Role of Advanced Glycation End Products in Assessment of Diabetes Mellitus as a Risk Factor for Retinal Vein Occlusion

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Abstract: Aim: In this study, we aimed to assess the correlation between diabetes mellitus (DM) and the retinal vein occlusion (RVO) based on skin autofluorescence (SAF) measurement, which reflects the accumulation of advanced glycation end products (AGE) in patients who have undergone an episode of central retinal vein occlusion (CRVO) or branch retinal vein occlusion (BRVO). Material and methods: In total, 23 patients (16 males, 7 females) with RVO were included in this study. Among these 23 participants, 12 (52%) had been diagnosed with CRVO and 11 (48%) with BRVO. The control group consisted of 14 healthy volunteers (11 females, 3 males). To calculate the risk of cardiovascular diseases (CVD) and DM, we conducted SAF examinations. We compared the SAF levels in three groups of patients: (1) with CRVO, (2) with BRVO, and (3) the control group. Basic demographic and clinical information and detailed history of the concurrent diagnoses of systemic diseases, such as systemic hypertension (HTN), DM, hyperlipidemia (HL), and heart diseases, were obtained. Results: In total, 10 (43.5%) patients were diagnosed with DM, 6 (55%) in the BRVO group and 4 (33%) in the CRVO group. The mean SAF value was significantly higher in the BRVO group than in the control group (2.64 a.u. and 2.35 a.u., respectively) (p = 0.023). More patients with risk of DM were identified in the CRVO group than in the BRVO group (p = 0.024). Conclusions: The advanced glycation end products (AGE) in the skin autofluorescence (SAF) is a viable method of evaluating the risk of DM in patients with RVO. We confirmed a correlation between RVO and DM, which was significantly pronounced in the CRVO form, although further carefully devised studies on the relationship between RVO and DM with a larger number of responders should be conducted in the future.

Keywords: retinal vein occlusion; diabetes mellitus; autofluorescence; advanced glycation end products

1. Introduction

Following diabetic retinopathy (DR), Retinal vein occlusion (RVO) is the most known cause of vision deterioration due to a retinal vascular disorder [1,2]. There are two major types of RVO: branch retinal vein occlusion (BRVO) and central retinal vein occlusion (CRVO). Hemi-CRVO (HRVO) has been considered by some authors as a subgroup of CRVO [3–5]. Although CRVO is less common than BRVO, it is associated with a worse visual prognosis, particularly in its ischemic form [4,6]. More than 16 million adults are affected by RVO, with the incidence of BRVO being 3- to 10-times higher than CRVO [5,7–9]. These numbers will undoubtedly rise because of the gradual elongation of the population’s lifespan. Therefore, an increased risk for both BRVO and CRVO will particularly concern...
older individuals compared to younger ones [1,10]. The pathogenesis of RVO is still not completely understood. Undoubtedly, arterial disturbances are a leading pathogenetic mechanism for RVO. In CRVO, because of the same adventitial sheath within the lamina cribrosa for the central retinal vessels, arterial stiffness affects neighboring veins. In BRVO, the compression of the veins at arteriovenous (AV) crossings, progressive changes concerning the venous walls, and the increased tendency to form blood clots are possible causes of the disorder [11].

Because of the varying anatomic relationships among retinal vessels, the risk factors for BRVO and CRVO may be different [10]. Three factors predispose individuals to thrombosis—damage of the endothelium, disturbed blood flow, and hypercoagulability—collectively defined as the Virchow’s triad, play a role in the pathogenesis of RVO [5,11,12]. Some authors have suggested that systemic diseases that elevate the risk of changes in the endothelium or disturbed blood flow, such as hypertension (HTN), dyslipidemia, diabetes mellitus (DM), and heart diseases, are strictly connected with retinal vascular occlusion [10]. Although HTN is consistently indicated as a significant risk factor for both BRVO and CRVO, its connection with the diabetic condition is still controversial, and different studies have reported varying results on this subject [13]. Some studies have indicated DM as a risk factor for CRVO, but not BRVO [6,14]. An interesting conclusion was drawn in the meta-analysis by Wang, which states that, in general, DM was not a risk factor for RVO in studies published before 2010. However, DM was attributed as a risk factor for RVO in studies published after 2010 [6]. The author implied that more recent studies might have been used more accurate methods. Moreover, the increasing number of studies, has made it possible to confirm a correlation between DM and RVO. However, no specific new methods of the detection of risk factors for DM and RVO have been pointed out in these published studies.

