

Measurement of advanced glycation endproducts in skin of patients with rheumatoid arthritis, osteoarthritis, and dialysis-related spondyloarthropathy using non-invasive methods

Tomoko Matsumoto · Toshiyuki Tsurumoto ·
Hideo Baba · Makoto Osaki · Hiroshi Enomoto ·
Akihiko Yonekura · Hiroyuki Shindo · Toshio Miyata

Received: 28 February 2007 / Accepted: 19 June 2007 / Published online: 25 July 2007
© Springer-Verlag 2007

Abstract Advanced glycation endproducts (AGEs) are the products of non-enzymatic glycation and oxidation of proteins and lipids. Low-turnover tissues such as articular cartilage seem to be susceptible to the accumulation of AGEs, which might lead to cartilage degradation. Recently, a non-invasive method for measuring skin AGE accumulation was developed by using the Autofluorescence Reader (AFR). To examine the usefulness of measuring skin AGE in patients with bone and joint diseases, we examined autofluorescence (AF) levels in skin of patients with osteoarthritis (OA), rheumatoid arthritis (RA), and dialysis-related spondyloarthropathy (DRSA). Ninety-three patients with RA, 24 patients with OA, and 29 patients with DRSA were examined, and 43 healthy volunteers were used as controls. Skin AF was assessed on the lower arm with the AGE-Reader. Mean AF was significantly higher in the patients with RA (median 2.13 and range 1.25–2.94) or with DRSA (median 2.21 and range 1.29–3.88) than in the patients with OA (median 1.63 and range 1.07–2.31) or in the controls (median 1.74 and range 1.10–2.46). There was no significant difference between OA and the controls, or between RA and DRSA. These findings suggest that differences of AGE accumulation in the skin might reflect the different pathologies of these diseases.

Keywords Advanced glycation endproduct · Rheumatoid arthritis · Osteoarthritis · Dialysis-related spondyloarthropathy · Skin autofluorescence

Introduction

Non-enzymatic glycation of proteins results in the formation of advanced glycation endproducts (AGEs), which have been shown to play a role in the pathogenesis of several diseases, such as diabetes mellitus, renal failure, and arteriosclerosis [1]. AGEs accumulated in bone, cartilage, synovia, or ligaments also induce various deleterious effects on musculoskeletal functions [2–4]. Spondyloarthropathy is a serious complication for long-term hemodialysis patients, and deposition of AGE β 2-microglobulin (β 2M) was found in the ligamentum flavum or vertebral bone of such patients [5, 6]. Pentosidine, a sensitive marker for AGEs, is present in serum, synovial fluid, and articular cartilage from patients with osteoarthritis (OA) and rheumatoid arthritis (RA) [7–9]. The accumulation of AGEs in articular cartilage affects chondrocyte metabolism and might lead to cartilage degradation [4, 10]. Clinically, OA and RA as well as destructive spondyloarthropathy (DSA) finally cause bone and joint destruction; however, little is known about how to protect against them.

Recently, a non-invasive method for measuring skin AGE accumulation was developed by using the Autofluorescence Reader (AFR). It has been reported that skin levels of AGEs obtained by biopsy correlated with those measured using the AFR [11], suggesting that autofluorescence (AF) of the skin might reflect the AGE levels in various tissues, including bone and cartilage. To examine the usefulness of measuring skin AGEs in patients with bone and joint diseases, we measured AF levels in the skin of

T. Matsumoto (✉) · T. Tsurumoto · H. Baba · M. Osaki ·
H. Enomoto · A. Yonekura · H. Shindo
Department of Orthopaedic Surgery,
Nagasaki University School of Medicine, 1-7-1,
Sakamoto, Nagasaki city 852-8501, Japan
e-mail: tomoko-m@nagasaki-u.ac.jp

T. Miyata
Institute of Medical Sciences and Department of Medicine,
Tokai University School of Medicine, Kanagawa, Japan

patients with OA, RA, and dialysis-related spondyloarthropathy (DRSA).

