

Fluorescent Advanced Glycation End Products in the Detection of Factual Stages of Cartilage Degeneration

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Received

Short title

Glycation detection in cartilage degeneration

Summary

Patients treated for knee disorders were included in this study. They were examined clinically (Lequesne and Tegner scores) as well as by standard X-ray. Patients underwent a surgical procedure, either arthroscopy or a knee replacement. At the initial phase of the surgery, a sample of cartilage was taken for laboratory examination. Progression of the disorder and the clinical examination was correlated with the factual condition of the cartilage using a novel fluorescence approach. The native fluorescence of cartilages was shown as a suitable and sensitive method for detection of the actual condition of cartilages. The correlation with X-ray examination and clinical status was found. Native fluorescence properties of cartilages from patients with chondropathy and osteoarthritis were described and found to be age-dependent. We also observed a higher concentration of advanced glycation end products due to inflammatory and/or degenerative processes in cartilage. In addition, acute pathological changes due to diseases such as meniscal lesions or anterior cruciate ligament rupture caused a significant increase of formation of advanced glycation end products even in the group of young patients. In fact, such an observation could be crucial and important for the detection of knee suspected for early meniscal and/or ACL lesion especially among young patients.

Key words

Nonenzymic glycation • Fluorescence detection • Cartilage • Knee joint

Abbreviations

OA, degenerative osteoarthritis; ME, meniscal lesions; ACL, anterior cruciate ligament; CH, chondropathy; S, synovitis; AGEs, advanced glycation end products; CML, carboxymethyllysine.

Introduction

Knee disorders and osteoarthritis of joints belong to most frequent diseases. Cartilage breakdown due to these diseases results in severe pain and disability. In addition to chondrocytes, cartilage comprises a highly hydrated proteoglycan matrix with a collagen network. Cartilage collagen fibers exhibit a very high tensile strength that increases with age (Parry *et al.* 1978, Jones 1991). Biomechanical properties of cartilage are closely related to collagen fibers distribution and orientation (Wu and Herzog 2002). The age-dependent changes of mechanical properties involve two distinct mechanisms of cross-linking: the enzymic cross-linking and a nonenzymic glycation. Enzymic cross-linking is controlled by lysyl oxidase, a copper-dependent enzyme. On the other hand, nonenzymic cross-linking, referred to also as glycation, results in the appearance of advanced glycation end products (AGEs). It also increases collagen stiffness and insolubility. Production of AGEs involves the reaction of lysine with glucose and then either oxidation of the complex that leads to formation of intermolecular cross-links, such as pentosidine, or conversion of a Schiff base (Amadori product) to carboxymethyllysine. Glyoxal and glucosone, products of oxidative degradation, can react with lysine and form AGEs (Dyer *et al.* 1991, Grandhee and Monnier 1991, Wells-Knecht *et al.* 1995, Bailey *et al.* 1998).

It has been reported that the production of AGEs can be modified in patients with chronic diseases, such as diabetes (Dyer *et al.* 1993, Schleicher *et al.* 1997, Santana *et al.* 2003), atherosclerosis (Hoshino *et al.* 1995), rheumatoid arthritis (Takahashi *et al.* 1997, Rodriguez-Garcia *et al.* 1998) and osteoarthritis (Chen *et al.* 1999). Age-dependent concentration of pentosidine, one of AGEs, has been reported also in cartilages (Sell and Monnier 1989). Immunocytochemical staining for carboxymethyllysine shows increased staining with age in cartilage (Schleicher *et al.* 1997). Verzijl *et al.* (2001) reported that the

pentosidine level increased in cartilage (aggrecan) but the rate of pentosidine accumulation is three-fold lower than in collagen (5-fold and 15-fold increases, respectively). The higher accumulation of pentosidine in collagen may result from higher turnover of aggrecan compared with collagen.

The aim of our study is to understand better the relationship between diseases and fluorescence properties of native fluorophores from cartilages of human patients. Both acute diseases, such as anterior cruciate ligament rupture, chondropathy or meniscectomy, and chronic processes, such as synovitis and osteoarthritis were investigated. The clinical evaluation scores were compared and correlated.