Because of such heterogeneity of opinions in the literature, we decided to seek a different way to assess this correlation. For this purpose, we measured the skin autofluorescence (SAF), which assesses the accumulation of advanced glycation end products (AGE) in patients who have undergone an episode of CRVO or BRVO.

AGEs comprise heterogeneous groups of molecules, which accumulate in plasma and tissues during the natural process of aging. Studies had shown that prolonged exposure to hyperglycemia and consequently AGEs formation are responsible for irreversible changes and leads to progression of diabetic complications [15,16]. The AGEs formation affects short-lived molecules such as circulating plasma proteins and lipids, as well as long-lived macromolecules, such as proteins in ocular lens [17,18]. AGEs are accounted for “metabolic memory”, which is responsible for chronic irreversible vascular abnormalities in diabetic patients, even with relatively good control of glucose level [16,19]. Its accumulation in ocular tissues due to aging process or accelerated in diabetic condition leads to various complications, including keratopathy, opacification of the lens and changes in vitreous which could partially explain vitreoretinal changes during diabetic retinopathy (DR) [17]. AGEs are also relevantly responsible for early retinal vascular changes in DR. In retina AGEs leads to pericyte loss and therefore breakdown of blood—retinal barrier and microvascular damage, as well as mediation of intracellular ROS generation and increased VEGF expression with consequently proliferative changes in DR [20].

AGEs is known to have a major role in the pathogenesis of chronic conditions, such as vasculopathy by the receptor-mediated mechanism. A receptor for AGEs (RAGE) plays an important role in inflammatory processes and tumor cell invasion [21,22]. Therefore, the increased concentration of AGE is associated with diseases such as DM, cardiovascular diseases, rheumatoid arthritis, and neurological/mental health conditions, such as depression and Alzheimer’s disease [23–25]. Moreover, because of the high anaerobic metabolism of glucose in cancer cells, the connection between the hyperglycemic condition and increased formation of AGEs occurs, which takes place in brain tumors [26]. AGEs formation occurs constantly within the body as it continues to age, and this process is greatly accelerated in individuals with diabetes [18,27–29]. The accumulation of AGEs leads to vascular wall
damage, contributing to the development of micro- and macrovascular disturbances both in type 1 and 2 diabetes [30–32].

In some authors’ opinions, the SAF assessment, as a non-invasive measurement, allows for the quantification of the AGEs load and shows a good predictive value (superior to the HbA1c) for the development of diabetic complications, microvascular and macrovascular disease, and cardiovascular and overall mortality [33].

In this study, we compared the values from the SAF examinations between both types of RVO and the control group.

2. Methods

The consecutive series of patients diagnosed with BRVO or CRVO and consulted at the University Hospital in Bydgoszcz, Poland and at the Outpatient Clinic in Bydgoszcz between January and May 2019 were taken into consideration in the study. Patients with significant other ocular disturbances in the affected eye, such as ocular trauma, retrobulbar external compression from Graves’ disease or orbital tumor, and optic nerve drusen, as well as some systemic diseases which may influence vessel condition, such as coagulation disorders or inflammatory systemic disorders, were excluded. These conditions were especially important in young individuals (less than 50 years old), who were considered for participation only if secondary pathologies, such as thrombophilia and uveitis, were excluded. To avoid possible false measurements of the SAF, participants with skin pigmentation, using skin sun creams, or with extensive scarring of the skin, were also excluded from the study. A small number of patients failed to arrive for the clinical examination. Ultimately, 23 subjects were enrolled in the study. The control group consisted of 14 healthy volunteers with no diagnosed general or ocular diseases and with normal values in laboratory tests.

