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Retinal vessel tortuosity measures and their applications

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A B S T R A C T

Structural retinal vascular characteristics, such as vessel calibers, tortuosity and bifurcation angles are increasingly quantified in an objective manner, slowly replacing subjective qualitative disease classification schemes. This paper provides an overview of the current methodologies and calculations used to compute retinal vessel tortuosity. We set out the different parameter calculations and provide an insight into the clinical applications, while critically reviewing its pitfalls and shortcomings.

1. Introduction

Cardiovascular disease (CVD) causes nearly half of all deaths in Europe (48%), whereas high blood pressure (BP) is the most common treatable risk factor for adverse CVD (Allender et al., 2008). There is an increased interest in measuring the ocular circulation as a means of directly assessing the microcirculation by non-invasive technologies (McClintic et al., 2010; Sasongko et al., 2010b). Caliber changes in retinal vessels (arteries and veins) have been shown to be valuable markers in the risk assessment of CVD and stroke (Doubal et al., 2009; McGeechan et al., 2009; Wong, 2004).

The retinal microvasculature is readily accessible for *in vivo* examination, allowing time and cost efficient imaging which is highly repeatable using photographic techniques (Couper et al., 2002; Sherry et al., 2002). The eye itself is one of the target organs affected by high BP and vascular dysregulation, hence reflects vascular damage of systemic origin (Lockhart et al., 2009). Therefore, one could argue that structural retinal vessel changes (i.e. diameters, tortuosity and branching patterns) can be used as surrogate indicators of CVD risk. Direct assessments of systemic macro-vascular function have been long established and include amongst others, arterial compliance measurements (Haluska et al., 2010) and flow mediated dilation of the brachial artery (Ghiadoni et al., 2012); but often give information only on arterial function while being costly, highly variable and require elaborate training and experience, as well as specialized software. In addition, these techniques neither provide information on other tissue sites nor on the microcirculation and often are unsuitable for regular screening due to the aforementioned reasons.

Although a considerable amount of epidemiological studies (Funagata Study, Rotterdam Study, SiMES,[1] ARIC,[2] MESA,[3] BDES,[4] BMES,[5] CHS[6]) have been conducted to assess the relationship between systemic disease and retinal vascular signs, such as retinal arteriolar narrowing, nicking, arterio-venous ratio and retinal vessel calibers (Nguyen et al., 2007), their findings rarely report on vessel tortuosity. Witt et al. (2006) report a strong correlation of risk of ischemic heart disease death and altered structural retinal vessel parameter, namely decreased vessel diameter and increased tortuosity, whereas others found associations of systemic disease and retinal measures to be dependent on age, gender and ethnicity (Nguyen et al., 2008; Sun et al., 2008).

In the early days, a plethora of subjective clinical grading schemes (Keith et al., 1939; Scheie, 1953; Leishman, 1957) had been introduced in order to classify general vessel appearance, including tortuosity, branching patterns and other retinal features. As any subjective grading, this has been highly dependent on grader experience (Kagan et al., 1966). The leap from subjective (visual grading) to objective assessment of vessel tortuosity did not happen until 1979, by Lotmar et al. (1979). Increased computer power, convenient image acquisition and sophisticated analysis have substantially enhanced objective techniques. However, due to a lack in standardizing image acquisition and parameter calculation as well as the complex image processing, they are rarely used in clinical environments.

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[1] Singapore Malay Eye Study.
[2] Atherosclerosis Risk in Communities Study.
[3] Multiethnic Study of Atherosclerosis.
[4] Beaver Dam Eye Study.
[5] Blue Mountains Eye Study.
[6] Cardiovascular Health Study.

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Increased tortuosity has been reported in a number of pathologies and genetic disorders such as systemic hypertension (Witt et al., 2006; Hughes et al., 2006, 2008; Thom et al., 2009; Cheung et al., 2011a,b), diabetic retinopathy (Sasongko et al., 2011), adolescent type 1 diabetes (Sasongko et al., 2010a; Benitez-Aguirre et al., 2011), plus disease in retinopathy of prematurity (ROP) (Capowski et al., 1995; Wallace et al., 2000, 2003; Gelman et al., 2005; Wallace et al., 2007b; Koreen et al., 2007; Gelman et al., 2007; Chiang et al., 2007) gestational diabetes mellitus (Boone et al., 1989), familial retinal arteriolar tortuosity (fRAT) (Sutter and Helbig, 2003; Nischler et al., 2011), chronic anemia (Incorvaia et al., 2003) and facioscapulohumeral muscular dystrophy (Longmuir et al., 2010).

