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〔Original Article〕

Skin autofluorescence, a measure of advanced glycation end products is associated with chronic low back pain

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Abstract

Low back pain is a globally debilitating health condition, but the mechanism of pain induction and perception in the context of low back pain remains unclear. Advanced glycation end-products（AGEs）have been reported as a possible biomarker of aging and we hypothesized that AGE accumulation is associated with chronic low back pain. The aim of this study was to determine whether AGEs measured by skin autofluorescence（SAF）can serve as a biomarker for chronic low back pain. 111 patients who visited the outpatient clinic were included in this prospective cohort study. They were divided into a chronic low back pain group（C group: 48 patients, mean age 52.2）and a group without low back pain（N group: 63 healthy volunteers, mean age 40.8）. SAF was measured as a parameter of AGEs using an autofluorescence reader. Measurements of low back pain visual analog scale（VAS）, presence of diabetes mellitus, and SAF were recorded, and correlations between VAS or diabetes mellitus and SAF were investigated. The C group had significantly higher SAF（2.20 vs 1.97, $P=0.033$）than the N group, whereas the SAF for diabetes mellitus patients was significantly higher（2.42 vs 1.97, $P=0.003$）than subjects without diabetes mellitus. SAF had no correlation with VAS（$P=0.190$）. From multiple logistic regression analysis, SAF（OR＝1.177, $P=0.04$）was identified as risk

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factors for chronic low back pain. SAF may serve as a biomarker for chronic low back pain.

***Key words*: skin autofluorescence, advanced glycation end-products, biomarkers, low back pain, diabetes mellitus**

Ⅰ. Introduction

Low back pain is a globally debilitating health condition, and chronic low back pain is a common health issue that has substantial financial and social costs. However, the mechanism of pain induction and perception in the context of low back pain remains unclear.

Low back pain is considered to involve multiple systems including muscles, nerves, the spine and intervertebral discs. Recently, several reports revealed that chemical stimuli and inflammatory mediators such as cytokines produce extracellular molecules such as high mobility group box 1 (HMGB1), which is activated by two receptor types: receptor of advanced glycation end-products (RAGE), and Toll-like receptor. Meanwhile, advanced glycation end-products (AGEs) such as pentosidine are modifications of proteins or lipids that become nonenzymatically glycated and oxidized[1]. AGEs accumulate in the elderly as well as in patients with diabetes mellitus (DM) or renal failure [2-5]. As such, AGEs can act as an aging marker.

RAGE belongs to the immunoglobulin superfamily of receptors[6], and localizes in tissues involved in ascending sensory pathways (e.g., skin, peripheral nerve, dorsal root ganglion, and spinal cord) as well as in endothelial cells, smooth muscle cells, monocytes, and macrophages[1].

RAGE plays an important pathological role in neuropathic pain and is expressed in response to injury, inflammation and diseases that affect sensory nerves[7].

Some reports show that AGE crosslinking can degrade the mechanical and biological functions of bone [8,9]. Recent studies suggest that AGEs accumulation is independently related to risk of bone fractures, diminished ability to walk and perform activities of daily living (ADL), declines in muscle properties and increased physical frailty. AGEs can thus be a potential risk factor and biomarker for decreased motor function [10-12], and represent a potential risk factor for frailty in the elderly[13]. AGEs may also promote bone degeneration or fracture, muscle stiffness, reduced muscle function, and neuropathy that are all associated with orthopedic pain including low back pain. Indeed, high serum levels of AGEs are reported to be associated with degenerative lumbar scoliosis[14].

The AGE reader has been developed to quantify skin autofluorescence (SAF), and has been proposed as a simple alternative to invasive measurement of AGE accumulation[15]. Several reports showed that the amounts of serum pentosidine and SAF are significantly increased in patients undergoing hemodialysis[15]and those with type 2 diabetes (T2DM)[16].

A study involving patients with DM showed that knee extension strength was negatively correlated with SAF, but not with skeletal muscle mass index[16]. Meanwhile, SAF was reported to be associated with low skeletal muscle mass index among middle-aged Japanese[17]. Together these findings suggest that the behavior of SAF in orthopedic patients could have diagnostic value.

We hypothesized that AGE accumulation is associated with chronic low back pain. The aim of the present study was to determine whether AGE levels revealed by SAF can serve as a biomarker for chronic low back pain.

Ⅱ. Methods

This is a prospective cohort study. The consecutive subjects all visited the outpatient clinic in our hospital.

