Dry eye disease is associated with retinal microvascular dysfunction and possible risk for cardiovascular disease

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ABSTRACT.

Purpose: **To explore the presence of microvascular endothelial dysfunction as a measure for early cardiovascular disease in individuals diagnosed with dry eye disease (DED) as compared to age-matched normal controls.**

Methods: **Systemic blood pressure, Body Mass Index, intraocular pressure, blood levels of glucose (GLUC), triglycerides, cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)] as well as retinal and peripheral microvascular function were assessed in twenty-five 35–50 year olds with diagnosed with DEDa (using the TFOS DEWS II criteria) and 25 age and sex-matched controls.**

Results: **After controlling all the influential covariates, individuals diagnosed with DED exhibited significant lower retinal artery baseline (p = 0.027), artery maximum diameter (p = 0.027), minimum constriction (p = 0.039) and dilation amplitude (p = 0.029) than controls. In addition, the time to reach the vein maximum diameter was significantly longer in the DED patients than in normal controls (p = 0.0052). Only in individuals diagnosed with DED, artery maximum constriction correlated statistically significantly and positively with HDL-C blood levels (p = 0.006). Similarly, artery slope$_{AD}$ correlated positively with T-CHOL and LDL-C (p = 0.006 & 0.011 respectively). Additionally, artery baseline diameter and maximum constriction were significantly and negatively correlated to T-CHOL/HDL-C ratio (p = 0.032 and p = 0.013 respectively) in DED individuals only.**

Conclusions: **Individuals with positive diagnosis of DED exhibit abnormal retinal microvascular function and possible higher risk for CVD.**

Key words: cardiovascular disease – dry eye disease – microvascular function – retinal vessels

Introduction

Dry eye disease (DED) represents a, multifactorial, chronic and debilitating pathology of the ocular surface characterized by loss of homeostasis of the tear film and accompanied by ocular symptoms. Tear film instability and hyperosmolarity, ocular surface inflammation and damage as well as neurosensory abnormalities play etiological roles in this disease (Craig et al. 2017).

In addition to other risk factors, DED has previously been associated with dyslipidaemia, a group of metabolic abnormalities characterized by any or a combination of the following: raised low-density lipoprotein cholesterol (LDL-C), raised total cholesterol (TC), raised triglycerides (TG) and low high-density lipoprotein cholesterol (HDL-C) (Musunuru 2010). Indeed, as lipid homeostasis is important for the stability of the tear film, the association between dyslipidaemia and DED is entirely justified. Moreover, disruptions of cholesterol biosynthesis are also associated with sebaceous/Meibomian gland (MG) dysfunctions (Bu et al. 2019), another cause of tear film instability and dry eye. Dyslipidaemia also represents a significant risk factor for cardiovascular disease (CVD) especially due to its contribution in the pathogenesis of atherosclerosis in medium-sized and large arteries but also at the microvascular level (Pereira 2012; Padró, Vilahur & Badimon 2018). This affects not only the anatomy of these vessels but, most importantly, their function. Indeed, at the functional level, it impairs endothelium-dependent vasodilation because of defects on nitric oxide (NO) bioavailability (Padró, Vilahur & Badimon 2018). This has catastrophic effects on the balance between the physiological vascular dilatory and constrictory states, which, in turn, will also affect other important circulatory

functions and, most importantly, vascular protection against oxidation, inflammation and thrombosis (Mudau et al. 2012). This imbalance characterises the so-called endothelial dysfunction (ED), an initial reversible step in the development of atherogenesis; nevertheless, it is also one of the most important stages in the development of CVD. Therefore, its identification, as early as possible, represents a key factor in CVD prevention (Mudau et al. 2012).

Besides having common risk factors, other relationships between DED and CVD are not clearly understood. However, as both CVD and DED disease are common and important health problems encountered frequently in the general population, a closer look at their other possible links is warranted. The present study explores the presence of microvascular ED (as a measure for early CVD) in individuals diagnosed with DED as compared to age-matched normal controls.

Methods

Study participants

Healthy individuals aged between 35 and 50-year-old were recruited for this case-control study through advertisements at the Vascular Research Laboratory, and the Dry Eye Clinic, Aston University (Birmingham, UK). Ethical approval was sought from the relevant local ethics committees, and written informed consent was received from all participants prior to study enrolment. The study was designed and conducted in accordance with the tenets of the Declaration of Helsinki, and all study-related procedures adhered to institutional guidelines.

