The relationship between advanced glycation end products and ocular circulation in type 2 diabetes

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Running head: AGEs and ocular circulation in diabetes

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Abstract

Aims: To determine whether skin autofluorescence (SAF) and serum pentosidine, biomarkers of advanced glycation end products (AGEs), were associated with ocular microcirculation in type 2 diabetes patients with early diabetic retinopathy (DR).

Methods: This study included 46 eyes of 46 type 2 diabetes patients with no DR or non-proliferative DR. SAF was measured with an autofluorescence reader. Optic nerve head (ONH) microcirculation, represented by mean blur rate (MBR), was measured with laser speckle flowgraphy. Overall MBR, vascular MBR, and tissue MBR were calculated in software. MBR, SAF, pentosidine levels, and clinical findings, including central macular thickness (CMT), were then compared.

Results: SAF in the diabetes patients was correlated with age ($P = 0.018$). Serum pentosidine were correlated with age, vascular MBR and tissue MBR ($P = 0.046$, $P = 0.035$, and $P = 0.01$, respectively). CMT was correlated with tissue MBR ($P = 0.016$), but not with vascular MBR or overall MBR. Separate multiple regression analyses of independent contributing factors revealed that age, SAF, serum pentosidine, duration of diabetes, and pulse rate contributed to tissue MBR ($P = 0.041$, $P = 0.046$, $P = 0.022$, $P = 0.011$ and $P = 0.036$, respectively), while SAF, HbA1c, pulse rate, tissue MBR, diastolic blood pressure, and creatinine contributed to CMT ($P = 0.005$, $P = 0.039$, $P < 0.001$, $P < 0.001$, $P = 0.022$ and $P = 0.001$, respectively).

Conclusions: Tissue MBR may be closely related to AGE levels and CMT in type 2 diabetes patients with early DR, suggesting that ocular circulation might be potential early biomarkers of DR.

Keywords: Skin autofluorescence; pentosidine; AGE; diabetic retinopathy; diabetic macular edema
Introduction

Diabetes mellitus affects over 350 million people worldwide, (Whiting et al. 2011) approximately one third of whom will go on to develop diabetic retinopathy (DR). This ocular condition has become a leading cause of visual disability among working-aged adults (Ruta et al. 2013). One of the most important contributors to the pathogenesis of DR is the accumulation of advanced glycation end products (AGEs) and associated decreases in microcirculation. The most difficult types of DR to treat successfully, even for experienced ophthalmologists, include proliferative DR (PDR) and diabetic macular edema (DME). The visual prognosis remains poor for most patients who develop these conditions, despite recent advances in medical and surgical therapies (Shimura et al. 2008; Schoenberger et al. 2011). One of these recent advances, the use of anti-vascular endothelial growth factors to treat DME, is affected by recurrence and significant numbers of non-responders in clinical use (Virgili et al. 2014). In order to prevent the development of PDR and DME in patients with diabetes, it is essential to understand the underlying pathogenesis of these conditions. Thus, it is important to find clinically useful new methods of evaluating and understanding the accumulation of AGEs, as well as measuring microcirculation decreases, in eyes with early DR.

AGEs, in addition to oxidative stress and inflammation, have been reported to play a role in the pathogenesis of various vascular diseases, including DR, that are associated with diabetes (Stitt 2001; Yamagishi 2011). Accumulation of AGEs in the ocular tissue, especially in the retina and cornea of patients with diabetes, has already been investigated (Hammes et al. 1999; Kaji et al. 2000). Serum levels of pentosidine, one of the most well understood AGEs, have also been reported to be higher in
subjects with type 2 diabetes (Daimon et al. 1999; Yoshida et al. 2005). It has been suggested that higher plasma levels of pentosidine may contribute to increased vascular rigidity of the retinal arteries in patients with type 2 diabetes and early DR (Sato et al. 2012). Furthermore, levels of skin autofluorescence (SAF), a non-invasive measurement parameter that has been introduced to assess tissue AGE levels, have been reported to be associated with various diabetes-related abnormalities and complications (Lutgers et al. 2009). Previously, biomarkers of systemic oxidative stress, such as SAF and serum pentosidine levels, have been reported to be associated with renal and cardiovascular diseases (Sugiyama et al. 1998; Lutgers et al. 2006; Koyama et al. 2007; Meerwaldt et al. 2007; Koyama et al. 2008; Tanaka et al. 2012). However, their association with clinical ophthalmological findings, such as ocular microcirculation and central macular thickness (CMT), is currently unclear, particularly in eyes with no DR (NDR) or non-proliferative DR (NPDR) (Hirano et al. 2014; Yasuda et al. 2015).