Methods

Selected diagnosis, surgery, sample collection

Patients in the study were administered according to the principles of the study design of the IGA grant No. 8122. They underwent a surgical procedure either arthroscopically or a total knee replacement. The diagnoses for the surgery were osteoarthritis, chondropathy, chronic synovitis with joint effusion and injuries (cruciate ligaments, meniscal injury, chronic instability after knee sprain). The samples for further experimental investigation were prepared from cartilage remnants after the surgical treatment designated to trash.

Clinical examination

All patients underwent clinical examination when Lequesne and Tegner scores were evaluated (Tegner and Lysholm 1985, Lequesne *et al.* 1987). The Outerbridge scale was used during the surgical procedure for detecting the severity of chondropathy (Outerbridge 1961).

X-ray examination

Routine radiographic examination (standard and lateral projections in the supine position) was done in all cases before the surgery, X-ray evaluation was performed according to the Jäger and Wirth score (Jäger and Wirth 1986) using a 4-grade scale (I – initial stage, prolonged and sharpened emminencia intercondylica, II – mild stage, decrease of joint space, subchondral sclerosis, light deformity of femoral condyles, III – expressed stage, severe osteophytes, sclero-cystic degeneration of subchondral bone, malalignment of joint axis, narrowing of joint space, IV – severe stage, disappearing joint space, severe osteophytes, subluxation of femur against tibia, general osteosclerotic damage of joint).

Surgical procedures and sample collection

Both surgical procedures (arthroscopy or total knee replacement) were conducted under general or spinal anesthesia in a regular operating room. After the cartilage evaluation during the initial phase of the surgery samples of cartilage were harvested from the non-weight-bearing zone of trochlea femoris. The sharp surgical double-spoon (conchotom) was used, so that the full thickness cartilage to the subchondral lamina was obtained in rice seed size ($2 \times 2 \times 2$ mm, about 100-200 mg). The procedure was performed under sterile conditions and the harvested sample from the joint was put into a syringe filled with physiological solution and transported to the laboratory in 10 ml of physiological solution, stored at room temperature for a maximum of 12 hours. Consequently the complete treatment procedure followed. In the post-operation period the wound healing was without any complication in all cases.

Cartilage cleavage and sample preparation

The cartilage delivered from operator was incubated in 1 % (w/w) acetic acid

overnight and lyophilized. Then, 0.5 g of the cartilage was cleaved by bacterial collagenase (collagenase:collagen = 1:100, W/W) in 50 ml of the incubation solution of 160 mM $(\text{NH}_4)_2\text{CO}_3$ and 1.3 mM CaCl_2 (pH 7.8) for 48 h. Afterwards the sample was used for fluorescence experiments. All measurement were recorded at 37 °C in 20 mM Tris-HCl (pH 7.4).

Fluorescence measurement

Steady-state excitation at $\lambda_{\text{ex}} = 335$ nm or $\lambda_{\text{ex}} = 370$ nm and emission at $\lambda_{\text{em}} = 400$ nm or $\lambda_{\text{em}} = 440$ nm spectra were recorded on the Perkin-Elmer LS50B spectrofluorimeter. For determination of the AGEs formation dependence on age, 600 μl of the incubation solution were diluted in 2 ml of 20 mM Tris-HCl (pH 7.4) and the fluorescence intensities of the solution at excitation/emission wavelengths of 335/400 nm and 370/440 nm were recorded at 37 °C. Both excitation and emission bandpasses were set to 5 nm, the signal was integrated for 5 s. The measured fluorescence intensity values were corrected for light scattering and normalized. To normalize native fluorescence intensity of the cartilages, the total protein concentration was determined for each sample and the relative fluorescence intensity to the protein concentration was estimated. All measurements were done and recorded at 37 °C.

Steady-state fluorescence intensity was corrected for protein concentration similarly as described by Amler *et al.* (1996).