Study exclusion criteria were defined as the positive diagnosis of hypertension, CVD, cerebrovascular disease, peripheral vascular disease, dyslipidaemia, diabetes, as well as other metabolic disorders or chronic diseases that required treatment. Individuals using any vasoactive medications were also excluded from the study. Potential participants were also screened for ocular diseases and were excluded from the study if they had a refractive error of more than ± 3DS and more than ± 1DC equivalent, intra-ocular pressure (IOP) >21 mmHg, cataract or any other media opacities, as well

as history of intra-ocular surgery or any form of retinal or neuro-ophthalmic disease affecting the ocular vascular system.

Quality of the retinal vascular images was assessed after the analysis and participants with poor image quality was excluded from the study.

General investigations

Standard anthropometric measures of height and weight were recorded to determine body mass index (BMI = weight/height). Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured using an automatic Blood Pressure monitor (UA-767; A&D Instruments Ltd, UK) to determine mean arterial pressure (MAP = 2/3 DBP + 1/3 SBP). Intra-ocular pressure (IOP) readings were obtained using non-contact tonometry (Pulsair; Keeler Ltd, UK).

In addition, blood and plasma samples drawn from the antecubital fossa vein were assessed immediately for fasting GLUC, TG, total cholesterol (T-CHOL), and HDL-C using the Reflotron Desktop Analyzer (Roche Diagnostics, UK). Low-density lipoprotein cholesterol (LDL-C) values were calculated as per the Friedewald equation (Friedewald, Levy & Fredrickson 1972).

DED diagnosis

All subjects underwent dry eye assessment using a digital slit lamp and the Keratograph K5m (Oculus, Wetzlar, Germany) including objective non-invasive breakup time, taking the average of three readings and ocular surface staining with fluorescein (i-DEW Flo, Mainline, Derby, UK) and lissamine green (GreenGlo, HUB Pharmaceuticals, Plymouth, MI, USA) using the TFOS DEWS II recommended methodology (Wolffsohn et al. 2017). Osmolarity was assessed in each eye using the Tearlab (Dallas-Fort Worth, TX, USA).

Diagnosis of dry eye was based on the latest TFOS DEWS II criteria (Wolffsohn et al. 2017) which involves a positive symptoms screening with the Ocular Surface Disease Index (cut-off >+13) and one or more of non-invasive tear breakup time (<10 s), hyperosmolarity (>+308 mOsm/l in the higher eye

or an intereye difference .8mIsm/l) and ocular surface staining (>5 corneal, >9 conjunctival punctate spots or lid margin (≥2 mm length and >+25% width).

Dynamic retinal microvascular function vessel analysis

Retinal microvascular function was assessed using the dynamic retinal vessel analyser (DVA, IMEDOS GmbH, Jena, Germany) in accordance with an established protocol (Nagel, Vilser & Lanzl 2004) Using a validated in-house algorithm, the following vessel reactivity and time-course parameters were determined: the average baseline diameter and range of maximum and minimum baseline vessel diameters (baseline diameter fluctuation, BDF); the maximum vessel dilation diameter during flicker stimulation expressed as a percentage change relative to baseline diameter (MD%) and the time taken in seconds to reach the maximum diameter (tMD); the maximum vessel constriction diameter during the postflicker recovery period expressed as a percentage change relative to baseline diameter (MC%) and the time taken in seconds to reach the maximum vessel constriction diameter (tMC); the overall dilation amplitude (DA) calculated as the difference between MD and MC; and the baseline-corrected flicker response (BCFR) used to describe the overall DA after normalizing for fluctuations in baseline diameters (DA-BDF). In addition, the arterial (A) and venous (V) dilation slopes ($Slope_{AD/VD}$ = (MD − baseline diameter)/tMD) and constriction slopes ($Slope_{AC/VC}$ = (MC − MD)/tMC) were also calculated (Fig. 1) (Shokr, Dias & Gherghel 2020).

