurtaran et al. reported significant differences of these 2 groups only in SCP layer. The previous study also described significant differences in mean VD in both SCP and DCP between early DN group and healthy individuals.[7] We also found that decreasing VD values significantly increases risk of developing any degree of DN. From the result of this present study and previous study[7], we propose that mean VD values of whole retina might be a useful tool in microalbuminuria screening. Further studies might be needed to identify the cutoff mean VD value that warrant detailed investigation. Additionally, the correlations between 24-hour urine albumin level and average VD values were found but the correlations were weak. Larger sample size might be able to reveal a stronger correlation and a urine albumin prediction formula can be developed.

Although the association between intercapillary area and eGFR was reported by Cheung et al.[31], we could only find significant difference between no DN group and late DN group and these differences were not presented when adjusted for confounding factors.

Previous study by Sng et al.[28] demonstrated a U-shaped relationship between the prevalence of CKD and quintiles of retinal vascular fraction dimension calculated from fundus photography. In our study, only fractal dimension of SCP from OCTA in no DN group were significantly different from late DN group. But we were able to identify that FD value less than 1.8401 significantly increases the risk of developing DN. However, different imaging protocol and algorithm can affect FD

values. More studies are needed to identify other factors that can affect vascular fractal dimension.

Similar to Cankurtaran et al.[7], we could not find any associations between mean FAZ area and DN. However, a prospective case-control study by Ahmadzadeh Amir et al.[34] described significantly larger FAZ area of SCP, DCP, and whole retina in participants with overt nephropathy compared with those without. Enlargement of FAZ is contributed by many factors apart from capillary endothelial dysfunction, such as capillary dropout and increased vascular endothelial growth factor. Even in healthy participants FAZ area was found to be different. FAZ area might be related to DR severity more than DN.[45]

Our study showed no statistically significant relationship between CRT and DN which is comparable to the result from Cankurtaran et al.[7] One possible explanation is that participants with diabetic macular edema or have received anti-endothelial vascular growth factors were excluded. The mean CRT might not reflect the true value among population in each group.

In contrast to Kocasarac et al.[36] who addressed significant thinner of CT in patients with DN compared to diabetic patients without DN and healthy control, we could not detect significant differences in subfoveal CT between the 3 groups. The correlation between 24-urine albumin and intercapillary area, fractal dimension, FAZ area, CRT, and subfoveal CT also could not be established. These results may be owing to the sample size of this present study that might be too small to yield the power needed to detect the differences in the OCT and OCTA characteristics other than VD.

Lastly, we could not observe significant correlation between 24-urine albumin level and VA, DR severity, and CRT. Once again, these findings could be from the exclusion of diabetic macular edema patients and patients who received anti-vascular endothelial growth factors or retinal laser therapy.

The differences in result from previous study might be due to the imaging protocol from each OCTA machine. The definition of vascular layers also varies between OCTA machines. Standardized protocol and segmentation are important in

establishing an effective screening tool. An automated image analysis software that incorporates into the OCTA machine can also be useful in further research.

Glomerular endothelium, basement membrane, and podocytes are important barriers to macromolecules. Dysfunction in one of these structures could result in protein loss in urine. Previous studies proved that microalbuminuria is significantly associated with major cardiovascular diseases and progressive kidney dysfunction and could be a sign of systemic endothelial dysfunction.[46] From the result of this study, VD values from OCTA could detect DN in the early microalbuminuria stage and potentially prevent serious morbidity and mortality from diabetes. This could be a new useful prediction model in diabetic patients. And with enough data, OCTA screening might be incorporate into routine DR screening to detect potential kidney complications and other systemic diabetic microvascular complications.

To our knowledge, this is a novel that aim to investigate relationship between vessel density values on OCTA and 24-hour urine albumin. One of the strengths of this study is that we conducted sample size calculation to ensure enough power to differentiate the result. Another strong point is the inclusion criteria of the study, especially the use of 24-hour urine albumin that reflects kidney functional evaluation used in real clinical setting. Additionally, most of the measurements in the present study were done by automated software thus bias was reduced.