Statistical analysis

Nonparametric regression analysis of variance for correlation and nonparametric Spearman coefficient for statistical significance were determined for each diagnosis. Statistical significance of the difference between more than two groups was determined by nonparametric Kruskal–Wallis ANOVA and between two groups by nonparametric Mann-

Whitney U Test. Biased samples were omitted. Values of p less than 0.05 were considered significant. Groups containing 2 patients were evaluated only by nonparametric regression analysis of variance.

Results

Group of patients, diagnosis and sample harvesting

A total of 125 patients (68 men and 57 women) were involved in our approved study. They were treated either for an acute injury of the knee or for chronic chondropathy or osteoarthritis. The age of patients ranged from 15 to 85 years (the mean age was 47.6 years).

Five main diagnoses, such as degenerative osteoarthritis, meniscal lesions, anterior cruciate ligament lesion, chondropathy and synovitis were followed (Table 1). Patients with chondropathy were taken as a control group to patients of similar age treated for other diagnoses (e.g. acute injury or OA).

Two sets of native fluorophores characterized the cartilage samples

The samples were digested and prepared for fluorescence studies as described in the Methods. Steady-state fluorescence spectra were measured. In fact, we detected at least two sets of native fluorophores in each sample, probably as a consequence of nonenzymic glycation products. A strong fluorescence intensity of the sample was observed at the excitation wavelength of $\lambda_{\text{ex}} = 335$ nm and the emission wavelength of $\lambda_{\text{em}} = 400$ nm (referred to as fluorescence 335/400 in this paper). Another fluorescence peak (referred to below as fluorescence 370/440) was observed at excitation wavelength of $\lambda_{\text{ex}} = 370$ nm and emission wavelength of $\lambda_{\text{em}} = 440$ nm. The measured fluorescence intensity values were corrected for light scattering and normalized. To normalize native fluorescence intensity of the cartilages,

the total protein concentration was determined for each sample and the relative fluorescence intensity to the protein concentration was estimated. All measurement were done and recorded at 37 °C.

Age-related increase of native fluorescence intensity from cartilages of chondropathy patients

Native fluorescence intensity of cartilage samples increased with age. The increase of fluorescence intensity with age was observed for both sets of fluorophores (for 335/400 fluorescence as well as for 370/440 fluorescence) and the average value confirmed for each measurement was determined from triplicates.

A significant (eleven-fold) increase of 335/400 fluorescence with age in the group with chondropathy (Fig. 1) was found. In fact, the obtained data could be described as a linear increase of the 335/400 fluorescence intensity with age (with the correlation coefficient $r = 0.943$). Similar behavior was observed for 370/440 fluorescence despite a lower correlation coefficient ($r = 0.86$). However, a different behavior could be observed for patients with meniscal lesions, anterior cruciate ligament lesion and synovitis. Our results show that both fluorescence intensities were significantly correlated with neither the objective findings nor the clinical tests (data not shown).

On the other hand, the 370/440 fluorescence intensity is significantly correlated with both objective findings and clinical tests. The p values showing significance of correlation were < 0.001 for all comparisons (Table 2).

Native fluorescence intensity in the cartilage from patients with osteoarthritis is significantly elevated but still retains the age-related dependence

Both fluorescence intensities (335/400 fluorescence as well as 370/440 fluorescence) were significantly elevated in the group of OA patients compared with the group of CH

patients (Figs 2A and 2B). Beside this important observation, fluorescence intensities, both of 335/400 fluorescence and 370/440 fluorescence, in the group of OA patients retained a significant correlation with age. In fact, a better correlation was found for the 370/440 fluorescence intensity (Fig. 3). The obtained data could be described as a linear increase as the best fitting of the measured data of the 370/440 fluorescence intensity with age even if the correlation coefficient was relatively low ($r = 0.606$).

Correlation between native fluorescence of cartilage and clinical examination

The native fluorescence intensities for both fluorescence maxima, i.e. 335/400 fluorescence as well as 370/440 fluorescence, were measured in triplicate. Correlation coefficients of significance between native cartilage fluorescence and clinical X-ray finding and three examinations are summarized in Table 2. A correlation between the 335/400 fluorescence and the X-ray pattern as well as between 370/440 fluorescence and the clinical examination were found. The p values are presented in parentheses in Table 2; they clearly suggest, however, a worse correlation of the 335/400 fluorescence with clinical examinations. The reason for this remains unknown and needs further examination. Though the correlation of the 335/400 fluorescence intensities with clinical examinations appeared to be significant, a correlation for 370/440 fluorescence is even more evident. The p values in parentheses show that corrected 370/440 fluorescence values can be very well correlated with X-ray findings as well as with all three clinical examinations.