The introduction of laser speckle flowgraphy (LSFG) and its most important measurement parameter, mean blur rate (MBR, an index of ocular blood flow), has made it easy to measure ocular microcirculation (Konishi et al. 2002; Watanabe et al. 2008; Sugiyama et al. 2010; Nagahara et al. 2011). LSFG has already been used to evaluate ocular hemodynamics in a number of clinical and experimental studies, and has proven particularly useful in studies of glaucoma (Sugiyama et al. 2010; Aizawa et al. 2011; Wang et al. 2012; Cull et al. 2013; Shiga et al. 2013; Tsuda et al. 2013; Aizawa et al. 2014; Kobayashi et al. 2014; Fan et al. 2015). MBR can also serve as a useful biomarker of positive postoperative visual outcomes in eyes with retinal diseases (Aizawa et al. 2014; Nitta et al. 2014; Kunikata et al. 2015; Saito et al. 2015).
Recently, it was reported that peripheral hemodynamics were negatively correlated with oxidative stress in healthy subjects, as well as in those with chronic heart failure (Nozawa et al. 2008; Inoue et al. 2012). In this study, we determined whether ocular microcirculation was associated with AGEs, an oxidative stress marker, as well as with other clinical findings, including CMT, in type 2 diabetes patients with early DR.

**Materials and Methods**

**SETTING AND DESIGN**

This was an institutional, cross-sectional study.

**PATIENTS**

The subjects included in this study comprised NDR or NPDR patients with type 2 diabetes, all of whom were observed at Tohoku University Hospital. All patients underwent a baseline ophthalmic examination, including measurement of visual acuity and intraocular pressure, a slit lamp examination, and a fundus examination, before the measurement of SAF and LSFG.

This study included DM patients (aged 20-80 Y.O.) who had HbA1c > 6.5% or were undergoing current pharmacological therapy. The exclusion criteria were the presence of: pancreatic diabetes, hepatic diabetes, gestational diabetes, secondary diabetes from endocrine disease or type 1 diabetes; ongoing hemodialysis; current malignant, inflammatory disease, chronic respiratory disease; and age-related macular degeneration, glaucoma, or retinal diseases other than NPDR.

The severity of DR was evaluated with indirect ophthalmoscopy and slit-lamp
biomicroscopy of the posterior segment with a +90 D lens (Volk Optical Inc., Mentor, Ohio, USA) according to the Early Treatment of Diabetic Retinopathy Study (ETDRS) criteria (Early Treatment Diabetic Retinopathy Study Group. 1991). This study was approved by the institutional review board of the Tohoku University Graduate School of Medicine. Informed consent for the participation in the research was obtained from each patient, and the research was conducted according to the provisions of the Declaration of Helsinki, 1995 (as revised in Edinburgh, 2000).