The lack of a standardized protocol and reliance on local (i.e. small area) metrics instead of more global parameters to achieve better risk stratification has led to these indices being used for research only. A further limiting factor is the fact that systemic parameters are rarely measured at the same time as tortuosity measures, hindering the assessment of its full potential as a surrogate marker for diagnostic purposes, progression monitoring and treatment efficacy evaluation. Instead of standardizing image acquisition, parameter calculation and analysis, a continuously increasing amount of tortuosity indices are being introduced. To give an overview of the most widely used indices and their potential value as a surrogate marker for systemic vascular disease we compiled this systematic review on retinal vessel tortuosity measures.

2. Materials and supplies

2.1. Search strategy

We searched the PubMed (Medline) and Web of Science databases from their respective inception date to June 2012 in order to identify relevant articles. The search queries for each database were conceptually similar but adjusted to each search engine accordingly. Combination searches of the keywords *tortuosit*** and *retina** or *fundus* occurring either in the title or the abstract were performed.

2.2. Inclusion/exclusion criteria

We included publications from any language. In case of non-English manuscripts, their translated abstracts were used to identify relevant articles. We selected studies dealing with the human retinal vasculature only, unless key definitions of tortuosity were given that apply to more than one vascular bed. At least one quantitative tortuosity index in the two dimensional space should be reported for the study to be included in our analysis. If data were duplicated or shared in more than one study, only the larger study was included. Case reports, pilot studies, review papers and animal studies were excluded from our analysis.

2.3. Data extraction and analysis

Results were scrutinized in the first instance based on their title and abstract in order to identify papers fulfilling the inclusion criteria. The full text of those publications was retrieved and reviewed independently by two authors (AK, RH). Their references were scanned and additional relevant articles were identified.

3. Detailed methods

Most studies analyzing vessel tortuosity are based on digital images of the retina and are subsequently post-processed in order to assess quantitative vascular tortuosity parameters. An overview of the various formulas used is shown in Table 1. In the following tables (Tables 2–5) a per software analysis is presented for each individual study that fulfilled the inclusion criteria. Details on population demographics and tortuosity indices are provided, including variables studied and resulting associations identified.

4. Potential pitfalls and trouble shooting

There are a wide variety of parameters and formulas available to calculate retinal vessel tortuosity. Unfortunately, there is no standard to date regarding image acquisition, measurement location and calculation. This makes direct comparison of tortuosity numerical values across studies at the least very difficult, if not impossible. Definitions used are some times not disclosed either (Stettler et al., 2009). In addition, software used to quantify retinal vessel tortuosity listed in this review, namely SIVA, CAIAR, ROPtool and RISA are custom made and not available in the public domain. Some of them are patent-protected too. Although there is a wide range of parameter formulas published (Table 1) there is no evidence that the more sophisticated approaches yield better clinical discrimination (Witt et al., 2006). Hence, the use of the distance factor (DF) formula offers a simple and quick method to assess vessel tortuosity, baring in mind its shortcomings at the same time.

4.1. Simple guide to measure retinal vessel tortuosity

The first step in quantifying retinal vessel tortuosity is to acquire an image of the retina. Nowadays the majority of retinal cameras in use are digital, making further image analysis easier. The fundus image must be of good contrast and in focus. When using mydriatic retinal cameras instillation of drops is required prior to obtaining an image. Depending on the camera angle chosen, area of interest and disease studied a single or multiple images (for assessment of larger areas) is necessary. Regarding the type of image obtained they can be either colored or monochromatic; the former should always be converted to monochromatic by extracting the green channel from the composite RGB image, because it offers the highest contrast. Some retinal cameras incorporate a green filter and therefore capture red-free images, with no need for further post-processing. The software listed above mostly apply an automated vessel detection algorithm while relying on manual observer verification. A simple and cost-efficient alternative to the customized software listed is manual or semi-automatic measurement of vessel tortuosity by using ImageJ (Abramoff et al., 2004). This freely available software allows the observer to create scripts and modify it according to analysis needs. Using ImageJ the observer can upload retinal images for tortuosity analysis of most current image formats (including TIFF, JPEG, BMP, etc.). Once uploaded one can manually measure tortuosity for example between branching points by selecting the vessel length between branches (arc length) and then selecting the shortest distance between these branches (chord length) to calculate simple tortuosity using the following formula

$$\text{Tortuosity} = \frac{\text{Arc length}}{\text{Chord length}} \tag{1}$$

(see also Table 1, Equation (1)). In case of multiple analysis and for larger study cohorts ImageJ can be customized and automated for further analysis by creating macros.