Basic demographic and clinical information were obtained all patients, including the date of the first occurrence of CRVO or BRVO, incidents of cystoid macular edema (CME), previous treatment of RVO (anticoagulants, intravitreal anti-VEGF, or steroid injections, laser therapy), sex, and eye laterality. Detailed information about the patients’ smoking status; sunlight exposure; concurrent diagnoses of systemic diseases, such as HTN, DM, hyperlipidemia (HL), heart diseases; and any ocular disturbances, such as glaucoma, were obtained. Retrospectively, the best corrected visual acuity (BCVA) before the episode of RVO and shortly after treatment was noted.

At the final follow-up, a clinical examination of all patients was performed at the same center (University Hospital in Bydgoszcz, Poland) between June and September 2019. The examination included a BCVA assessment (using Snellen charts, decimal fraction), intraocular pressure (IOP), slit-lamp biomicroscopy (with an evaluation of the lens status, presence of abnormal pupil reactions, neovascularization of the iris, and iridocorneal angle), indirect ophthalmoscopy (due to possible neovascularization of the optic disk or elsewhere and/or vitreous hemorrhage), and optical coherence tomography (OCT, Nidek RS-3000) for CME detection.

The metabolic status of each individual was assessed. For this purpose, laboratory examinations were performed, and blood glucose levels, Hb1Ac, and lipid profiles were assessed. The diagnosis of diabetes mellitus was based on the patient’s medical history with the use of diabetic medication. The metabolic tests were carried out to better assess the metabolic status of each individual, apart from each patient’s medical history with antidiabetic treatment. By assessing metabolic status, we were able to ensure that our study groups (with DM and without DM) were variable.

2.1. SAF Measurement Technique

To calculate the risk of cardiovascular diseases (CVD) and diabetes, we conducted SAF examinations. To this end, the AGE Reader diagnostic device, manufactured by Diagoptics, was employed.

The examination technique is straightforward and non-invasive. The patient places their forearm onto the device. Then, AGE molecules are excited through ultraviolet light
type B (UVB). The photons initially absorbed by the AGEs are emitted in a process called auto-fluorescence and counted by the built-in photomultiplier. The AGE Reader illuminates a skin surface of ~4 cm$^2$, guarding against the surrounding light, with an excitation light source with a peak intensity at ~370 nm. The emission light and reflected excitation light from the skin are measured with a spectrometer in the 300–600 nm range using a glass fiber. The SAF was expressed by arbitrary units (a.u.).

The higher the SAF signal, the greater risk of developing diabetic and cardiovascular changes. We compared the SAF levels in the 3 groups of patients: (1) with CRVO, (2) with BRVO, and (3) the control group (healthy subjects with no diagnosed DM and CVD).

The study was approved by the Institutional Review Board and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all individuals.

2.2. Statistical Analysis

The normality of data was assessed using the Shapiro-Wilk test. For normal distribution, the independent t-test was used to compare both groups. A chi-square test was used to compare the categorical data. For comparing three or more groups, the ANOVA test was used. $p \leq 0.05$ was considered statistically significant.

3. Results

3.1. Baseline Characteristics

In total, 23 patients (16 males, 7 females) with RVO were included in this study. Among these 23 participants, 12 (52%) were diagnosed with CRVO and 11 (48%) with BRVO. Among the 12 individuals in the CRVO group, 4 (33.3%) had the ischemic form of CRVO and 8 (66.7%) had non-ischemic CRVO. The control group consisted of 14 healthy control subjects (3 males, 11 females). The mean age of all the RVO participants was 61.4 ± 14.8 years (range: 36–79 years). The mean ages of the CRVO, BRVO, and control groups were 55.7 ± 7.5 (range: 36–77), 67.7 ± 7.8 (range: 50–79), and 57.8 ± 13.3 (range: 41–73) years, respectively. We noticed a significant difference in the age of the participants in the CRVO and BRVO groups ($p = 0.024$). The morbidity rate was higher in males. There was no significant difference in the distribution of eye laterality. The basic demographics of the analyzed groups are shown in Table 1.