**MEASUREMENT OF PHYSICAL AND OPHTHALMOLOGICAL FINDINGS**

Systolic blood pressure and diastolic blood pressure (SBP and DBP) were measured after the patients had rested in a sitting position for 10 min. Measurements were made in the left brachial artery at the height of the heart by an automated blood pressure monitor (HEM-759E, Omron Corporation, Kyoto, Japan). LSFG was then used to measure optic nerve head (ONH) circulation. The subjects did not fast before collection of the blood samples. Hemoglobin A1c (HbA1c), triglyceride, total cholesterol, creatinine, and pentosidine were measured with automated standardized laboratory techniques. Ophthalmological examinations included fundus photography and the measurement of visual acuity, IOP, spherical equivalent (SE) and diopter (D). Optical coherence tomography (OCT) was used to measure central macular thickness (Topcon 3D OCT-2000, Topcon, Tokyo, Japan) and subfoveal choroidal thickness (OCT Spectralis, Heidelberg Engineering, Germany). Only the right eye of each patient was used in the analysis of the data.

**MEASUREMENT OF SKIN AUTOFLUORESCENCE**
SAF was measured with an AGE Reader (DiagnOptics BV, Groningen, Netherlands), which has been described in detail in previous reports. (Meerwaldt et al. 2004; Koetsier et al. 2010; Arsov et al. 2013) SAF is expressed in arbitrary units (AU). In this study, as in our previous report, care was taken to measure SAF at the same location, the inner forearm, in each patient (Yasuda et al. 2015).

LASER SPECKLE FLOWGRAPHY MEASUREMENT

Before the LSFG measurement, the pupils of each subject were dilated with 0.5% tropicamide and 0.5% phenylephrine hydrochloride. The details of the underlying principles of LSFG (Softcare, Fukutsu, Japan) have been described in previous reports (Tamaki et al. 1995; Isono et al. 2003). Briefly, LSFG measures the speckle pattern that arises when laser irradiation is scattered by the ocular fundus tissue. The LSFG software calculates MBR based on the light intensity of this speckle pattern, on a point-by-point basis. The software also automatically divides a heartbeat map of the ONH into large vessel and capillary areas, and blood flow parameters are assessed separately for the vessel area (referred to as vascular MBR), the tissue area (tissue MBR) and the total area of the ONH (overall MBR). Statistical analyses in this study were based on ONH values that represented the average of three LSFG measurements.

STATISTICAL ANALYSIS

Variables were expressed as the median (interquartile range: IQR). Spearman’s rank correlation test was used to estimate relationships between variables. Multiple linear regression analyses were performed to determine independent variables affecting
tissue MBR and CMT. The statistical analyses were performed with R software (version 3.2.0, R core team). Differences were considered significant at $P < 0.05$.

**Results**

The clinical characteristics of both the diabetes patients and the non-diabetic controls are shown in Table 1. This study included 46 patients with type 2 diabetes, with a median age of 53 years (IQR 39-61).

A single regression analysis revealed that SAF in the diabetes patients was correlated with age ($r = 0.347, P = 0.018$, Table 1) but not with any other clinical findings. Single regression analysis also revealed that serum pentosidine concentration was correlated with vascular MBR, tissue MBR and age ($r = -0.312, P = 0.035$, $r = -0.374, P = 0.01$, and $r = 0.295, P = 0.046$, respectively, Table 1 and Figure 1), but not with any other clinical findings. However, SAF and serum pentosidine did not correlate to each other ($r = -0.091, P = 0.545$, Table 1).

HbA1c was not correlated with overall MBR, vascular MBR, or tissue MBR ($r = -0.085, P = 0.573$, $r = -0.185, P = 0.218$ and $r = -0.086, P = 0.572$, respectively, Figure 2) or with SAF or serum pentosidine ($r = -0.005, P = 0.973$ and $r = -0.055, P = 0.715$, respectively).

CMT was correlated with tissue MBR ($r = 0.355, P = 0.016$, Figure 3), but not with vascular MBR or overall MBR ($r = 0.026, P = 0.862$ and $r = 0.192, P = 0.201$, respectively, Figure 3).