Patients and methods

Patients

Ninety-three patients with RA (mean age 61 and range 22–83 years) fulfilling the 1987 ACR criteria [12], 24 patients with OA of 11 hips or of 13 knees (mean age 69 and range 47–87 years), and 29 patients with DRSA (mean age 62 and range 50–82 years) were examined. Forty-three healthy volunteer are used as a control (mean age 40 and range 23–72 years). RA patients were classified into three groups according to the degree of joint destruction: slight: radiologically normal or only a few small joints were damaged. Moderate: one or two major joints were damaged. Severe: more than three major joints were severely damaged. All OA patients included in this study were in the advanced stage of the disease, showing severely damaged joints. The duration of hemodialysis in patients with DRSA was 18.2 ± 9.9 years. Although all 29 patients with DRSA were affected neurologically, only 24 severe cases were treated by surgery. Radiologically, destruction of the spine (DSA) was found in 20 patients, while nine patients were normal (non-DSA).

Skin autofluorescence

Skin AF was assessed on the ventral site of the lower arm with an AGE-Reader (DiagnOptics BV, Groningen, The Netherlands). In brief, the AGE-Reader consists of a tabletop box containing a black light excitation light source (peak wavelength ~ 360 nm). Light emitted from the skin is measured with an integrated spectrometer. AF was calculated by dividing the average light intensity emitted per nm over the 420–600 nm range by the average light intensity emitted per nm over the 300–420 nm range.

Statistical analysis

Non-parametric methods were used to analyze the data. Analysis of statistical correlation was performed using the Spearman test of rank correlation. The Kruskal–Wallis test and the Mann–Whitney *U*-test were used to analyze differences between groups.

Results

Autofluorescence was significantly higher in the patients with RA (median 2.16 and range 1.45–2.94) or with DRSA

(median 2.21 and range 1.29–3.88) than in the patients with OA (median 1.63 and range 1.07–2.31) or in the control group (median 1.74 and range 1.10–2.46). There was no significant difference between patients with OA and the control group. Although some patients with DRSA showed high levels of AF, there was no significant difference between the RA and DRSA groups (Fig. 1) Relationships between age and AF levels were obtained in the OA ($r = 0.54$ and $P < 0.01$) and RA ($r = 0.25$ and $P = 0.015$) groups (Fig. 2), but not in the DRSA or control group (data not shown).

In DRSA, there was no relationship between duration of hemodialysis and AF. However, patients with DSA tended to have higher AF (median 2.418 and range 1.288–3.878) than radiologically normal cases (median 1.903 and range 1.487–3.007), although the difference was not significant (Fig. 3).

In RA, there was no relationship between AF and serum CRP levels or the duration of disease. Patients with severe joint destruction tended to show higher AF (median 2.31 and range 1.45–2.94) compared to those with slight joint destruction (median 2.127 and range 1.55–2.68) or moderate (median 2.120 and range 1.50–2.82), although the difference was not significant (Fig. 4).

Discussion

In this study, we have shown that patients with DRSA or RA had higher AF levels compared to those with OA or healthy controls. These findings suggest that long-term hemodialysis as well as RA increase the systemic accumu-