|                          | Overall RVO Group (n = 23) | CRVO Group (n = 12) | BRVO Group (n = 11) | Control Group (n = 14) |
|--------------------------|---------------------------|---------------------|---------------------|------------------------|
| Number of patients       | 23                        | 12                  | 11                  | 14                     |
| Age (years)              | Mean (SD)                 | 61.4 (±14.8)        | 55.7 (±7.5)         | 67.7 (±7.8)            | 57.8 (±13.3)          |
|                          | Range                     | 36–79               | 36–77               | 50–79                  | 41–73                 |
| Sex, n (%)               | Male                      | 16 (69.6)           | 9 (75)              | 7 (63.6)               | 3 (21.4)              |
|                          | Female                    | 7 (30.4)            | 3 (25)              | 4 (36.4)               | 11 (78.6)             |
| Eye, n (%)               | Right                     | 10 (43.5)           | 5 (41.7)            | 5 (45.5)               | n/a                   |
|                          | Left                      | 13 (56.5)           | 7 (58.3)            | 6 (54.5)               | n/a                   |

3.2. RVO Characteristics Acquired from Medical Documentation and at Presentation

The mean duration time from the incident of RVO was 24 months (range: 1–72). Of the 23 subjects, only 10 (43.5%) had confirmed anticoagulant treatment during the RVO episode. Cystic macular edema (CME) had previously been found in 18 patients (78.3%), 8 patients (66%), and 10 patients (91%) in the overall RVO group, CRVO group, and BRVO group, respectively. Intravitreal anti-VEGF agents were administered in 14 (60.9%) cases with CME, and in 6/14 (54.5%) eyes, the injections were repeated more than 3 times (pre-
dominantly in the CRVO group). No patient had a steroid injection in CME management. Micropulse laser therapy was performed in nine (39.1%) eyes in the overall RVO group, although in eight eyes, it was because of BRVO, representing 72.7% eyes of the BRVO group. Panretinal photocoagulation was performed in eight (34.8%) eyes, predominantly in the CRVO group (six cases, representing 50% of the eyes of the CRVO group). The estimated mean BCVA before the incident of RVO was 0.7, 0.4, and 0.8 for the overall RVO group, CRVO group, and BRVO group, respectively. After the treatment of RVO, the mean BCVA in the early follow-up (1–3 months) was 0.3, 0.07, and 0.5 for the overall RVO group, CRVO group, and BRVO group, respectively.

BCVA improved significantly during the time following any form of RVO. In the final clinical assessment, the mean BCVA was 0.5 in the overall RVO group, 0.4 in CRVO group, and 0.7 in BRVO group. There was noticeably less improvement in the final BCVA in ischemic CRVO eyes compared to the non-ischemic form of CRVO. Intraocular pressure (IOP) was in the normal range in all groups. There was no abnormal pupil reaction or symptoms of neovascularization in any part of the eye. In two (8.7%) eyes, vitreous hemorrhage was observed, with both cases being from the CRVO group. Based on the OCT scans, CME was detected in seven eyes (30.4%). Three of them had suffered a CRVO episode, and four had suffered a BRVO episode. The mean central subfield thickness in the OCT scans was 321.5 ($\pm$152) $\mu$m, 363.7 ($\pm$194) $\mu$m, and 272.5 ($\pm$70) $\mu$m in the overall RVO group, CRVO group, and BRVO group, respectively.