Separate multiple regression analyses of independent contributing factors revealed that age, SAF, pentosidine concentration, duration of diabetes and pulse rate contributed to tissue MBR ($P = 0.041$, $P = 0.046$, $P = 0.022$, $P = 0.011$ and $P = 0.036$, respectively).
respectively, Table 2), and SAF, HbA1c, pulse rate, tissue MBR, diastolic blood
pressure and creatinine concentration contributed to CMT ($P = 0.005$, $P = 0.039$, $P <
0.001$, $P < 0.001$, $P = 0.022$ and $P = 0.001$, respectively, Table 3).

**Discussion**

We set out to investigate the relationship between biomarkers of systemic oxidative
stress, including SAF and serum pentosidine, and ocular microcirculation
(represented by MBR), as well as other clinical parameters, in patients with type 2
diabetes and early DR. We found that SAF and serum pentosidine were correlated
with age, and that serum pentosidine was correlated with vascular MBR and tissue
MBR. Furthermore, CMT was positively correlated with tissue MBR, but not with
vascular MBR or overall MBR. A series of multiple regression analyses of different
independent contributing factors revealed that age, SAF, serum pentosidine, duration
of diabetes and pulse rate contributed to tissue MBR, while SAF, HbA1c, pulse rate,
tissue MBR, diastolic blood pressure and creatinine concentration contributed to
CMT.

Although it is suspected that the accumulation of AGEs is associated with poor
control of diabetes and renal failure, a direct relationship has not been established,
nor has a definitive relationship been established between AGEs and age. However,
SAF has been found to be relatively high in patients with type 2 diabetes, having a
median value of more than 2.0 AU (Tanaka et al. 2012). This finding was confirmed
in our current data. Our data also supported previous observations that age is
positively correlated with SAF (Lutgers et al. 2006; Meerwaldt et al. 2007; Tanaka et
al. 2012) and that patients with type 2 diabetes have a relatively high median serum
level of pentosidine; more than 30 microgram/ml (Yoshida et al. 2005). Our results
generally fit the pattern of these previous results, but we found that while serum
pentosidine levels were positively correlated with age, they were not correlated with
HbA$_1$c or creatinine. This contrasts with a previous study showing that serum
creatinine and HbA$_1$c influenced plasma pentosidine levels in diabetes (Sugiyama et
al. 1998). This discrepancy might have arisen because of differences in the
distribution of patients between this and previous studies: our study specifically
selected subjects with NDR or NPDR, and accordingly included only a small number
of patients with renal insufficiency (only 5 of 46 patients had a creatinine level more
than 1.0 mg/dl, and none had a level more than 2.0 mg/dl). By contrast, the earlier
study had a high rate of PDR combined with renal insufficiency (Sugiyama et al.
1998).

Among biomarkers of systemic oxidative stress, SAF and serum pentosidine have
both been reported to reflect AGE levels. Nevertheless, the direct relationship
between these two measurement parameters remains unclear. As these measurements
are highly promising additions to clinical care, elucidating their relationship is an
important goal. Measuring serum pentosidine and SAF is easy, non-invasive, and
possible even in an outpatient setting, in contrast to previous techniques for
measuring the accumulation of AGEs in human subjects. In addition to pentosidine,
other blood biomarkers of AGEs, such as N(ε) -(carboxymethyl)lysine (CML) and
N(ε) -(carboxyethyl)lysine (CEL), have been reported to be associated with DR
(Hammes et al. 1999; Berner et al. 2012). We chose to measure pentosidine because
it was the most commonly used biomarker of diabetic nephropathy in the Japanese
clinical context of this study. Furthermore, pentosidine levels increase with DMR
severity (Salman et al. 2009), suggesting that pentosidine can be considered a good
template of AGE levels. Additionally, pentosidine has been reported to
accumulate in the lamina cribrosa in human subjects (Albon et al. 1995), suggesting
that serum pentosidine and SAF are likely also associated with blood flow in the
ONH. Our finding that SAF was not correlated to serum pentosidine agrees with the
results of a study of end-stage renal disease patients undergoing hemodialysis (Ueno
et al. 2011) while it disagrees with reports that examined skin biopsies from diabetes
(Meerwaldt et al. 2004) and hemodialysis patients (Meerwaldt et al. 2005). These
studies found that SAF was strongly correlated with skin pentosidine levels. Thus,
we consider it likely that systemic measurements of serum pentosidine do not
directly reflect local measurements of AGEs in the skin, and that serum pentosidine
measurements should thus be interpreted separately from measurements of AGEs in
the skin, even in early DR.