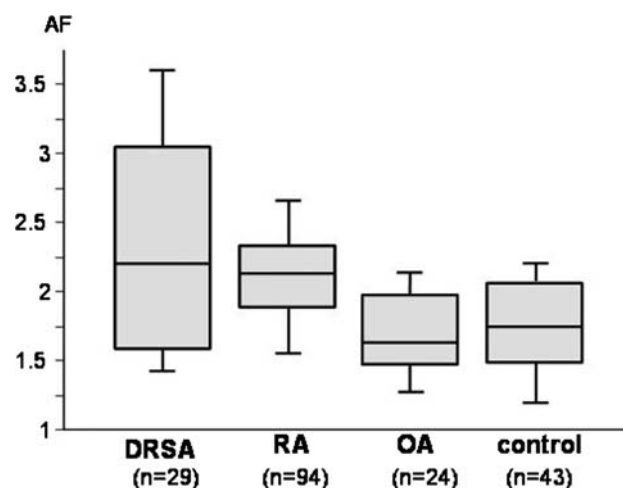


Fig. 1 Autofluorescence (AF) values of patients with dialysis-related spondyloarthropathy (DRSA), rheumatoid arthritis (RA) or osteoarthritis (OA), and of healthy controls. Box plots show the 25th and 75th percentiles. Horizontal lines within the boxes indicate the median. Vertical bars indicate the 5th and 95th percentiles

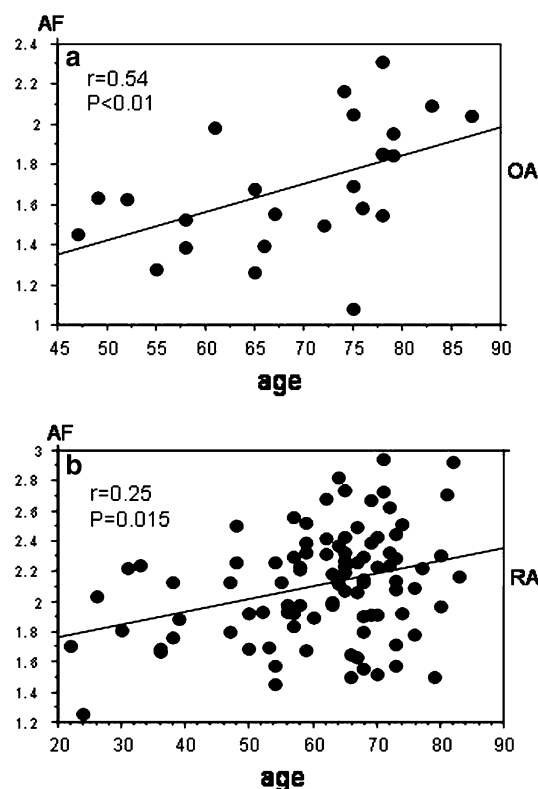


Fig. 2 Association between autofluorescence (AF) and age of patients with osteoarthritis (a) or with rheumatoid arthritis (b). The Spearman rank correlation coefficient (r) and P -value are given

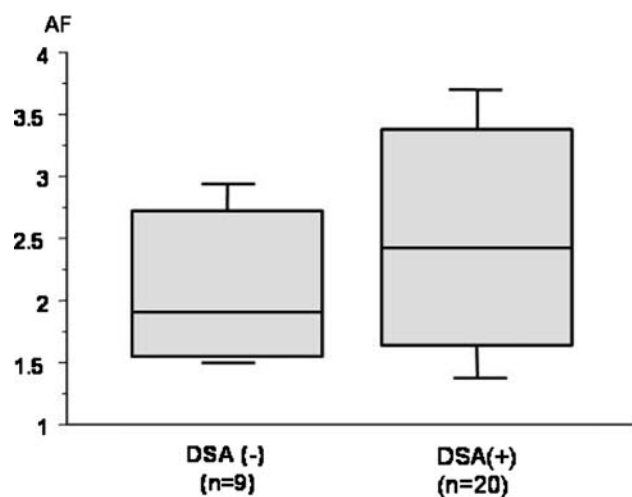


Fig. 3 Comparison of autofluorescence (AF) values of patients with dialysis related spondyloarthropathy with and without destructive spondyloarthropathy (DSA). Box plots show the 25th and 75th percentiles. Horizontal lines within the boxes indicate the median. Vertical bars indicate the 5th and 95th percentiles

lation of AGE, while deposition of AGEs in OA might be restricted to the affected joint. The finding that AF levels in OA were the same as those in the controls despite the fact that the mean age of patients in the OA group was much