3.3. Risk Factors

Among the 23 participants, 10 (43.5%) were diagnosed with DM, 6 (55%) in the BRVO group and 4 (33%) in the CRVO group. All diabetic patients had at least one other metabolic risk factor, such as HTN (9/10 (90%) cases of DM patients) or HL (3/10 (30%) cases of diabetic subjects). Overall, hypertension was noted in 16 (69.6%) participants. In the CRVO and BRVO group, hypertension was noted in seven patients (58.3%) and nine (81.8%) patients, respectively. Hyperlipidemia was observed in five (21.7%), two (16.7%), and three (27.3%) subjects in the overall RVO group, CRVO group, and BRVO group, respectively. Two (8.7%) patients reported a past stroke incident. Neither was diagnosed with DM, and one of them was treated on account of HTN. Only one (4.3%) participant from the CRVO group reported smoking. Out of all the patients, glaucoma was detected in five (21.7%) eyes, four (33.3%) of which were in the CRVO group and only one (9.1%) of which was in the BRVO group.

Regarding the patients’ metabolic outcomes, the mean fasting glucose level in the group with DM and in the group without DM was 116 (range 79–240) mg/dL and 91.6 (range 77–101) mg/dL, respectively. The mean Hb1Ac was 6.1% (range 5.4–9.9) in the group with DM and 5.2% (range 4.8–6.0) in the group without DM. The mean total cholesterol was 171.9 (range 129–193) mg/dL and 183.4 (range 125–273) mg/dL in group with the DM and without DM, respectively.

3.4. Advanced Glycation Endproducts and RVO

Six (55%) patients in the BRVO group and four (33%) patients in the CRVO group were diagnosed with DM. There were no significant differences in the number of patients between these two groups ($p = 0.414$). The mean SAF values did not differ between the BRVO and CRVO groups (2.64 a.u. and 2.63 a.u., respectively) ($p = 0.484$).

In the CRVO group, 10 (91%) out of 11 patients evaluated with SAF had DM/CVD based on the AGEs values. In the BRVO group, four (44%) out of nine patients evaluated with SAF had a first-degree risk of DM/CVD based on the AGEs values. There was a significant difference between these two groups ($p = 0.024$).

The mean SAF value in the control group was 2.35 $\pm$ 0.35 a.u., which is significantly lower compared to the BRVO group and overall RVO group (Table 2).
Table 2. Mean SAF value correlation between the BRVO group, CRVO group, and control group.

| Group                  | Mean SAF (a.u.) ± SD | Study Group | Mean SAF (a.u.) ± SD | p   |
|------------------------|----------------------|-------------|----------------------|-----|
| Control group (2.35 ± 0.35) |                      | CRVO (2.63 ± 0.55) |            | 0.064 |
|                        |                      | BRVO (2.64 ± 0.28) |            | 0.023 |
|                        |                      | BRVO + CRVO (2.64 ± 0.44) |      | 0.025 |

4. Discussion

Data from recent studies suggest that 48% of RVO is connected with NTH, 20% with HLD, and 5% with DM [5,34]. Whereas HTN and glaucoma are commonly indicated as risk factors for CRVO, the influence of other general diseases on CRVO appearance is unclear [35–43]. Some studies have shown DM to be an important risk factor of CRVO, whereas others were unable to find a significant correlation between DM and CRVO [14,36–39,43,44]. The Eye Disease Case-Control Study Group assessed the risk factors for both central and branch retinal vein occlusion in a large multicenter study. They found an increased risk of BRVO in patients with a history of cardiovascular disease or systemic hypertension, but not in patients with DM [45]. They noted a cardiovascular risk profile for patients with BRVO and indicated that 50% of them may be attributable to hypertension. On the other hand, the authors of The Eye Disease Case-control Study Group confirmed an increased risk of CRVO in patients with DM apart from other diseases, such as systemic hypertension and open-angle glaucoma. However, they emphasized that the history of treatment of DM and higher blood glucose levels, as well as cardiovascular disease, electrocardiographic abnormalities, higher alpha-globulin levels, and lower albumin-globulin ratios, were associated with an increased risk only for the ischemic form of CRVO [37].