The most interesting finding of the current study was the negative correlation of
AGE markers, particularly serum pentosidine, with ONH blood flow in NPDR eyes.
This is understandable, despite the inclusion in our study of only NDR or NPDR
patients, because serum levels of AGEs have previously been reported to be related
to diabetic complications, including DR (Ono et al. 1998; Ono et al. 1998). AGE
accumulation, a natural consequence of aging that can be accelerated by
hyperglycemia, may play a role in vascular complications of diabetes (Brownlee et al.
1984) by encouraging the production of key cytokines related to the development of
DR (Matsumoto et al. 2002). The vascular rigidity of the retinal arteries has been
reported to increase with higher plasma levels of pentosidine in type 2 diabetes
patients with early DR (Sato et al. 2012). Therefore, combined with previous findings
that AGEs are involved with the pathogenesis of DR (Stitt 2010), the results of our study suggest that AGEs, including serum pentosidine, may be promising biomarkers of ONH ischemia in diabetes patients with early DR. Consequently, anti-AGE therapies, administered after a clinical finding of elevated AGEs and decreased ocular blood flow, may hold promise as future treatments to prevent the progression of DR in patients at an early stage.

In contrast to serum pentosidine, we found that HbA1c was not correlated with ONH blood flow. Although HbA1c is commonly used as an indicator of glycemic control and a biomarker of diabetic vascular complications, it has been reported that HbA1c does not correlate closely with the presence or progression of DR (Monnier et al. 1999; Tanaka et al. 2012; Yasuda et al. 2015). Moreover, skin AGE levels have been shown to have a closer relationship with diabetic vascular complications and to have a better predictive value than HbA1c (Genuth et al. 2005), suggesting that SAF may also be of considerable value in obtaining information on retinal damage. The difference we observed between SAF and HbA1c in their correlation with ONH blood flow may be due to the fact that HbA1c reflects the average blood glucose level, which indicates only the short-term effects of glycemic control, while skin levels of AGEs reflect the long-term effects of hyperglycemia, thus representing a form of metabolic memory (Genuth et al. 2005). Thus, a multilateral approach that incorporates the distinctive advantages of both serum pentosidine, for its high correlation with ocular blood flow, and SAF, for its ability to reflect the long-term accumulation of AGEs and the non-invasiveness of its measurement technique, may be the most accurate way to predict ONH blood flow and the course of DR.

Another important result of the current study was the positive correlation of CMT
with ONH blood flow. Measuring CMT is of great practical significance in DR patients, because DME, a condition which has become the leading cause of legal blindness in industrialized countries, is the main cause of severe central visual impairment in NPDR patients (Saaddine et al. 2008; Bandello et al. 2010). AGEs are thought to be one of the most important causes of DME, but a recent report showed that neither SAF, HbA1c or self-assessed DM duration correlated with DME prevalence or severity (Hirano et al. 2014). In our previous work on ocular blood flow in DME, we found that MBR in the ONH was negatively correlated with CMT after the intravitreal administration of bevacizumab ($r = -0.80$) (Nitta et al. 2014). While there were no clinically significant DME patients in our current study, we made the interesting finding that CMT was positively correlated with tissue MBR in the ONH. This suggests that ocular ischemia might cause decreased CMT, even in early DR eyes. This new finding on tissue MBR in early DR eyes is important, because as our recent work has shown, tissue MBR in the ONH accurately reflects regional blood flow and has validity for inter-individual comparisons regardless of patient ethnicity (Aizawa et al. 2014). Thus, though CMT is likely affected by multiple factors, the current study shows that AGE-induced ocular ischemia, i.e., low tissue MBR, is a possible cause of retinal cell loss and consequent decreased CMT.