The study was approved by the Research Ethics Committee in our facility, and was conducted in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (IRB approval code: 2428). Written informed consent was

obtained from all individual patients.

In total, 111 orthopedic outpatients (54 males, 57 females, mean age 45.7 ± 18.9 years old) were enrolled and divided into two groups: Group C, which included 48 patients with chronic low back pain (20 males, 28 females, mean age 52.2 ± 19.4 years old) and Group N, which included 63 healthy volunteers without low back pain (34 males, 29 females, mean age 40.8 ± 17.0 years old). Patients having pain lasting more than 3 months were classified as chronic. Subjects younger than 20 years old were excluded.

SAF was measured as a parameter of AGEs using an AGE Reader (DiagnOptics BV, Groningen, Netherlands), which is a non-invasive tool that uses the fact that several AGEs exhibit autofluorescence to estimate the skin AGE accumulation[15]. The technical details have been described in detail in previous reports. SAF is expressed in arbitrary units (AU). SAF measurements were performed at room temperature on the ventral side of the forearm while the subject was seated.

Clinical symptoms, presence of DM as a lifestyle disease, and SAF were measured. History of disorders or diseases was self-reported. Clinical symptoms were evaluated using the visual analog scale (VAS) score for low back pain from 10 (extreme amount of pain) to 0 (no pain).

Study items were SAF of each group, with or without DM, and correlations between SAF with VAS. Measurements were analyzed using a Wilcoxon/Kruskal-Wallis test to assess differences between groups. We calculated Pearson correlation coefficients to assess the correlation between VAS score for low back pain and SAF as a biochemical marker. All data are expressed as the mean standard ± deviation (SD). $P<0.05$ was considered significant.

To determine SAF and DM as an independent variable in predicting occurrence of chronic low back pain, we performed multiple logistic regression, using chronic low back pain as a dependent variable with SAF and DM.

Ⅲ. Results

SAF readings were significantly higher for the C group than the N group ($P=0.033$; Fig. 1). However, we detected no correlation between AGEs with VAS for low back pain (Fig. 2). SAF and VAS also showed no correlation in C group.

The total number of subjects with DM was 24. Of these, C group had 14 and N group had 10 patients. Diabetic patients in both the C and N groups had significantly higher SAF readings compared to patients in the same group who did not have DM (all: $P=0.003$, C group: $P=0.047$, N group: $P=0.034$; Fig. 3).

Based on multiple logistic regression analysis, SAF (OR = 1.177, $P=0.04$) was identified as risk factors for chronic low back pain independent of DM (Table 1).

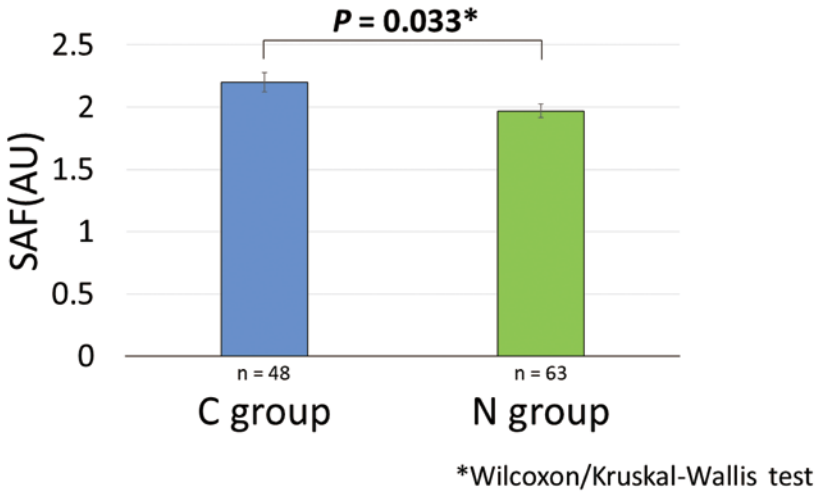

Fig. 1 Skin autofluorescence (SAF) in the study groups. The SAF for the C group was significantly higher than the N group (2.20 ± 0.075 and 1.97 ± 0.054, respectively; $P<0.05$).