Digital thermal monitoring

The peripheral microvascular function was assessed using VENDYS 5000 BCE digital thermal monitoring (DTM) system (Endothelix, Inc, Houston, TX, USA) according to an established protocol (Schier et al. 2013). The following parameters were measured and calculated (Fig. 2): temperature rebound (TR): maximum temperature (T_{MAX}); minimum temperature (T_{MIN}); adjusted TR (aTR); and area under the curve TR. The post-occlusive aTR determined by the software algorithm
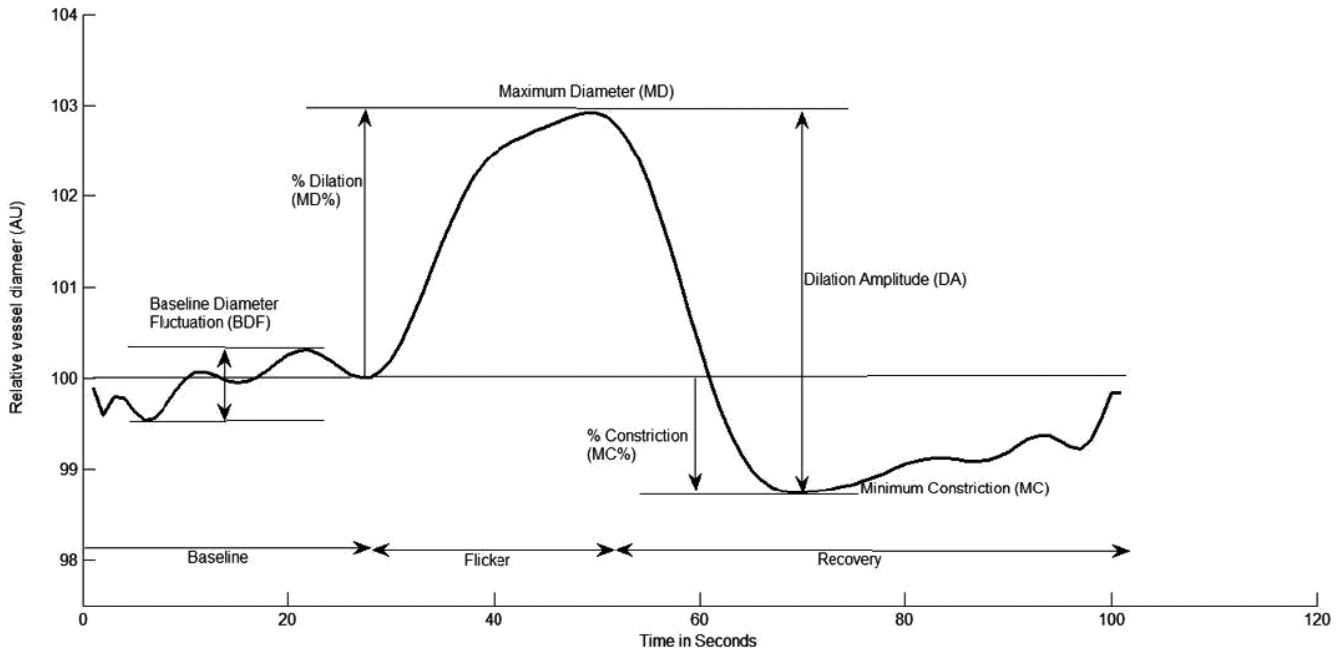
Fig. 1. Graphical presentation of the dynamic vessel response profile displaying the parameters calculated and used in analysis. (DA) calculated as (MD − MC). (MD%) calculated as the percent increase from baseline to MD. (MC%) calculated as the percent constriction below baseline following MD.

is directly associated with the extent of the subjects vascular reactivity. An aTR below 1 was considered poor cardiovascular reactivity (high risk), whereas an aTR between 1 and 2 was considered intermediate vascular reactivity (medium risk). An aTR of >2 was considered a sign for normal peripheral vascular reactivity (Karimzad, Shokr & Gherghel 2019).

Statistical analysis

Based on previous studies, a change of 30% with a SD of 2.5% in retinal vessel reactivity was shown to be significant. As the study design was multi-factorial in nature it was calculated that $n = 25$ in each group was sufficient to provide 90% power with an alpha of 0.05.