4.2. Parameter calculation

One of the physical principles believed to govern the architecture of mammalian vascular trees is its optimum arrangement so that the cost of work for blood delivery through it, is minimized (Murray, 1926). Healthy retinal blood vessels are fairly straight

Table 1
Definitions of tortuosity indices used across literature. DF: distance factor; CAIAR: computer-aided image analysis of the retina.

#	Tortuosity index (notation or unit)	Formula or expression	References
1	$\frac{\text{Arc length}}{\text{Chord length}}\ (\text{DF})$	$\dfrac{\int_a^b \sqrt{(x'(t))^2+(y'(t))^2}\mathrm{d}t}{\sqrt{(x(a)-x(b))^2+(y(a)-y(b))^2}}$	Benitez-Aguirre et al. (2012, 2011), Zepeda-Romero et al. (2011), Mahal et al. (2009), Hughes et al. (2008), Chiang et al. (2007), Koreen et al. (2007), Ferrara et al. (2007), Hughes et al. (2006), Gelman et al. (2005), Eze et al. (2000), Smedby et al. (1993)
2	$\frac{\text{Arc length}}{\text{Chord length}}-1(\tau_1)$	$\dfrac{\int_a^b \sqrt{(x'(t))^2+(y'(t))^2}\mathrm{d}t}{\sqrt{(x(a)-x(b))^2+(y(a)-y(b))^2}}-1$	Thom et al. (2009), Hughes et al. (2009), Tapp et al. (2007), Witt et al. (2006), Dougherty and Varro (2000), Hart et al. (1999, 1997)
3	Total curvature (τ_2)	$\int_a^b \left\|\frac{x'(t)y''(t)-x''(t)y'(t)}{[(y'(t))^2+(x'(t))^2]^{3/2}}\right\|\mathrm{d}t$	Bhuiyan et al. (2010), Hart et al. (1999, 1997), Smedby et al. (1993)
4	Total squared curvature (τ_3)	$\int_a^b \frac{[x'(t)y''(t)-x''(t)y'(t)]^2}{[(y'(t))^2+(x'(t))^2]^3}\mathrm{d}t$	Hart et al. (1999, 1997)
5	$\frac{\text{Total curvature}}{\text{Arc length}}(\tau_4)$	$\dfrac{\int_a^b \left\|\frac{x'(t)y''(t)-x''(t)y'(t)}{[(y'(t))^2+(x'(t))^2]^{3/2}}\right\|\mathrm{d}t}{\int_a^b \sqrt{(x'(t))^2+(y'(t))^2}\mathrm{d}t}$	Hart et al. (1999, 1997)
6	$\frac{\text{Total squared curvature}}{\text{Arc length}}(\tau_5)$	$\dfrac{\int_a^b \frac{[x'(t)y''(t)-x''(t)y'(t)]^2}{[(y'(t))^2+(x'(t))^2]^3}\mathrm{d}t}{\int_a^b \sqrt{(x'(t))^2+(y'(t))^2}\mathrm{d}t}$	Crosby-Nwaobi et al. (2012), Li et al. (2012), Cheung et al. (2011a), Lim et al. (2011), Cheung et al. (2011b), Koh et al. (2010), Sasongko et al. (2012a,b, 2011, 2010a), Hughes et al. (2009), Witt et al. (2006), Hart et al. (1999, 1997)
7	$\frac{\text{Total curvature}}{\text{Chord length}}(\tau_6)$	$\dfrac{\int_a^b \left\|\frac{x'(t)y''(t)-x''(t)y'(t)}{[(y'(t))^2+(x'(t))^2]^{3/2}}\right\|\mathrm{d}t}{\sqrt{(x(a)-x(b))^2+(y(a)-y(b))^2}}$	Hart et al. (1997, 1999)
8	$\frac{\text{Total squared curvature}}{\text{Chord length}}(\tau_7)$	$\dfrac{\int_a^b \frac{[x'(t)y''(t)-x''(t)y'(t)]^2}{[(y'(t))^2+(x'(t))^2]^3}\mathrm{d}t}{\sqrt{(x(a)-x(b))^2+(y(a)-y(b))^2}}$	Tam et al. (2011), Hart et al. (1999, 1997)
9	Integrated curvature (rad/pixel)	$\dfrac{\sum\theta}{\sqrt{(x(a)-x(b))^2+(y(a)-y(b))^2}}$	Zepeda-Romero et al. (2011), Chiang et al. (2007), Koreen et al. (2007), Gelman et al. (2005)
10	Smooth tortuosity index	$\dfrac{\text{Arc length}}{\text{Cubic}-\text{spline interpolated curve length}}$	Wallace et al. (2009, 2007a)
11	CAIAR tortuosity index	Series of 12 tortuosity indices based on changes in subdivided chord lengths. See appendix in Wilson et al. (2008) for details.	Owen et al. (2011, 2009), Wilson et al. (2008)
12	Tortuosity coefficient 02	Sum of second differences of the vessel midline coordinates divided by the sampling interval	Dougherty and Varro (2000)
13	Tortuosity coefficient 01	Sum of differences of the vessel midline coordinates divided by the sampling interval	Eze et al. (2000)
14	Standard deviation tortuosity	Standard deviation of distribution of the vessel's midline incremental lateral displacements sampled at constant increments	Wenn and Newman (1990)