Significantly increased native fluorescence intensity was observed in the cartilage of patients with osteoarthritis, meniscal lesions, anterior cruciate ligament lesion and synovitis

A correlation of the fluorescence intensity of samples from patients with chondropathy and osteoarthritis with age was observed and described in this study. The groups of patients

with osteoarthritis, meniscal lesions, anterior cruciate ligament lesion and synovitis were not affected by age. However, a significantly higher 335/400 and 370/440 fluorescence intensities were found in the group of patients with meniscal lesions, anterior cruciate ligament lesion and synovitis compared to the group of CH patients (Figs 2A and 2B). Such an increase was observed even in the group of youngest patients (15–25 years old). These patients were diagnosed with anterior cruciate ligament lesion and exhibited a significantly higher fluorescence than chondropathy patients did.

Discussion

Clinical tests of Outerbridge, Tegner and Lysholm are at least partly substitutable

Frequent diseases, such as chondropathy or injuries of the knee joint, can affect the intraarticular environment and result in articular degeneration. Those sequels can be verified according to various classification schemes.

To the most commonly accepted systems describing the severity of the hyaline cartilage damage belong those from Outerbridge (1961) and from Noyes and Stabler (1989). There are, however, certain limitations and deficiencies in both systems. Outerbridge divided the changes into four grades. Noyes and Stabler use simple terms for describing articular cartilage abnormalities – the description of the articular surface, the extent (depth) of involvement, the diameter of the lesion, and the location of the laesion. According to this system, in spite of being somewhat qualitative and subjective, one can record the observed articular cartilage changes.

Clinical examination describes the functional outcome of the joint. There are many schemes and evaluation systems that can mutually differ. The algofunctional index (Lequesne *et al.* 1987) and the score (Tegner and Lysholm 1985) belong to the most commonly used

schemes for the evaluation of the functional status. The prevalence of osteoarthritis is supposed to increase with age.

A correlation and substitutability among the clinical tests and X-ray findings was observed during the study. Based on our results we concluded that a significant mutual correlation exists between the individual clinical tests of Outerbridge (1961) and of Tegner and Lysholm (1985). These clinical tests are supposed to be mutually substitutable at least for the diagnosis of degenerative osteoarthritis, meniscal lesions, rupture of anterior cruciate ligament, chondropathy and synovitis (Table 2).

In addition, the results of X-ray examination significantly correlated with clinical tests. The p value <0.001 indicated a highly significant correlation among the clinical tests as well as with X-ray examination. Such a correlation indicates that the clinical tests are well chosen and also that the subjective complaints and ailments of the majority of patients reflected the *objective diagnosis*. The p values presented in Table 2 also suggest, however, a somewhat worse correlation with the clinical examination according to Tegner and Lysholm (1985). The reason why this test differed from the other two could be of potential interest in the future.

Both the 335/400 and 370/440 fluorescence intensities are suitable markers for estimation of cartilage wastage and can characterize patients with osteoarthritis, meniscal lesions, anterior cruciate ligament lesion or synovitis

We found a mutual correlation among the clinical tests and X-ray findings. However, the highest interest was to observe a correlation of clinical tests with the cartilage native fluorescence intensity. Such information would be valuable for the real biological status determination of cartilage without reference to age.

We found in digested articular cartilages two groups of native fluorophores with fluorescence maxima at $\lambda_{em} = 400$ nm and $\lambda_{em} = 440$ nm (excitation at $\lambda_{ex} = 335$ nm and $\lambda_{ex} =$

370 nm, respectively). The ratio of the 335/400 nm fluorophore concentration with respect to protein increased linearly with age. This is demonstrated in the group of patients with chondropathy which is, in fact, reported to be an initial ailment of cartilage. This group substituted in our study the control group of healthy patients due to a simple reason: the group of healthy patients could be insignificant from a statistical point of view. The relative 335/400 fluorescence intensity was age-related.