All data are reported as mean (SD) unless otherwise indicated. The Shapiro–Wilk test was used to determine the distribution of the data. Univariate associations were determined using Pearson's (normally distributed data) or Spearman's method (non-normally distributed data), and forward stepwise regression analyses were performed to test the influences of
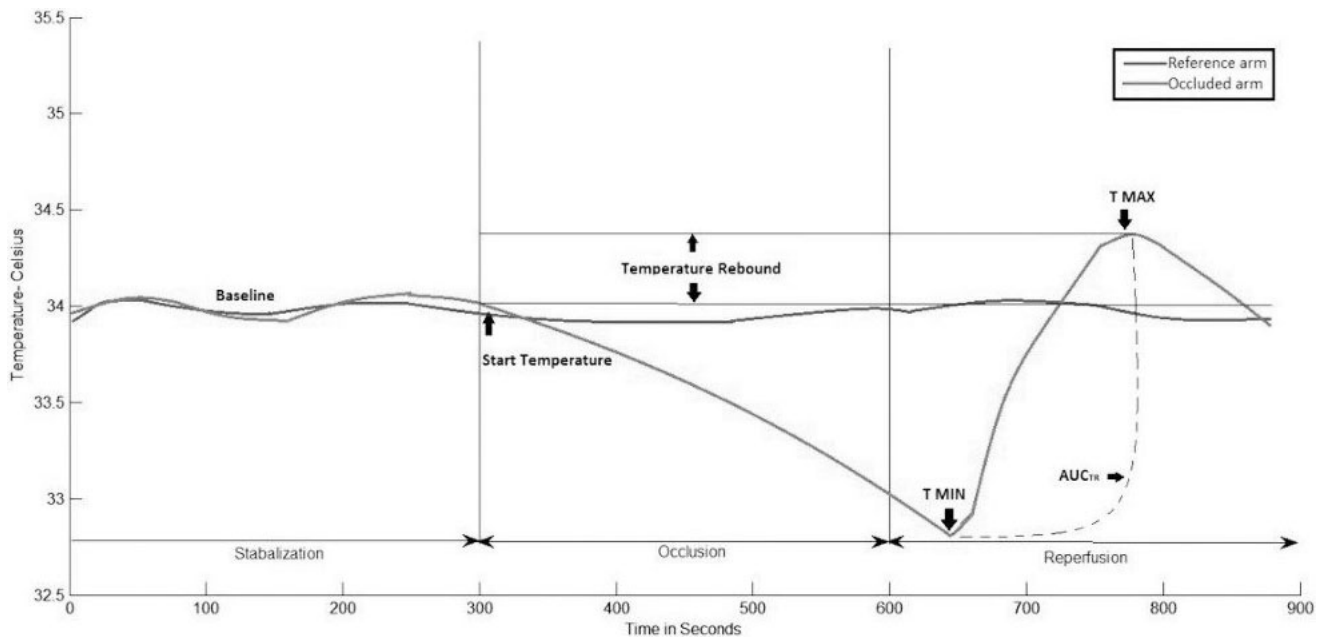
Fig. 2. Graphical representation of the Digital Thermal Monitor software analysis; AUCTR = area under the curve temperature rebound; T_{MAX} = maximum temperature, T_{MIN} = minimum temperature.

systemic and circulating markers on the measured variables. In multivariate regression models the β coefficient value was considered to answer the question of which of the independent variables has a greater effect on the dependent variable as β refers to the SD change in the dependent variable per SD increase in the predictor variable, and is particularly useful when variables are measured in different units. Differences between groups were subsequently assessed using one-way analysis of variance (ANOVA) or analysis of covariance (ANCOVA).p-values of <0.05 were considered significant. All analyses were performed using Statistica® (version 13.3; StatSoft Inc., Tusla, OK, USA) software.

Results

A total number of 66 participants were initially screened for study inclusion, of which 16 individuals were excluded based on the quality of retinal vascular image analysis. The remaining 50 participants were included in the final analysis and classified into two study groups based on a positive diagnosis for DED: 25 individuals (12 men and 13 women) and negative diagnosis: 25 individuals (15 men and 10 women).

General characteristics of the study population are presented in Table 1. There were no significant differences in age, sex, BMI, HR, IOP, GLUC, TG, and LDL-C, between the study groups (all p > 0.05). Although still within the normal range, the individuals with DED had a statistically significant higher T-CHOL (5.0 versus 4.44 mmol/l) and lower HDL-C (1.21 versus 1.58 mmol/l) values than those without DED (p = 0.0356 and 0.0137 respectively), albeit close to the upper limit for these parameters (Table 1).

There were no significant differences between the study groups with regard to the peripheral microvascular function parameters as measured using the DTM method (all p > 0.05, Table 1). In addition, after controlling all the influential covariates identified using multivariate analysis, there were no significant differences between the study groups with regards to the retinal microvascular parameters BDF, BCFR, MD%, MC%, tMD and tMC, Slope$_{AD}$ and Slope$_{AC}$ (all p > 0.05, Table 2). However, individuals diagnosed with DED exhibited

significant lower artery baseline diameter, artery MD, MC and DA than individuals in the non-dry eye group (p = 0.0269, p = 0.0273, p = 0.0386 and p = 0.0291, respectively, Fig. 3). In addition, vein tMD was significantly longer in the DED patients

than in normal controls (p = 0.0052; Table 3).