Stem et al. assessed the correlation between the components of the metabolic syndrome (HTN, DM, and HLD) individually and in combination with the risk of CRVO. They concluded that there was no increased risk of CRVO in the case of patients with DM alone (no HTN or HLD). When all three metabolic syndrome components were present, the risk of developing CRVO increased to 58% [13]. The risk of CRVO was connected with the severity of DM in individuals. For those with end-organ damage from DM, the risk of CRVO increased to 53% compared to those without DM. In the case of those without end-organ damage, there was not a noticeable risk for CRVO. Similar conclusions were drawn by Newman-Kasey et al. with respect to BRVO [10]. Although there was no association between DM without end-organ damage (“uncomplicated” cases of DM) and BRVO (p = 0.2), subjects with end-organ damage from DM (“complicated” cases) had a 36% increased hazard of developing BRVO (p < 0.0001) compared to those without DM. These studies may explain the differences between the final conclusions found in the literature. Authors have suggested that, when studying individuals with various stages of diabetes, it is possible that patients with uncomplicated DM mask the significant effect of complicated DM on CRVO risk.

Meanwhile, a recent nationwide, retrospective, matched cohort study, which included 240,761 DM patients, established that individuals with new-onset DM have a significantly increased risk of RVO [46]. These results seem to confirm an increasing association between DM and RVO over time.

In relation to general risk factors, our study confirmed an important role of hypertension in developing RVO. HTN was the most common risk factor in both groups of RVO, although HTN was significantly more frequent in the BRVO group (in 81.8% in BRVO compared to 58.3% in CRVO group). These findings agreed with data from other authors [14,38,45]. Similar to data from the literature, in this study, hyperlipidemia was noted in approximately 20% of RVO cases (16.7% and 27.3% in the CRVO and BRVO groups, respectively) [14,34,37]. However, the incidence of DM in both BRVO and CRVO groups differed from other publications [34]. In their meta-analysis, O’Mahoney et al. noted that DM was slightly more prevalent among the 2877 cases with any form of RVO than the 13,225 unaffected controls, and the PAR% for DM among individuals with any form of RVO...
was 4.9%. Furthermore, although CRVO was significantly associated with the presence of DM, BRVO was not [14]. The incidence of DM in our study was much higher than in other publications. DM diagnosis was noted in 43.5% of the overall RVO group. Moreover, patients from the BRVO group were diagnosed with DM more often than from the CRVO group (54.5% and 33%, respectively).

In the case of chronic hyperglycemia, AGE formation is accelerated. Hyperglycemia is responsible for protein glycation, which interferes with normal protein function [22,47]. The results of AGEs are influenced by the direct damage to the protein structure and by binding RAGE, which eventually leads to oxidative stress and inflammation. Therefore, hyperglycemia plays a significant role in AGEs accumulation not only in diabetic conditions, Therefore, hyperglycemia plays a significant role in AGEs accumulation not only in diabetic condition, but also in other diseases [21,26].

Under the chronic hyperglycemic condition in DM, with concomitant hyperlipidemia, oxidative/carbonyl stress, and disturbed renal function, the accumulation of AGEs occurs as one of the major pathogenetic mechanisms responsible for end-organ damage in diabetic patients. AGE-related crosslinking and receptor-mediated cellular activation lead to the loss of elasticity of the vascular wall and inflammation, which can result in microvascular and macrovascular complications. Therefore, AGEs are perceived as potential biomarkers for the progression of possible changes in DM [48–50].

Apart from their correlation with general neurodegenerative diseases, AGEs are also responsible for pathophysiologic ocular changes, such as cataract formation, diabetic retinopathy (DR), glaucoma, and age-related macular degeneration [17,51–53]. Some authors have also indicated a correlation between the SAF values and the severity of DR in type 2 diabetes. They found positive significance between the SAF and the severity of DR, suggesting the SAF as a surrogate biomarker for the non-invasive evaluation of DR [54,55].