Our investigation into the correlation between CMT and skin AGEs was inspired by recent findings that AGEs can cause neural cell apoptosis (Ren et al. 2015), and that eyes with NDR or with NPDR undergo reductions in retinal thickness (Carpineto et al. 2016; Chen et al. 2016). These studies suggest that retinal degeneration might occur even in eyes with early DR. Furthermore, skin AGEs, which can be non-invasively assessed by measuring SAF, have been reported to increase in DM
patients (Tanaka et al. 2012; Januszewski et al. 2012; Yasuda et al. 2015), and to correlate with the severity of DR (Tanaka et al. 2012; Yasuda et al. 2015). Thus, we believe that the CMT and SAF, as well as MBR, all of which are non-invasive, quickly measurable parameters, may be promising early biomarkers of the risk of diabetic changes. However, our findings on the relationship between CMT and skin AGEs in early DR were unclear. We performed a number of separate multiple regression analyses, which revealed that SAF negatively contributed to tissue MBR and positively contributed to CMT. By contrast, a single regression analysis found that SAF was not correlated with CMT.

Our study was limited by its cross-sectional design, its small sample size, and the lack of healthy control subjects. Thus, while we could confirm a close relationship between tissue MBR, AGEs and CMT in eyes with early DR, we could not make more detailed conclusions. Although it is likely that accumulated AGEs cause decreases in both ocular blood flow and CMT, the underlying mechanism may be complicated. Separate multiple regression analyses showed that SAF made a negative contribution to tissue MBR, but a positive contribution to CMT. Nevertheless, our findings on the relationship of ocular blood flow and AGEs in diabetes patients should be reliable, since that they were based on carefully selected subjects. We focused on type 2 diabetes because of its increasing prevalence worldwide. Previous studies have generally treated type 1 and type 2 diabetes as separate topics, because these disease types have different pathological mechanisms and conditions. Our conclusions were given additional strength by our exclusion of patients undergoing hemodialysis, because of the possibility that the level of HbA1c could be underestimated due to anemia and the short lifespan of red blood cells (Peacock et al.
We also excluded patients with inflammatory, malignant or respiratory diseases from this study, as these conditions can affect the accumulation of skin AGEs, regardless of diabetic status. Thus, we consider that our study, with its clear focus on diabetes, obtained sufficient evidence to support our conclusions.

In conclusion, we found that serum pentosidine levels were negatively correlated with vascular and tissue MBR in the ONH, and that tissue MBR was correlated with CMT. Furthermore, multiple factors, including SAF and serum pentosidine, negatively contributed to tissue MBR, while factors including tissue MBR positively contributed to CMT. Thus, SAF and serum pentosidine may have potential as biomarkers of ONH ischemia in diabetes patients with early DR. Finally, our results suggest that tissue MBR is closely related to AGE levels and CMT in type 2 diabetes patients with early DR, suggesting that ocular circulation might be a potential biomarker of early DR, as a supplement to AGE levels. Further investigation is needed to confirm a causal relationship between AGEs and ocular disease, but both AGEs and ocular blood flow may be considered promising new surrogate markers of DR severity and progression.
Acknowledgement

Informed consent for treatment and participation in the research for this study was obtained from all patients. The institutional review board of Tohoku University Graduate School of Medicine approved this study. The principal investigators, Dr. Kazuki Hashimoto, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the analysis. No authors have any potential conflicts of interest to disclose. The authors would like to express our gratitude to Dr. Hideyuki Onami (Department of Ophthalmology, Iwaki Kyoritsu General Hospital, Fukushima, Japan) for their support of this paper. This paper was supported in part by JST grants from JSPS KAKENHI Grants-in-Aid for Scientific Research (B) (T.N. 26293372), for Scientific Research (C) (H.K. 26462629), and for Exploratory Research (T.N. 26670751). The funders had no role in the design or conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication.
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Figure legends

Figure 1

Relationship between serum pentosidine levels and blood flow in the optic nerve head

Left: Serum pentosidine levels were correlated with tissue mean blur rate (MBR) ($r = -0.374, P = 0.01$).