Only in individuals diagnosed with DED, artery MC correlated statistically significantly and positively with HDL-C blood levels (p = 0.006). Similarly, artery slope$_{AD}$ correlated

Table 1. General characteristics of the study population.

	Dry eye diagnosis	None dry eye	p-value
Number	25	25	–
Sex	12 Male : 13 Female	15 Male : 10 Female	–
Age (years)	44.1 (2.5)	37.6 (2.5)	0.073
BMI (kg/m^2)†	26.03 (1.03)	26.71 (1.03)	0.645
SBP (mmHg)	116.90 (4.96)	120.5 (4.96)	0.611
DBP (mmHg)	68.94 (3.43)	71.3 (3.43)	0.629
HR (bpm)	62.14 (2.10)	66.00 (2.98)	0.366
IOP	13.48 (0.42)	13.31 (0.44)	0.776
GLUC (mmol/l)	4.81 (0.15)	4.54 (0.16)	0.228
TG (mmol/l)	1.01 (0.07)	0.94 (0.07)	0.502
T-CHOL (mmol/l)	5.0 (0.172)	4.44 (0.19)	0.036*
HDL-C (mmol/l)	1.21 (0.109)	1.58 (0.09)	0.014*
LDL-C (mmol/l)	3.15 (0.23)	2.81 (0.23)	0.301
T-CHOL/HDL-C	4.36 (0.377)	3.086 (2.34)	0.020*
aTR	1.52 (0.14)	1.76 (0.14)	0.237

Data are presented as mean (SD) unless otherwise indicated.
aTR = adjusted temperature rebound, BMI = body mass index, DBP = diastolic blood pressure, GLUC = glucose, HDL-C = high-density lipoprotein cholesterol, HR = heart rate (in beats per minute), IOP = intraocular pressure, LDL-C = low-density lipoprotein cholesterol, SBP = systolic blood pressure, T-CHOL = total cholesterol, TG = triglycerides.
* Significant p-values are indicated where p < 0.05 was considered significant.
† Calculated as weight in kilograms divided by height in metres squared.

Table 2. Summary of retinal arterial vascular functional parameters.

	Mean (SD)		
Parameter	Dry eye	None-dry eye	p-value
Artery baseline	110.59 (2.52)	112.72 (2.51)	0.027*
Artery-BDF	5.47 (0.47)	5.434 (0.47)	0.954
Artery-DA†	8.29 (0.77)	10.73 (0.77)	0.029*
Artery-BCFR‡	3.84 (0.51)	4.33 (0.51)	0.502
Artery-MD	118.75 (2.64)	123.28 (2.85)	0.027*
Artery-tMD	16.73 (0.92)	15.67 (0.92)	0.414
Artery-MD%	4.95 (0.54)	5.56 (0.53)	0.434
Artery-MC	109.50 (2.38)	111.67 (2.38)	0.039*
Artery-tMC	23.95 (1.59)	25.71 (1.58)	0.436
Artery-MC%	−3.05 (0.38)	−3.63 (0.38)	0.287
Artery-Slope$_{AD}$§	0.41 (0.04)	0.39 (0.043)	0.764
Artery-Slope$_{AC}$¶	−0.52 (0.08)	−0.41 (0.085)	0.361

Unless otherwise indicated, all values are expressed in arbitrary units, which approximately correspond to micrometres (μm) in a normal Gullstrand eye.
Baseline = baseline diameter, BCFR = baseline-corrected flicker response, BDF = baseline diameter fluctuation, DA = dilation amplitude, MC = Maximum constriction, MC% = percentage constriction below baseline, MD = artery maximum dilation, MD% = percentage change in diameter from baseline to maximum dilation, Slope$_{AC}$ = slope of arterial constriction, Slope$_{AD}$ = slope of arterial dilation, tMC = reaction time to maximum constriction diameter from maximum dilation diameter, tMD = reaction time to maximum dilation diameter.
* Significant p-values are indicated where p < 0.05 was considered significant.
† Calculated as MD − MC.
‡ Calculated as DA − BDF (Nagel, Vilser & Lanzl 2004).
§ Calculated as (MD − baseline)/tMD (Mroczkowska et al. 2012).
  Calculated as (MC − MD)/tMC (Mroczkowska et al. 2012).

positively with T-CHOL and LDL-C (p = 0.006 & 0.011 respectively). Additionally, artery baseline diameter and MC were significantly and negatively correlated to T-CHOL/HDL-C ratio (p = 0.032 and p = 0.013 respectively) in DED individuals only (Fig. 4).