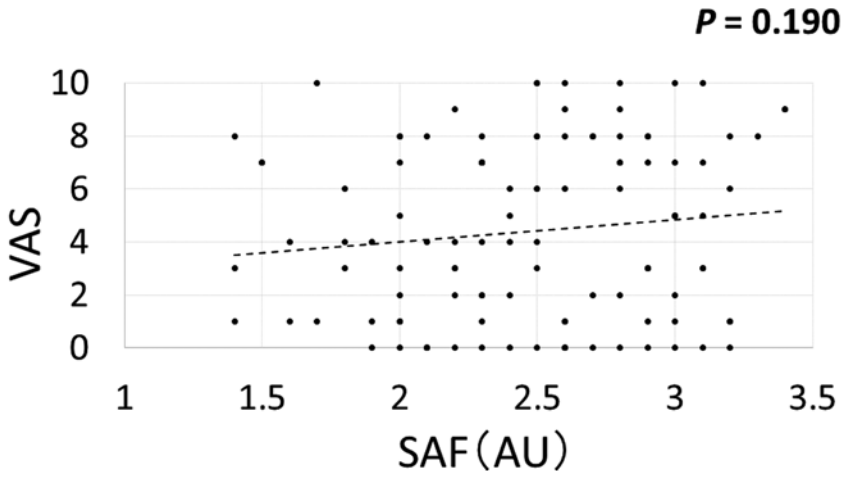

Fig. 2 Correlation of skin autofluorescence（SAF）with visual analog scale（VAS）for low back pain. No significant correlation was noted between SAF and low back pain VAS（$P = 0.190$）.

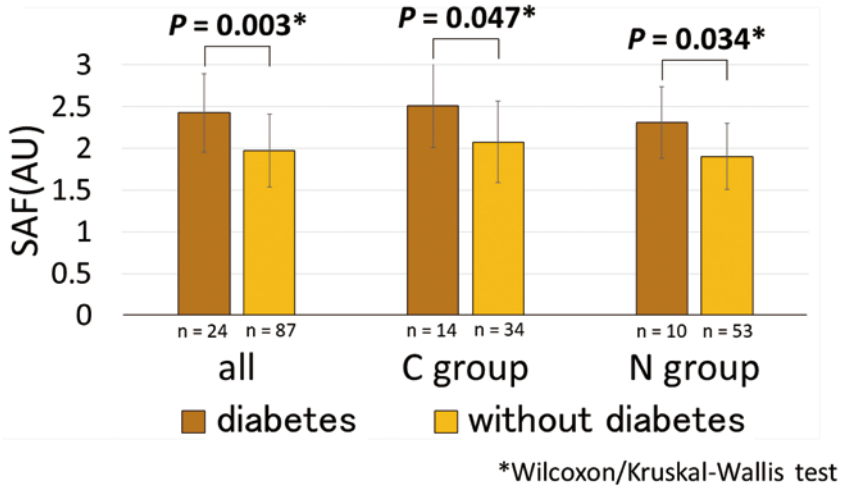

Fig. 3 Skin autofluorescence（SAF）in subjects with and without diabetes mellitus. SAF values were significantly higher in subjects with diabetes mellitus than those without diabetes mellitus in both the C and N groups（all subjects: $P = 0.003$, C group: $P = 0.047$, N group: $P = 0.047$）.

Table 1 Multiple logistic regression analysis: factors associated with chronic low back pain

Variables	OR	(95%CI)	*P* value
SAF	1.177	(0.010 - 0.422)	0.04
DM	1.091	(-0.147 - 0.336)	0.439

OR; odds ratio, CI; confidence interval

Ⅳ. Discussion

AGEs are now frequently used as an aging marker. AGE formation is associated with the rate of protein turnover via glycoxidation, the degree of hyperglycemia, and the extent of oxidant stress in the environment[2-5]. Early glycation and oxidation processes result in the formation of Schiff bases and Amadori products. Further glycation of proteins and lipids causes molecular rearrangements that lead to the generation of AGEs [2]. Diabetic patients often have high concentrations of AGEs, but AGEs also form during the aging process. Once formed, AGEs are considered to be irreversible [4]. As such, the accumulation of AGEs can reflect a history of diseases related to lifestyle such as DM wherein chronic high blood sugar produces glycation and oxidative stress.

The formation and accumulation of AGEs on long-lived proteins affects their structure and function by promoting binding to specific receptors that can enhance cytokine production and activate transcription factors[18].