when observed in a close-up (e.g. area of 1 disk diameter (DD)) and gently curved when observing the bigger picture (e.g. area of 2–3 DD). In numerous pathological conditions, as previously mentioned, vessels may malform and become tortuous, either locally, or over an extended area, or both. In clinical practice, the gold standard of severity assessment of the vessels' structural departure from normality is visual grading carried out by experienced examiners. Classification is made on a subjective scale: no tortuosity, mild, moderate or severe. Clearly, this is far from being flawless. Outcomes may fluctuate along various levels of experience amongst graders, it is time-consuming and tedious to assess a series of retinal photographs and possible variations of background contrast or illumination can alter the grader's perception of tortuosity.

Attempts to objectively quantify the clinician's intuitive notion of tortuosity are ongoing but far from being concluded. The majority of literature reporting on objective tortuosity parameters is based on measures initially discussed by Smedby et al. (1993) and further expanded by Hart and colleagues (Hart et al., 1997, 1999). Due to its calculation simplicity, the relative length increase over a straight vessel, which is commonly referred to as the distance factor (DF) is the most widely used tortuosity index (or variants thereof (τ_1)). Intuitively, in the case of a perfectly straight vessel segment it equals to the minimum value of 1. Thus, the more

Table 2
Studies using the Singapore I vessel assessment (SIVA) software, reporting on tortuosity index outcomes. (MA)BP: (mean arterial) blood pressure; DF: distance factor; SiMES: Singapore Malay Eye Study; PDR: proliferative diabetic retinopathy; AL: axial length; SE: spherical equivalent; DR: diabetic retinopathy; A: arteriolar tortuosity; V: venular tortuosity; AV: arteriolar and venular tortuosity combined; ↑: positive association; ↓: negative association; —: no association; *: borderline trend.

Authors (year)	Sample size (age)	Tortuosity index	Variable(s) studied	Association(s)
Li et al. (2012)	665 pregnant women (18–46)	τ_5	Blood pressure	— A — V
Benitez-Aguirre et al. (2012)	511 Type-1 diabetics (12–20)	DF	Incident renal dysfunction	↓V
Crosby-Nwaobi et al. (2012)	60 Type-2 diabetics: 30 no retinopathy (median: 64.5), 30 PDR (median: 63)	τ_5	Progression to PDR	↓A ↓V
Sasongko et al. (2012a)	944 Type-1 diabetics (12–20)	τ_5	Retinopathy and early kidney dysfunction	↑A
Sasongko et al. (2012b)	224 diabetics: 85 Type-1, 139 Type-2 (18–70)	τ_5	Serum apolipoproteins levels (AI and B), age and sex adjusted	AI: ↓A — V, B: ↑ *A — V
Benitez-Aguirre et al. (2011)	736 Type-1 diabetics (12–20)	DF	Incident retinopathy	↑A
Cheung et al. (2011a)	1913 non-diabetics from SiMES (40–80)	τ_5	Blood pressure	↑V with elevated BP
Cheung et al. (2011b)	2250 non-diabetics, 664 diabetics from SiMES (40–80)	τ_5	Aging and increasing MABP	Aging: ↓A ↓V, MABP: ↓A ↑V
Lim et al. (2011)	2218 non-diabetics, 664 diabetics from SiMES (40–80)	τ_5	Longer AL and myopic SE	AL: ↓A ↓V, SE: ↓A — V
Sasongko et al. (2011)	224 diabetics, 103 non-diabetics (18–70)	τ_5	Diabetes and DR	Diabetes: ↑A ↑V, mild and moderate DR: ↑A
Koh et al. (2010)	2023 non-diabetics, 618 diabetics from SiMES (40–80)	τ_5	Neuroretinal rim area	↓A ↓V with a thinning rim
Sasongko et al. (2010a)	944 Type-1 diabetics (12–20)	τ_5	Glycated hemoglobin levels (A1C)	↑A with higher levels