Discussion

This study examined the link between DED and CVD risk, as assessed using known circulatory markers as well as measurements of microvascular function at the retinal and peripheral level. Through this approach, it identified that individuals diagnosed with DED exhibit abnormal retinal, but not peripheral microvascular function and these abnormalities correlate with plasma levels of circulating cholesterols. To our knowledge, this is the first study to reveal that middle-aged individuals with DED have increased retinal microvascular dysfunction compared to normal, sex and age-matched controls.

Similar to other research (Dao et al. 2010; Wang et al. 2012; Chun et al. 2013), this study also demonstrated that individuals with DED exhibit higher, albeit at the upper normal levels of circulating T-CHOL when compared to those without DED. It has been previously proposed that the relationship between hypercholesterolemia and DED can be explained as increased levels of

Table 3. Summary of retinal venous vascular function parameters.

Parameter	Mean (SD)		p-value
	Dry eye	None-dry eye	
Vein-baseline	133.62 (3.92)	139.94 (3.92)	0.260
Vein-BDF	5.37 (0.43)	5.23 (0.43)	0.822
Vein-DA[†]	9.11 (0.61)	8.96 (0.62)	0.867
Vein-BCFR[‡]	3.73 (0.43)	3.72 (0.43)	0.988
Vein-MD	140.04 (4.58)	149.92 (4.58)	0.134
Vein-tMD	19.46 (0.70)	16.40 (0.70)	0.005*
Vein-MD%	4.94 (0.38)	4.50 (0.38)	0.415
Vein MC	130.94 (4.35)	140.96 (4.35)	0.110
Vein-tMC	33.49 (1.61)	33.29 (1.61)	0.930
Vein-MC%	−2.0 (0.19)	−1.76 (0.19)	0.371
Vein-slope$_{AD}$[§]	0.49 (0.07)	0.48 (0.07)	0.908
Vein-slope$_{VC}$[¶]	−0.52 (0.08)	−0.41 (0.086)	0.361

Unless otherwise indicated, all values are expressed in arbitrary units, which approximately correspond to micrometres (μm) in a normal Gullstrand eye.
Baseline = baseline diameter, BCFR = baseline-corrected flicker response, BDF = baseline diameter fluctuation, DA = dilation amplitude, MC = Maximum constriction, MC% = percentage constriction below baseline, MD = vein maximum dilation, MD% = percentage change in diameter from baseline to maximum dilation, Slope$_{VC}$ = slope of venous constrictionSlope$_{VD}$ = slope of venous dilation, tMC = reaction time to maximum constriction diameter from maximum dilation diameter, tMD = reaction time to maximum dilation diameter.
* Significant p-values are indicated where p < 0.05 was considered significant.
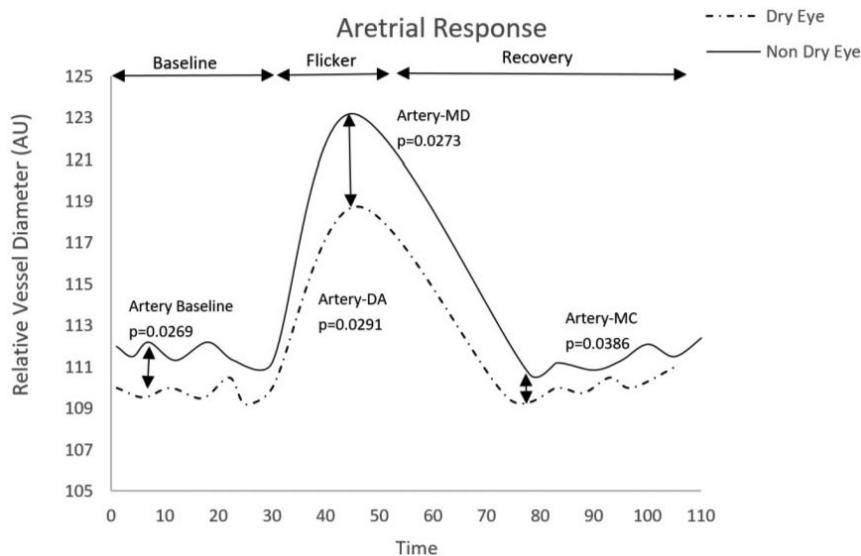[†] Calculated as MD − MC.
[‡] Calculated as DA − BDF (Nagel, Vilser & Lanzl 2004).
[§] Calculated as (MD − baseline)/tMD (Mroczkowska et al. 2012).
 Calculated as (MC − MD)/tMC (Mroczkowska et al. 2012).