There are several possible mechanisms through which AGEs could contribute to chronic low back pain. Accumulation of AGEs in bone collagen matrix has been associated with brittleness of collagen fibers and impaired mechanical functions of cortical and trabecular bone[19,20]. AGEs are now thought to be a risk factor of bone fracture in osteoporosis and DM. High levels of AGEs are also associated with diminished muscle function[10,11]and loss of muscle mass[12]. AGEs may also play a role in sarcopenia that is mediated by RAGEs that upregulate inflammation and endothelial dysfunction in the microcirculation of skeletal muscle [21].

Meanwhile, Eguchi et al. reported that high serum levels of pentosidine are associated with severity of degenerative lumbar scoliosis in older women and suggested that AGEs can be a potential biomarker for lumbar scoliosis and kyphotic deformity[14].

Some reports described a relationship between AGEs and neurodegeneration or inhibition of neuronal regeneration[22]. Emerging data from expression and localization studies indicate a role for RAGE in states of sensory nerve hyper-excitability that are associated with peripheral inflammation or direct nerve damage. RAGEs localize on peripheral nerves and show increased expression following trauma or disease[23,24]. RAGEs are also expressed in non-neuronal cell types that interact with sensory neurons including Schwann cells, endothelial cells, smooth muscle cells, monocytes and macrophages[25].

AGEs that play a role in bone degeneration or fracture, decreased muscle functions, spinal malalignment, degenerative lumbar scoliosis and neurodegeneration may contribute to low back pain.

Here we describe a new simple method to measure AGEs using SAF. Although various instruments to measure SAF have been developed, the use of SAF to assess AGE levels is controversial. Some reports found no correlation between SAF and serum AGEs[26-28]. On the other hand, several reports showed that both serum pentosidine and SAF were significantly increased in patients with T2DM[16]and those undergoing hemodialysis[15]. Thus, the dynamics of SAF and serum AGEs remain unclear.

We have reported that women with osteoporotic vertebral compression fractures had increased SAF compared to women without fractures[29], and suggested that SAF may be a biomarker for the reduction of physical function and bone fracture associated with aging.

In this study, we showed that SAF as a measure of AGE accumulation correlated with chronic low back pain and DM, SAF was identified as risk factors for chronic low back pain independent of DM from multiple logistic regression analysis, indicating that SAF could serve as a biomarker for chronic low back pain. However, we found no correlation between SAF and low back pain VAS, suggesting that the dynamics of SAF and AGEs may be unrelated to the severity of low back pain. As part of treatment strategies for chronic back pain, patients having both higher AGE levels and DM may have greater benefits if exercise and lifestyle guidance interventions are begun at an early stage.

This study does have some limitations. Our study group included only 48 patients with low back pain and thus our findings require confirmation in a larger population. Selection of our study subjects was based only on data related to a self-reported disorder and a differential diagnosis of low back pain was not considered. Moreover, only the presence of DM was noted and disease severity and duration were not considered. History of DM was self-reported, with no evidence of described hemoglobin A1C levels. Those data are not available because the subjects were low back pain outpatients in our clinic and blood tests were not performed in all cases. We made comparisons only among patients who had visited an orthopedic outpatient clinic and did not examine a healthy population. The age difference between C group and N group was 11.4 and this may be a confounding factor. Last, this was a cross-sectional rather than longitudinal study.

Age, DM and AGEs accumulation are considered to have positive correlation and to be potentially predominant bias for investigating the relationship between AGEs and pain. To eliminate these biases, it will be necessary to conduct age-adjusted comparative

studies or larger and longer-term surveys; for example, prospective longitudinal cohort study with evidence of measures taken to validate comorbidities.

In conclusion, the levels of AGEs revealed by skin autofluorescence were significantly higher in subjects who had chronic low back pain and DM patients in particular showed significantly higher amounts of AGEs. From multiple logistic regression analysis, SAF was identified as risk factors for chronic low back pain independent of DM, indicating that SAF could serve as a biomarker for chronic low back pain. We saw no correlation between SAF and low back pain VAS, suggesting that there is no association between SAF dynamics and low back pain severity, but if the number of subjects in the study increases, there may be a correlation between these items. Confirmation of these results in a larger patient population is needed.

Contributors

T. U, Y. E, K. I, Y. S, S. M, M. I, M. N, T. S, M. S, M. S, K. E, S. O and S. O made substantial contributions to the conception or design of the work and wrote the manuscript.

H. K, K. K, R. H, F. H and T. F provided discussion and intellectual input into the manuscript.

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Conflict of interest

S.O. is a member of the Editorial Board of the Chiba Medical Journal.

Ethical approval

Not applicable.

Data availability

Not applicable.

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