Table 3
Studies using the computer-aided image analysis of the retina (CAIAR) software, reporting on tortuosity index outcomes. CHASE: child heart and health study in England; LDL: low-density lipoprotein; ROP: retinopathy of prematurity; A: arteriolar tortuosity; V: venular tortuosity; AV: arteriolar and venular tortuosity combined; ↑: positive association; —: no association.

Authors (year)	Sample size (age)	Tortuosity index	Scope of study	Association(s)
Owen et al. (2011)	872 children from CHASE (10–11)	Mean change in subdivided chord lengths	Cardiovascular risk factors: blood pressure, triglyceride and cholesterol levels	↑A with increasing values
Ghodasra et al. (2010)	30 preplus ROP: 19 regressed, 11 progressed infants	One of the 12 CAIAR indices (no extra info)	Regressed vs. progressed	— A — V — AV
Owen et al. (2009)	387 vessel segments from 28 eyes of 14 children taken during CHASE (10)	12 indices (u_1–u_{12}) based on changes in subdivided chord lengths and Hart's τ_6, τ_7	CAIAR validation study	u_2 (AV) showed optimal agreement with subjective grades
Wilson et al. (2008)	16 computer-generated sinusoidal model vessels and 75 retinal vessels from 10 (selected) preterm infants	12 indices (u_1–u_{12}) based on changes in subdivided chord lengths and Hart's τ_6, τ_7	CAIAR feasibility study	CAIAR output correlates moderately with experts' grading

tortuous the vessel the larger its tortuosity index. DF is a dimensionless measure making it advantageous over inter-studies or inter-eyes comparisons. Nevertheless, its use has been criticized as it may well capture a false representation of a vessel's tortuosity, heavily underestimating its value (Fig. 1).

Contemporary studies either directly use one of these metrics (DF, τ_1–τ_7) to quantify tortuosity in the clinical setting or define their own tortuosity index comparing its performance with Hart's best performing one: the total squared curvature (τ_3) (Trucco et al., 2010). Also, Hart et al. report that from all 7 indices they studied, the ones closer to an ophthalmologist's notion of tortuosity turned out to be τ_3 and τ_4. But, the majority of subsequently published studies implemented the use of the total squared curvature normalized over the arc length (τ_5) instead (Table 1). To the best of our knowledge, it has not been demonstrated whether τ_5 is superior in performance against others. Whereas most studies focused on assessing the vasculature near the optic nerve head and the large vessel arcades branching off temporally, one study has expanded the use of tortuosity measures (using τ_7) to evaluate the parafoveal capillary network (Tam et al., 2011).

4.3. Standardization approach

In order to compare tortuosity parameters, a more standardized approach is necessary. A good example for the benefits of standardization is the arterio-venous-ratio (AVR) (Hubbard et al., 1999)

Table 4
Studies using the ROPtool software, reporting on tortuosity index outcomes. ROP: retinopathy of prematurity.

Authors (year)	Sample size	Tortuosity index	Scope of study	Main outcome
Wallace et al. (2009)	58 images, 7 infants	Smooth tortuosity index	Monitoring plus disease in ROP over time	ROPtool tracks tortuosity changes successfully across visits
Wallace et al. (2007a)	185 images, 117 infants	Smooth tortuosity index	ROPtool validation study	ROPtool shows much better sensitivity and only slightly worse specificity compared to 3 ROP experts

Table 5
Studies using the retinal image multiscale analysis (RISA) software, reporting on tortuosity index outcomes. CVD: cardiovascular disease; ROP: retinopathy of prematurity; IHD: ischemic heart disease; EHT: essential hypertension; MHT: malignant hypertension; SAA: serum amyloid A; CRP: C-reactive protein; IC: integrated curvature; TI: tortuosity index; A: arteriolar tortuosity; V: venular tortuosity; ↑: positive association; ↓: negative association; —: no association; *: borderline significance.