Center: Serum pentosidine concentration was correlated with vascular MBR ($r = -0.312, P = 0.035$).

Right: Serum pentosidine concentration was not correlated with overall MBR ($r = -0.227, P = 0.129$).

Figure 2

Relationship between HbA1c and blood flow in the optic nerve head

Left: HbA1c was not correlated with tissue mean blur rate (MBR) ($r = -0.086, P = 0.572$).

Center: HbA1c was not correlated with vascular MBR ($r = -0.185, P = 0.218$).

Right: HbA1c was not correlated with overall MBR ($r = -0.085, P = 0.573$).

Figure 3

Relationship between central macular thickness (CMT) and blood flow in the optic nerve head

Left: CMT was correlated with tissue MBR ($r = 0.355, P = 0.016$).

Center: CMT was not correlated with vascular MBR ($r = 0.026, P = 0.862$).

Right: CMT was not correlated with overall MBR ($r = 0.192, P = 0.201$).
Figure 1. Correlations between pentosidine and MBR

- Tissue MBR (A.U.): $r = -0.374$, $P = 0.010$
- Vascular MBR (A.U.): $r = -0.312$, $P = 0.035$
- Overall MBR (A.U.): $r = -0.227$, $P = 0.129$
Figure 2. Correlations between HbA1c and MBR

- Tissue MBR (A.U.): $r = -0.086$, $P = 0.572$
- Vascular MBR (A.U.): $r = -0.185$, $P = 0.218$
- Overall MBR (A.U.): $r = -0.085$, $P = 0.573$
Figure 3. Correlations between CMT and MBR

- Tissue MBR (A.U.): $r = 0.355$, $P = 0.016$
- Vascular MBR (A.U.): $r = 0.026$, $P = 0.862$
- Overall MBR (A.U.): $r = 0.192$, $P = 0.201$
Table 1. Characteristics of diabetic patients, with factors possibly related to oxidative stress parameter

