学 位 論 文 の 要 旨

氏名　高柳 佑士

学 位 論 文 名　Evaluation of Relevance Between Advanced Glycation End Products and Diabetic Retinopathy Stages Using Skin Autofluorescence

発 表 雑 誌 名　Antioxidants
（巻，初頁～終頁，年）　(**9**, 1100, 2020)

著 者 名　Yuji Takayanagi, Mikihiro Yamanaka, Jo Fujihara, Yotaro Matsuoka, Yuko Gohto, Akira Obana, Masaki Tanito

INTRODUCTION

Diabetic retinopathy (DR) has been recognized as a major vascular complication of diabetes mellitus (DM), and disease progression often results in devastating visual loss. Although several previous studies have shown that hyperglycemia, smoking, hypertension, and other factors are related closely to diabetic angiopathy, these traditional factors do not fully explain the development of the vascular complications of DM and the involvement of other factors likely to promote this process.

The accumulation of advanced glycation end products (AGEs) in tissues increases in DM. AGEs can modify the functional properties of intracellular proteins such as antioxidant enzymes, induce crosslinking of collagen leading to increased stiffness of the blood vessels, and interact with a receptor for AGEs (RAGE), which activates proinflammatory signaling pathways. Therefore, AGEs are thought to play an important role in the pathogenesis of diabetic microangiopathy, particularly in the progression of DR. However, the relevance between AGEs levels and DR stages and the clinical utility of skin autofluorescence (sAF) measurements as a disease marker for DR are largely unknown.

In this article, we investigated the potential roles of AGEs during progression of DR. Since non-invasively measured sAF can easily estimate the levels of AGEs accumulation, we examined the demographic differences and AGE scores measured by sAF in each DR stages and evaluated the clinical relevance of AGEs in patients with DM, especially those with proliferative diabetic retinopathy (PDR). We provided a novel approach to identify patients at risk for DR progression.

MATERIALS AND METHODS

2.1. Subjects

The current study adhered to the tenets of the Declaration of Helsinki, and was approved by the institutional review boards of Matsue Red Cross Hospital and the Seirei Hamamatsu General Hospital. This study was conducted at the Matsue Red Cross Hospital (No. 303, issued

on 21 September 2016), Iinan Hospital (No. 309, issued on 18 November 2016), and Seirei Hamamatsu General Hospital (No. 2198, issued on 20 July 2016). All participants gave written informed consent for inclusion in the study.

We included 394 eyes of 394 Japanese subjects (172 men, 222 women; mean age ± standard deviation (SD), 68.4 ± 13.7 years), i.e., subjects with DM (n = 229) and non-diabetic controls (n = 165). In the patients with DM, if both eyes were eligible for inclusion in the study, the eye with the worse DR stage was included; if both eyes had the same stage, the eye with the worse best-corrected visual acuity (BCVA) was included; if both eyes had the same BCVA, the right eye was included. The control subjects were 20 years and older, had no history and clinical signs of DM, did not use glaucoma medications, and the previous highest IOP obtained by any type of tonometer was 20 mmHg or lower; the eyes with better BCVA were included in the analysis. Eyes with retinal diseases other than DR and those with glaucoma other than NVG were excluded from this study.

The subjects were interviewed about a history of DM, systemic hypertension, insulin use, and current smoking habits. In patients with DM, the most recent blood hemoglobin A1c (HbA1c) levels were collected during the chart reviews. The participants underwent examinations including measurement of the best-corrected visual acuity (BCVA), intraocular pressure (IOP), slit-lamp microscopy, gonioscopy, fundoscopy, and fundus photograph images. The lens status (phakia/pseudophakia) observed during slit-lamp examination and the previous highest IOPs were obtained from the medical records.

The patients with DM were divided into groups based on those with no DR (NDR, n = 101) and DR (n = 128). DR included simple DR (SDR, n = 36), pre-proliferative DR (PPDR, n = 25), and PDR (n = 67). PDR was further divided into those with (n = 22) and without (n = 45) neovascular glaucoma (NVG). The DR stages were diagnosed based on bilateral funduscopic findings using the Davis classification of DR, specifically, the presence of retinal dot hemorrhages and hard exudates are signs of SDR; that of soft exudates, venous beading, and intraretinal microvascular abnormalities are signs of PPDR; and that of retinal neovascularization and vitreous or preretinal hemorrhages are signs of PDR [14]. NVG was diagnosed based on elevated IOP above 21 mmHg measured by Goldmann applanation tonometry and neovascularization of the iris and of the angle detected by slit lamp and gonioscopic examinations.

2.2. Measurement of AGEs in the Fingertip Skin

To estimate the AGEs, the participants underwent measurements of the sAF levels using the AGEs Sensor (Air Water Biodesign Inc., Kobe, Japan). The sAF levels were measured using the middle finger of the non-dominant hand in which the least skin melanin is present. The sAF values were obtained with the excitation and emission wavelengths of 365 nm and 440 nm,

respectively, which were correlated positively with the level of the hyperglycemia-associated AGEs, Nδ-(5-hydro-5-methyl-4-imidazolone-2-yl)-ornithine. The measurements of sAF were carried out consequently three times in each patient at the first visit, and the mean values were applied to the analyses.

RESULTS AND DISSCUSSION

Compared to controls (0.52 ± 0.12 a.u.), the AGEs scores were significantly higher in patients with DM (0.59 ± 0.17 a.u., $p < 0.0001$), NDR (0.58 ± 0.16 a.u., $p = 0.0012$), and DR (0.60 ± 0.18 a.u., $p < 0.0001$). The proportion of patients with PDR was significantly higher in the highest quartile of AGEs scores than the other quartiles ($p < 0.0001$). Compared to those without PDR (SDR and PPDR), those with PDR were younger ($p = 0.0006$), more were pseudophakic ($p < 0.0001$), had worse BCVA ($p < 0.0001$), had higher IOP ($p < 0.0001$), and had higher AGEs scores ($p = 0.0016$). Multivariate models also suggested that younger age, male gender, pseudophakia, worse BCVA, higher IOP, and higher AGEs scores were risk factors for PDR. The results suggested that AGEs scores were higher in patients with DM and were independently associated with progression of DR. In addition, more PDR was seen in the highest quartile of AGEs scores.

The current results showed that AGEs were an independent factor for development of DR. In fact, the AGEs scores were significantly higher in patients with DR and those with DM compared to the control group, and the higher AGEs score was correlated with the progression of DR. In addition, AGEs were an independent risk factor for PDR; multivariate analyses were performed to adjust for background characteristics. Previous reports have shown that sAF was well correlated with tissue accumulation of AGEs, past glycemic control in patients with DM [13], and the severity of DR [19]. The results of our study were consistent with those of previous studies, and the evidence we report suggested that AGEs would be a key exacerbating factor for the progression of DR.

The second clinical suggestion we provide here is that the highest quartile of the AGEs scores was associated with a higher proportion of DR, especially PDR. Previous reports have shown a clear correlation between the prevalence of DR and serum hydroimidazolone levels, which is one of the most abundant AGEs in vivo, and the highest levels of hydroimidazolone in patients with PDR [26]. This evidence is particularly consistent with the current results. It is important that only the highest quartile group had a significantly higher proportion of PDR. This observation suggested that the level of AGEs might not be correlated linearly with the severity of the DR stages; AGEs might have been physiologically significant in the development of PDR when the levels of AGEs become markedly elevated.

CONCLUSION

The current results suggested that AGEs scores estimated by finger-tip sAF were higher in patients with DM and independently associated with the progression of DR. In addition, the highest quartile of AGE scores had a higher proportion of patients with DR, in particular PDR.