Authors (year)	Sample size (age)	Tortuosity index	Scope of study or variable(s)	Association(s) or main outcome
Zepeda-Romero et al. (2011)	10 preterm infants	DF, IC	Corneal compression artifact	IC: ↓A rest: —
Mahal et al. (2009)	51 Type-2 diabetics: 22 European, 29 Afro-Caribbean	DF	Ethnicity	— A — V
Hughes et al. (2009)	167 healthy controls (45–74)	τ_1, τ_5	CVD risk factors	— A — V
Stettler et al. (2009)	159 hypertensives with type-2 diabetes, 552 without	no info	SAA and CRP inflammatory markers: diabetics vs. non-diabetics	SAA, CRP (non-diabetics): ↑A rest: —
Thom et al. (2009)	Hypertensives: 373 amlodipine-based regimen, 347 atenolol-based regimen	τ_1	Atenolol vs. amlodipine	amlodipine: ↓A — V
Hughes et al. (2008)	25 untreated hypertensives (24–71)	DF	Lisinopril vs. amlodipine	— A — V
Koreen et al. (2007)	20 Selected images, 11 experts	DF, IC	RISA diagnostic evaluation	Arteriolar IC showed the highest diagnostic accuracy and agreement with experts
Tapp et al. (2007)	166 children	τ_1	Birth weight	↑A with lower birth weight
Hughes et al. (2006)	20 normotensives, 20 essential hypertensives, 20 malignant hypertensives	DF	EHT, MHT vs. controls	MHT: ↑*A ↑*V, EHT: — A — V
Witt et al. (2006)	124 IHD and 28 stroke deaths, 528 healthy controls (45–74)	τ_1, τ_5	IHD, stroke deaths vs. controls	IHD deaths: ↓A rest: —
Gelman et al. (2005)	16 preterm infants	DF, IC	RISA diagnostic evaluation	Both arteriolar and venular IC showed the highest diagnostic accuracy
Swanson et al. (2003)	42 preterm infants	1/DF	No ROP, mild ROP, severe ROP	↑A with ROP severity

which describes the relative size (diameter) of the summarized central retinal artery equivalent over its venous counterpart. Research groups, using the SIVA (Fig. 2), the CAIAR and the ROPtool software, have already adopted a very similar grid so as to limit the measurement area (see Tables 2–4). The grid approach appears to be preferable because, on average, it is possible (regardless of anatomical variations) to measure at least four arteries and four veins.

However, despite these developments, there are still issues which remain to be addressed. These include hardware (camera type, calibration differences in resolution (CCD sensor) and image formatting) and software used in image acquisition and analysis (vessel detection and segmentation algorithm, resolution cut-offs) (Patton et al., 2006).

Image acquisition (mode and time; mydriatic vs. non mydriatic) and camera angle settings are an additional source of bias between studies. The camera field angle used to acquire retinal pictures impacts mainly on the measurement area but is equally important when defining a vessel detection threshold, which is dependent on imaging resolution.

Should an enlarged grid be used for tortuosity evaluation then even so it will still contain predominantly parent vessels, thus lacking information on the smaller arterioles and venules and subsequently the capillary network. A type-specific analysis may be more elusive to assess the pathological origin based on the vascular tree: i.e. arteries, arterioles, pre-capillaries, post-capillaries, venules, as there is possibly a time sequence depending on the vessel construction as to which one will be first compromised where the most affected site subsequently will add more strain to the system. Larger vessels may withstand pressure changes longer than smaller ones but once the resistance system (i.e. the capillary network) is compromised they may more rapidly deteriorate. Hence, having an analysis approach which takes into account these mechanisms will provide a more substantial insight into the time sequence of structural damage at the retinal level.

4.4. Clinical applications

Structural changes in retinal vessel caliber and appearance are highly linked to underlying systemic vascular pathology. Retinal vessel parameters have been shown to be linked to Left Ventricular Hypertrophy (LVH) in African Americans (Gabriella et al., 2008) as well as to hypertension (Gepstein et al., 2012; Cheung et al., 2011a), diabetes (Tsai et al., 2011), atherosclerosis (Klein et al., 2000) and renal disease (Grunwald et al., 2012; Ooi et al., 2011; Yau et al., 2011).