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<tr>
<td>Age (years)</td>
<td>53 (39-61)</td>
<td>0.347</td>
<td>0.018</td>
<td>0.295</td>
<td>0.046</td>
</tr>
<tr>
<td>Sex (M / F)</td>
<td>29 / 17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>General findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index</td>
<td>27.4 (24.0-31.0)</td>
<td>-0.011</td>
<td>0.940</td>
<td>0.128</td>
<td>0.395</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>124 (119-130)</td>
<td>0.134</td>
<td>0.371</td>
<td>-0.085</td>
<td>0.572</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>75 (70-81)</td>
<td>0.106</td>
<td>0.481</td>
<td>0.123</td>
<td>0.412</td>
</tr>
<tr>
<td>Pulse rate (beat/min)</td>
<td>78 (70-85)</td>
<td>0.113</td>
<td>0.451</td>
<td>-0.274</td>
<td>0.064</td>
</tr>
<tr>
<td>Hemoglobin A1c (%)</td>
<td>9.3 (8.5-10.7)</td>
<td>-0.005</td>
<td>0.973</td>
<td>-0.055</td>
<td>0.715</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>156 (113-208)</td>
<td>-0.071</td>
<td>0.637</td>
<td>-0.136</td>
<td>0.364</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>184 (160-209)</td>
<td>-0.006</td>
<td>0.966</td>
<td>-0.126</td>
<td>0.403</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.71 (0.53-0.82)</td>
<td>0.164</td>
<td>0.273</td>
<td>0.102</td>
<td>0.497</td>
</tr>
<tr>
<td>Pentosidine (μg/mL )</td>
<td>0.032(-0.028 - 0.040)</td>
<td>-0.091</td>
<td>0.545</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SAF</td>
<td>2.2 (1.8-2.6)</td>
<td>-</td>
<td>-</td>
<td>-0.091</td>
<td>0.545</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>5 (2-13)</td>
<td>0.255</td>
<td>0.085</td>
<td>-0.035</td>
<td>0.816</td>
</tr>
<tr>
<td>Ophthalmological findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual acuity (logMAR)</td>
<td>-0.079 (-0.176-0.020)</td>
<td>0.134</td>
<td>0.372</td>
<td>0.255</td>
<td>0.086</td>
</tr>
<tr>
<td>IOP (mmHg)</td>
<td>16 (14-18)</td>
<td>0.066</td>
<td>0.660</td>
<td>-0.122</td>
<td>0.418</td>
</tr>
<tr>
<td>SE (Diopter)</td>
<td>-1.38 (-2.72-0)</td>
<td>-0.099</td>
<td>0.510</td>
<td>0.19</td>
<td>0.203</td>
</tr>
<tr>
<td>CMT (μm)</td>
<td>241 (222-255)</td>
<td>0.231</td>
<td>0.122</td>
<td>-0.251</td>
<td>0.092</td>
</tr>
<tr>
<td>Choroidal thickness (μm)</td>
<td>265 (219-329)</td>
<td>-0.243</td>
<td>0.103</td>
<td>-0.172</td>
<td>0.251</td>
</tr>
<tr>
<td>Ocular blood flow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall MBR</td>
<td>21.6 (18.5-24.0)</td>
<td>-0.187</td>
<td>0.211</td>
<td>-0.226</td>
<td>0.129</td>
</tr>
<tr>
<td>Vascular MBR</td>
<td>40.6 (35.4-44.9)</td>
<td>-0.219</td>
<td>0.142</td>
<td>-0.312</td>
<td>0.035</td>
</tr>
<tr>
<td>Tissue MBR</td>
<td>11.1 (9.3-12.6)</td>
<td>-0.081</td>
<td>0.591</td>
<td>-0.374</td>
<td>0.010</td>
</tr>
</tbody>
</table>

SBP: systolic blood pressure, DBP: diastolic blood pressure, SAF: Skin autofluorescence, IOP: Intraocular pressure, SE: Spherical equivalent, logMAR: logarithm of the minimum angle, CMT: central macular thickness, MBR: Mean blur rate, r: Spearman's correlation coefficient
Table 2. Multiple regression analysis of factors contributing to MBR (Tissue)

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue MBR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.317</td>
<td>0.041</td>
</tr>
<tr>
<td>Skin autofluorescence</td>
<td>-0.276</td>
<td>0.046</td>
</tr>
<tr>
<td>Pentosidine</td>
<td>-0.300</td>
<td>0.022</td>
</tr>
<tr>
<td>Diabetes duration</td>
<td>0.368</td>
<td>0.011</td>
</tr>
<tr>
<td>Pulse rate</td>
<td>0.285</td>
<td>0.036</td>
</tr>
<tr>
<td>High-density lipoprotein</td>
<td>-0.239</td>
<td>0.060</td>
</tr>
</tbody>
</table>

β: standardized regression coefficient. Adjusted R-squared = 0.365.
MBR: Mean blur rate
<table>
<thead>
<tr>
<th></th>
<th>( \beta )</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin autofluorescence</td>
<td>0.316</td>
<td>0.005</td>
</tr>
<tr>
<td>Hemoglobin A1c</td>
<td>0.233</td>
<td>0.039</td>
</tr>
<tr>
<td>Pulse rate</td>
<td>-0.432</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tissue MBR</td>
<td>0.524</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.263</td>
<td>0.022</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.393</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\( \beta \): standardized regression coefficient. Adjusted \( R \)-squared = 0.512.

CMT: central macular thickness. MBR: Mean blur rate