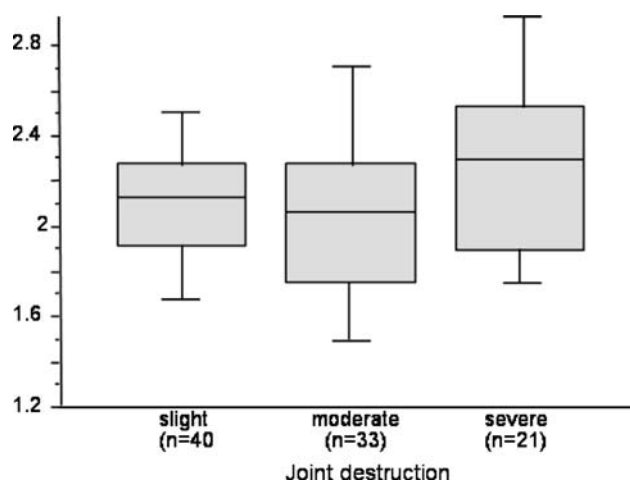


Fig. 4 Comparison of autofluorescence (AF) values of patients with rheumatoid arthritis with various degree of joint destruction: slight, moderate, and severe. Box plots show the 25th and 75th percentiles. Horizontal lines within the boxes indicate median. Vertical bars indicate the 5th and 95th percentiles

higher than that of the controls was unexpected. In DRSA, some patients showed high AF, but some showed low AF, resulting in a wide range of AF values and consequently in the lack of a significant difference between RA and DRSA. It is possible that the dark skin color of patients with long hemodialysis might have affected the AF of the skin and contributed to the variability of AF.

It was reported that AGE modification of $\beta 2M$ occurred during long-term hemodialysis [13], and that AGEs that accumulated in the tissue around bones induced inflammation and damaged the bone [14, 15]. Consistent with these reports, we showed here that the DSA group tended to have higher AF levels comparing to those in the non-DSA group. In a previous study, we demonstrated immunohistologically that the deposition of AGE $\beta 2M$ in the ligamentum flavum was higher in the DSA group [5]. These findings suggest that AF also reflects the accumulation of AGEs in the spinal tissue.

Rheumatoid arthritis is a state of oxidative stress associated with chronic inflammation. Although pentosidine levels in plasma were reported to correlate with CRP levels [7, 8, 16], there was no relationship between AF and serum CRP levels in this study. However, RA patients with severe destruction of joints tended to show higher levels of AF compared to those in patients in the groups with slight or moderate destruction, suggesting that AGEs might be related to the joint damage.

Receptor for AGE (RAGE) is present in articular cartilage and is increased with aging and in OA [10, 17], and it has been reported that AGE stimulates RAGE on chondrocytes and synoviocytes, inducing catabolic activity and cartilage degradation [4, 10]. These findings suggest that AGE accumulation in the joints also plays a role in the develop-

ment of OA, although the AF in the OA group was the lowest in this study. There might be some differences in the local or systemic pattern of the distribution of AGE, according to the type of disease.

This is the first report to examine the AF of Japanese patients with DRSA, RA, and OA using the AGE-Reader. AGE accumulation in the skin in part reflects the different pathologies of these diseases. However, there are some limitations to this study, including the following. First, the skin color of patients might affect the AF, because the AGE-Reader is not reliable for dark brown skin. Second, the AF value showed such high variation in each disease that a larger number of patients should be included in a larger study to enable better statistical analysis.

This simple, rapid, and non-invasive method for measurement of skin AF could be one of the useful methods for assessing bone and joint diseases.