However, this study still had some limitations. Primary limitation of the study is its cross-sectional design which can only establish the association, not causal or chronological relationship. We also did not recruit healthy individual as a control group. The causal and temporal relationship between retinal and kidney lesions is yet to be explored. Secondly, VD measurements were done in the whole image area, not separately measured in parafoveal or perifoveal areas. But as reported by Cankurtaran et al.[7], the significance of each area in SCP and DCP was similar. Due to limitation of OCTA machine, we had to use another software to analyze the images. And there is no consensus about standard binarization algorithm, thus VD values can vary among studies. Using the manufacturer's software in the machine which is proper calibrated might improve the accuracy. Another minor point is that DR severity grading was done using fundus photographs which the field of view was

limited but it can be useful in the generalization to telemedicine setting. Finally, due to missing and inaccurate data, we did not include history of DM duration in the analysis. But DR severity might partially reflect the DM duration.

CHAPTER VI Conclusion

The result of this study suggests the retinal microcirculation changes occur in concert with analogous pathological changes in kidney. Moreover, these retinal biomarkers especially vessel density value, can be detected by OCTA in the early stage of the disease. Vessel density values of deep capillary plexus and the whole retina were significantly associated with diabetic nephropathy status. Decreasing vessel density value also significantly associated with increasing 24-hour urine albumin. More detailed studies are needed to explore these relationships in a larger different population to determine a standardize protocol and cutoff values in a prediction model. However, we could not determine the significant relationship between fractal dimension, intercapillary area, foveal avascular zone area, central retinal thickness, and subfoveal choroidal thickness and diabetic nephropathy status. Moreover, correlation between 24-hour urine albumin and visual acuity, DR severity, and central retinal thickness could not be established

APPENDIX A

Case Record From

Case Record Form **Participant ID** | | | |

Age ..... Sex ☐ M ☐ F BP ...../..... Wt. ..... kg Ht. ..... cm

Ethnicity ☐ Thai ☐ Thai-Chinese ☐ Caucasian ☐ .....

Underlying ☐ DM ☐ HT ☐ DLP ☐ Thyroid ☐ .....

Current medication .....

SCr ..... mg/dL BUN ..... mg/dL HbA1c ..... %

24-hour urine albumin ..... mg 24-hour urine creatinine ..... mg

Group ☐ **No DN** ☐ **Early DN** ☐ **Late DN**

Eye examination

Characteristic	OD	OS
BCVA (letter score)		
IOP		
Cornea		
A/C		
Lens		
Fundus		
DR severity		
VD Whole layer		
VD SCP		
VD DCP		
Intercapillary area		
FAZ area		
FD		
Subfoveal CT		
CRT		
Axial length		

APPENDIX B

Certificate of Approval from the Institutional Review Board

COA No. 549/2021

IRB No. 263/64

INSTITUTIONAL REVIEW BOARD

Faculty of Medicine, Chulalongkorn University

1873 Rama 4 Road, Pathumwan, Bangkok 10330, Thailand, Tel 662-256-4493

Certificate of Approval

The Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, has approved the following study which is to be carried out in compliance with the International guidelines for human research protection as Declaration of Helsinki, The Belmont Report, CIOMS Guideline and International Conference on Harmonization in Good Clinical Practice (ICH-GCP)

Study Title	: Optical Coherence Tomography Angiography Biomarkers in Diabetic Nephropathy Patients in Thailand: A Diabetic Eye and Kidney Diseases (DEK-D) Study
Study Code	: -
Principal Investigator	: Nuntachai Surawatsatien, M.D.
Affiliation of PI	: Department of Ophthalmology, Faculty of Medicine, Chulalongkorn University.
Review Method	: Expedited
Continuing Report	: At least once annually or submit the final report if finished.

Document Reviewed :

1. Research Proposal Version 2.0 Date 8th April 2021
2. Protocol Synopsis Version 1.0 Date 8th March 2021
3. Information sheet for research participant Version 2.0 Date 8th April 2021
4. Consent to participate in the project for volunteers Version 1.0 Date 8th March 2021
5. Case Record Form Version 1.0 Date 8th March 2021

Approval granted is subject to the following conditions: (see back of this Certificate)

6. Curriculum Vitae and GCP Training
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- Assoc.Prof. Nattachai Srisawat, M.D., Ph.D.
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- Major Thanapong Somkijrungroj, M.D.
- Apivat Mavichak, M.D.
- Wijak Kongwattananon, M.D.
- Disorn Suwajanakorn, M.D.

Signature .....
(Emeritus Professor Tada Sueblinvong MD)
Chairperson
The Institutional Review Board

Signature .....
(Associate Professor Onanong Kulaputana MD, PhD)
Member and Assistant Secretary, Acting Secretary
The Institutional Review Board

Date of Approval : April 23, 2021
Approval Expire Date : April 22, 2022

Approval granted is subject to the following conditions: (see back of this Certificate)

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