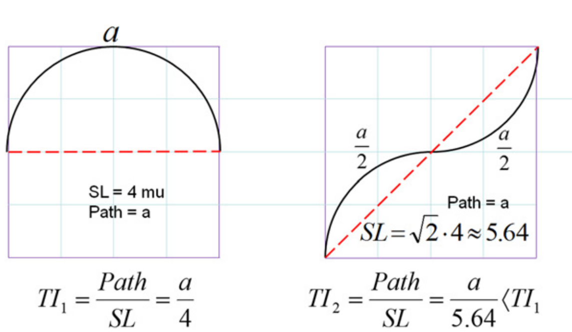

Fig. 1. A vessel that bends gradually yields higher tortuosity comparing to one that bends more frequently when the arc over chord length tortuosity definition is used (see Table 1). SL: straight line (chord) length, path: path (arc) length, TI: tortuosity index, mu: measuring units. Adapted from Aslam et al. (2009).

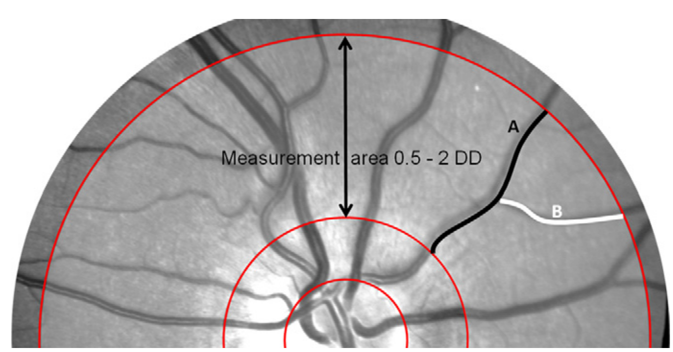

Fig. 2. Schematic representation of the grid approach for standardization of the area within tortuosity is measured in studies using the Singapore I vessel assessment (SIVA) software. Vessel A and B showing example of parent vessel used for analysis. DD: disk diameter. For references, see Table 2.

Although tortuosity measurements can be obtained from both, arteries and veins, up to now it has been used more frequently for arterial assessment. Hence, literature provides more information on arterial tortuosity changes in the retina linked to systemic disease than those of veins. Initially, tortuosity measurements were introduced to describe retinal abnormalities in pre-term babies (Capowski et al., 1995). Within this clinical presentation a form of standardized analysis has been reached, in that studies examining ROP use the same analysis (Smooth Tortuosity Index) and software (ROPtool) approach.

In the last decade, tortuosity measurements have been increasingly used as surrogate markers for systemic vascular disease and to assess and establish the extent of vascular disease upon the ocular circulation. The eye in particular offers the possibility to non-invasively study the microcirculation *in vivo*. Tortuosity is virtually independent from the variations of the systemic circulation throughout the day and during the cardiac cycle (Chen et al., 1994; Hao et al., 2012), rendering it a robust measure. Although there are a wide array of parameter calculations published (Table 1) to date, it is often not feasible to compare studies, unless they used the same definition and materials. However, associations between retinal tortuosity measurements and systemic and ocular abnormalities have been demonstrated in numerous studies, regardless of the parameter calculation applied.

Whether structural changes in the vasculature of the fundus take place first and then functional changes come as a result or vice versa and which one of the two is more crucial, it remains to be identified. To date, functional retinal vessel assessment has also been linked to systemic vascular disease (Heitmar and Summers, 2012), similar to structural retinal changes. Although, there appears to be a better consensus toward a standardized assessment protocol, there is also limited information published regarding longitudinal studies, reproducibility and intra-ocular variability. Both functional and structural measures deserve further merit and potentially supplement each other, but for ease of data acquisition, cost of equipment and data storage, structural parameters such as tortuosity indices, vessel calibers, branching angles, length to diameter ratios, fractal dimensions and junctional exponents might be more suitable.

In order for tortuosity indices to become more clinically useful a consensus on its calculation is desired. Besides standardization, studies validating ocular markers against systemic markers along with information on reproducibility and age dependency for different ethnicities are required to be able to use tortuosity for diagnostic purposes.

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