cholesterol in the meibomian lipid would increase its melting point, thus leading to increased viscosity and plugging of the meibomian orifice (Butovich, Millar & Ham 2008). It is also important to note that individuals with DED included in our study also exhibited statistically significant lower, albeit still normal

levels of HDL-C and higher T-CHOL/HDL-C ratio than the normal controls group. It is well known that HDL-C transports cholesterol from the tissues to the liver to be disposed, making it beneficial in the prevention of CVD. Moreover, decreases in HDL-C have also been linked with ED and a

Fig. 3. Comparison of retinal arterial response profile across groups. AU = arbitrary units, BDF = baseline diameter fluctuation calculated as the maximum range in vessel diameter during first 30 s of baseline readings, MD% = calculated as the percentage change in vessel diameter from baseline to maximum following onset of flicker, slope$_{AC}$ = calculated as (MC – MD)/(tMC), tMC = time to reach maximum constriction post flicker, tMD = time to reach maximum diameter during flicker.
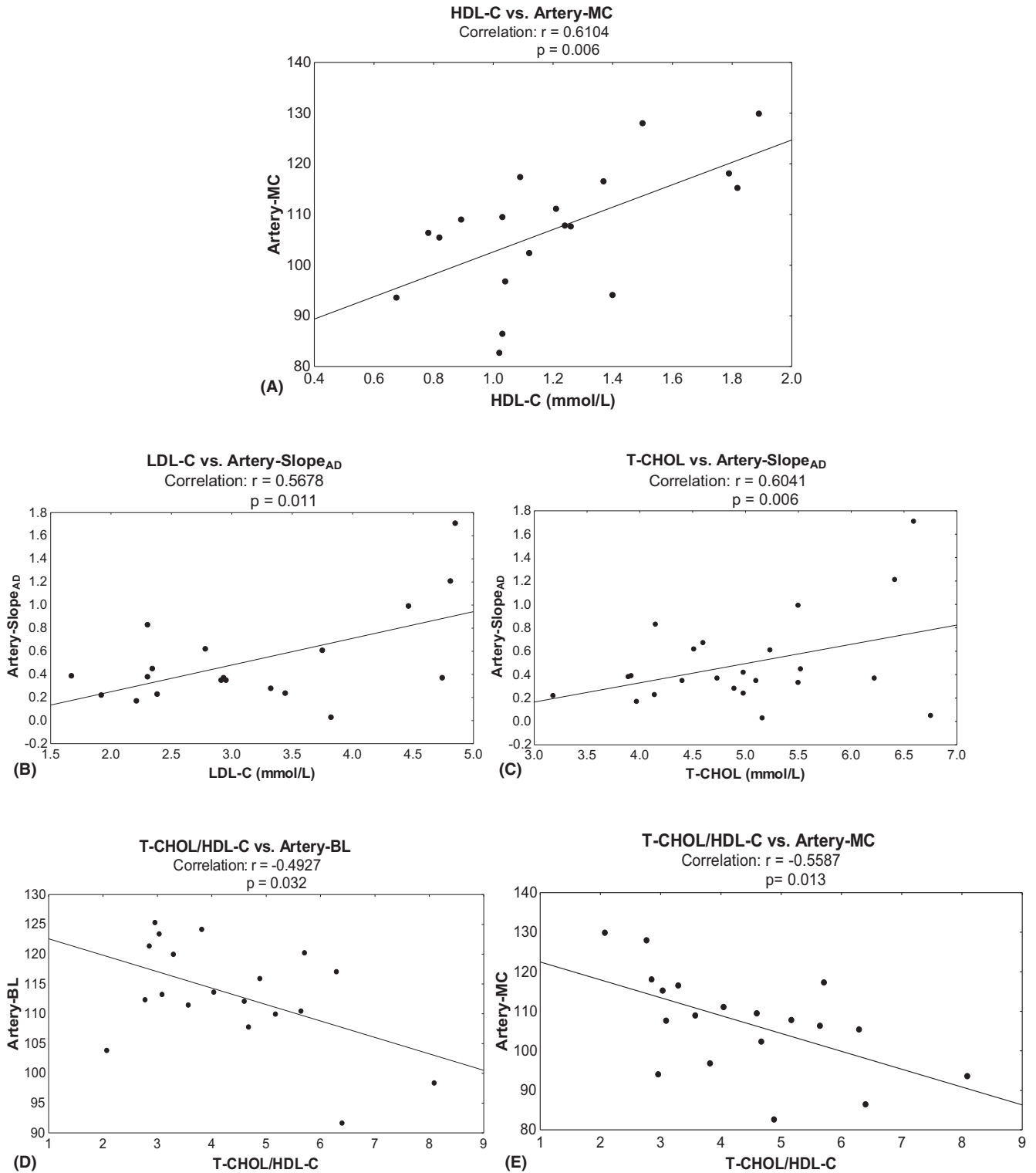
Fig. 4. Correlation between retinal arterial responses and systemic blood lipids. Arterial maximum constriction, Artery- MC = Arterial maximum constriction, Artery- MC, Artery- SlopeAD = slope of arterial dilation, Artery-BL = artery baseline diameter, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, T-CHOL = total cholesterol, T-CHOL/HDL = total cholesterol/high density lipoprotein ratio. Normalized unit values used on y-axes.