References

1. Ramasamy R, Vannucci SJ, Yan SS, Herold K, Yan SF, Schmidt AM (2005) Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. *Glycobiology* 15:16R–28R
2. Drinda S, Franke S, Ruster M, Petrow P, Pullig O, Stein G, Hein G (2005) Identification of the receptor for advanced glycation end products in synovial tissue of patients with rheumatoid arthritis. *Rheumatol Int* 25:411–413
3. Saudek DM, Kay J (2003) Advanced glycation endproducts and osteoarthritis. *Curr Rheumatol Rep* 5:33–40
4. Steenvoorden MM, Huizinga TW, Verzijl N, Bank RA, Ronda HK, Luning HA, Lafeber FP, Toes RE, DeGroot J (2006) Activation of receptor for advanced glycation end products in osteoarthritis leads to increased stimulation of chondrocytes and synoviocytes. *Arthritis Rheum* 54:253–263
5. Inatomi K, Matsumoto T, Tomonaga T, Eto M, Shindo H, Hayashi T, Konishi H (2004) Histological analysis of the ligamentum flavum of patients with dialysis-related spondyloarthropathy. *J Orthop Sci* 9:285–290
6. Nokura K, Koga H, Yamamoto H, Kimura A, Tamai H, Yazaki S, Suzuki N, Miyazaki S, Niwa T (2000) Dialysis-related spinal canal stenosis: a clinicopathological study on amyloid deposition and its AGE modification. *J Neurol Sci* 178:114–123
7. Chen JR, Takahashi M, Suzuki M, Kushida K, Miyamoto S, Inoue T (1999) Comparison of the concentrations of pentosidine in the synovial fluid, serum and urine of patients with rheumatoid arthritis and osteoarthritis. *Rheumatology (Oxford)* 38:1275–1278
8. Miyata T, Ishiguro N, Yasuda Y, Ito T, Nangaku M, Iwata H, Kurokawa K (1998) Increased pentosidine, an advanced glycation end product, in plasma and synovial fluid from patients with rheumatoid arthritis and its relation with inflammatory markers. *Biochem Biophys Res Commun* 244:45–49
9. Senolt L, Braun M, Olejarova M, Forejtova S, Gatterova J, Pavelka K (2005) Increased pentosidine, an advanced glycation end product, in serum and synovial fluid from patients with knee osteoarthritis and its relation with cartilage oligomeric matrix protein. *Ann Rheum Dis* 64:886–890
10. Loeser RF, Yammani RR, Carlson CS, Chen H, Cole A, Im HJ, Bursch LS, Yan SD (2005) Articular chondrocytes express the receptor for advanced glycation end products: potential role in osteoarthritis. *Arthritis Rheum* 52:2376–2385
11. Meerwaldt R, Graaff R, Oomen PHN, Links TP, Jager JJ, Alderson NL, Thorpe SR, Baynes JW, Gans ROB, Smit AJ (2004) Simple non-invasive assessment of advanced glycation endproduct accumulation. *Diabetologia* 47:1324–1330
12. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 31:315–324
13. Niwa T (2001) Dialysis-related amyloidosis: pathogenesis focusing on AGE modification. *Semin Dial* 14:123–126
14. Hou FF, Miyata T, Boyce J, Yuan Q, Chertow GM, Kay J, Schmidt AM, Owen WF (2001) beta(2)-Microglobulin modified with advanced glycation end products delays monocyte apoptosis. *Kidney Int* 59:990–1002
15. Miyata T, Maeda K (1995) Pathogenesis of dialysis-related amyloidosis. *Curr Opin Nephrol Hypertens* 4:493–497
16. Hein GE, Kohler M, Oelzner P, Stein G, Franke S (2005) The advanced glycation end product pentosidine correlates to IL-6 and other relevant inflammatory markers in rheumatoid arthritis. *Rheumatol Int* 26:137–141
17. Verzijl N, DeGroot J, Bank RA, Bayliss MT, Bijlsma J, Floris PJG, Lafeber FPJG, Maroudas M, TeKoppele JN, TeKoppele JM (2001) Age-related accumulation of the advanced glycation end-product pentosidine in human articular cartilage aggrecan: the use of pentosidine levels as a quantitative measure of protein turnover. *Matrix Biol* 20:409–417