Measuring glycated hemoglobin (HbA1c), a marker of chronic hyperglycemia, is a recommended diagnostic examination for DM and is used universally to diagnose diabetes in patients with risk factors. It also helps to identify those at higher risk for developing DM in the future [15]. Whereas as the normal value of HbA1c is considered <6.0%, patients with levels ranging from 6.0% to 6.5% are at a 10-times higher risk of developing DM. Glycemic exposure, defined as HbA1c, plays significant role in the development of microvascular complications in DM. However, while HbA1c indicates the glycemic status, AGE levels are a better predictor for diabetic complications, as shown by the findings of the DCCT study [16]. The significance of AGE levels was especially notable in the intensive glycemic control group, implying that even with effective glycemic control, diabetic complications might still progress. Furthermore, the DCCT/EDIC Research Group established that AGEs assessed by the skin intrinsic fluorescence (SIF) measurement correlate with the mean HbA1c over time [56].

We did not found any notion of a correlation between AGEs and RVO in the literature. Thus, we decided to investigate the significance of accumulation of AGEs in RVO patients with respect to DM.

In this study, the mean SAF value was 2.64 a.u. in the BRVO group and 2.63 a.u. in the CRVO group. The lack of difference in the mean SAF value may come as surprising, considering the 10-year difference in the mean age between the CRVO and BRVO groups, as age is one of the factors elevating the SAF value [57]. These results suggest that in the CRVO group, with CRVO generally affecting younger people, there is an accelerated accumulation of AGEs in the skin.

The mean SAF values in the CRVO group with DM (n = 4) and without DM (n = 6) were 2.98 a.u. and 2.44 a.u., respectively. These values differed greatly from the mean SAF values in the BRVO group with DM (n = 5) and without DM (n = 4), which were 2.6 a.u. and 2.7 a.u., respectively. These results may suggest that patients with CRVO are at a much higher risk of developing DM than patients with BRVO. We should emphasize the significant age difference between these two types of RVO. The mean age in the CRVO group was lower. In a healthy population, a lower age should be related to lower mean
SAF values. Our results may indicate that in CRVO patients, the process of AGE synthesis advances faster than in BRVO patients.

A higher percentage of patients with an increased risk of DM in the CRVO group compared to the BRVO group, based on the SAF measurement, may also indicate the faster process of AGE synthesis. In the CRVO group 10 (91%), 11 patients evaluated with SAF had a risk of DM based on the AGEs values. In the BRVO group 4 (44%), 9 patients evaluated with SAF had a risk of DM. There was a significant difference between these two groups ($p = 0.024$). It is worth noting that the mean SAF value in patients with an increased risk of DM did not differ between two groups. From a clinical point of view, these results could indicate that among patients with an increased risk of DM, there is a higher tendency of developing CRVO rather than BRVO, and that RVO may emerge in younger individuals.

The mean SAF value in diabetic patients was higher in the CRVO group compared to the BRVO group (2.98 a.u. and 2.6 a.u., respectively). Unfortunately, the CRVO group with DM was too small ($n = 4$) to assess statistical significance. Therefore, the observed tendency should be verified in a larger group of patients. It should also be noted that in the CRVO group, the standard deviation in the SAF values was relatively high compared to the BRVO and control groups (Table 2). The considerable dispersion of these results may indicate that not every patient with CRVO is at the same risk level of DM development. From a clinical point of view, it seems justified to assess the SAF in patients with CRVO in order to identify those at risk of DM.

Apart from the fact that the incidence of DM in our study was higher than in other studies, we found a significant correlation between RVO and DM in the SAF measurement, which confirms the findings from other recent studies.

We are aware of several concerning limitations of our study. First, our sample size was relatively small in comparison to other publications. Second, the follow-up of RVO was diverse. In addition, apart from laboratory tests, we did not perform a detailed assessment of the cardiovascular condition of the studied group. However, our main goal was to indicate a different way to find an association between DM and RVO. More well-devised studies on the relationship between RVO and DM with a larger number of responders should be undertaken in the future.

In conclusion, measuring the advanced glycation end products (AGE) in the skin autofluorescence (SAF) is useful method of evaluating the risk of DM development in patients with RVO. We confirmed the correlation between retinal vein occlusion and diabetes mellitus, which was significantly pronounced in the CRVO form.

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