reduction in the bioavailability of NO (Campbell & Genest 2013). In addition, T-CHOL/HDL-C ratio has previously been specified as a better indicator of premature CVD risk in than T-CHOL levels (Tewari et al. 2005), making these observations very important.

The present study revealed a novel finding that, independent of other characteristic, individuals with DED exhibit abnormal retinal arterial and venous microvascular dysfunction. To date, little research looked at microvessels in individuals with DED and only at the level of conjunctival vessels. It is

interesting to mention that patients with DED have been previously shown to exhibit abnormal microvascular response and reduced blood flow at the conjunctival vessels level after trigeminal stimulation, suggesting that these patients suffer of an imbalance in the autonomic nervous system (ANS) (Chen et al. 2017). Indeed, conjunctival vessels have a dual autonomic innervation (Ruskell 1985). Moreover, DED has been proposed to be associated with various ANS dysfunctions as autonomic nerves are abundant in MG tissue and play an important role in regulating the secretory activities of MG in animals (LeDoux et al. 2001; Li et al. 2012). Endothelial dysfunction (ED) and ANS imbalance often co-exist in the development of various CVD processes (Amiya, Watanabe & Komuro 2014). At the retinal microvascular level, in the absence of autonomic innervation, metabolic and myogenic stimuli are more involved in retinal autoregulation of the microvascular calibre (Kur et al., 2012). Although the function of retinal microvessels are not under the influence of ANS, this study suggests that both the ANS and endothelial dysregulation co-exist in individuals with DED and the results of this imbalance are evident and can be measurable at different vascular levels. In addition, abnormalities in the retinal venous functionality have also been found in the DED group. Since retinal veins typically incite a more passive regulatory contribution to increases in blood flow whether this observation may reflect some kind of reconciliation of alterations in arterial outflow to the venous side via downstream autoregulatory mechanisms (Kotliar et al. 2004) is unclear at present.

The positive correlation between the retinal microvascular function parameters and the levels of circulating HDL-C in DED individuals reinforces the fact that the HDL-C has an important role in prevention of CVD. Indeed, retinal vascular calibres have been shown to be independently associated with risk factor variables such as age, blood pressure, HDL-C, and LDL-C. Our DED group, however, also exhibited negative relationship between T- CHOL/HDL-C ratio and retinal artery MC. As this circulatory parameter is a strong indicator of risk for CVD (Davidson et al. 2008; Agirbasli et al. 2015), this

observation is very important and, in addition to the above mentioned micro-circulatory abnormalities, points to the fact that DED individuals could exhibit higher risk for CVD than age- and sex-matched normal individuals. Further follow-up studies, to confirm the actual development of CVD in these individuals, are warranted.

Conclusion

Significant attenuations in retinal vascular function exist and can be detected in persons diagnosed with DED. Moreover, these abnormalities correlate with known circulatory markers for CVD. Functional retinal assessments could therefore be useful for early vascular screening, possibly contributing to a reduced risk for CVD morbidity in these individuals.

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