According to reports the production of AGEs can be modified in patients with chronic diseases, such as diabetes, atherosclerosis, and osteoarthritis in many tissues (Dyer *et al.* 1993, Chen *et al.* 1999). Accumulation of AGEs especially in articular cartilage has been described during development of osteoarthritis (DeGroot *et al.* 2001). A nonenzymic cross-linking has been reported to increase collagen stiffness and insolubility due to formation of nonenzymic glycation products (Bailey *et al.* 1998) which were also responsible for the appearance of native fluorophores. Increasing the amount of native fluorophores with age caused, naturally, higher fluorescence intensity observed in our study. Consequently, it can be concluded that the accumulation of AGEs increased with age. This is in agreement with the earlier observation of increased pentosidine level in cartilage aggrecan (Uchiyama *et al.* 1991, Verzijl *et al.* 2001).

The linear age-dependent increase of 335/400 fluorescence is in good correlation with the observation of healthy tissues (Sell and Monnier 1989, Uchiyama *et al.* 1991). In contrast, a significantly lower amount of nonenzymic glycation products was formed in aggrecan of OA patients. It can be supposed that the age-related increase in cartilage AGEs levels may be responsible, at least in part, for the age-related decline in the synthetic capacity of cartilage and concentration of the nonspecific glycation products increases in OA patients with age. Interestingly, other diagnoses exhibited no differences in all age-dependent groups. It should be mentioned that OA patients exhibited a significantly higher fluorescence intensity levels

compared with the group of chondropathy patients. The generally higher fluorescence which further increased with age probably reflected a higher amount of nonspecific glycation products caused by degenerative changes in OA cartilage and, on top of it, due to ageing which can independently increase the amount of AGEs.

The results also support the view that an increased level of degradation products in ACL is similar to the level of OA changes at higher age. ACL in young patients caused an increased formation of nonenzymic glycation products in comparison with the chondropathy group. Moreover, no correlation of nonenzymic glycation product with age in other diagnoses indicated that the acute processes may stimulate a higher nonenzymic glycation products formation in young patients. Thus, it can be concluded that hyaline cartilage in the knee joint after repeated inflammations or injuries contains a concentration of AGEs which is comparable to the concentration in healthy patients of a higher age. In fact, such an observation could be crucial and important for the detection of knee suspected for early meniscal and/or ACL lesion especially among young patients.

Acknowledgements

This work was supported by the Internal Grant Agency of the Ministry of Health of the Czech Republic grant (IGA) No. 8122 - 3/2004, the Czech Science Foundation grant No.

1ET400110403, the Science Foundation of the Charles University in Prague grant No. 200053 and the Ministry of Health grant No. 210060.

References

- AMLER E, ABBOTT A, MALAK H, LAKOWICZ J, BALL WJ, JR.: The carbohydrate moieties of the beta-subunit of Na⁺, K(+) -ATPase: their lateral motions and proximity to the cardiac glycoside site. *Biophys J* **70**: 182-193, 1996.
- BAILEY AJ, PAUL RG, KNOTT L: Mechanisms of maturation and ageing of collagen. *Mech Ageing Dev* **106**: 1-56, 1998.
- CHEN JR, TAKAHASHI M, SUZUKI M, KUSHIDA K, MIYAMOTO S, INOUE T: 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, 1999.
- DEGROOT J, VERZIIL N, JACOBS KM, BUDDE M, BANK RA, BIJLSMA JW, TEKOPPELE JM, LAFEBER FP: Accumulation of advanced glycation endproducts reduces chondrocyte-mediated extracellular matrix turnover in human articular cartilage. *Osteoarthritis Cartilage* **9**: 720-726, 2001.
- DYER DG, BLACKLEDGE JA, THORPE SR, BAYNES JW: Formation of pentosidine during nonenzymatic browning of proteins by glucose. Identification of glucose and other carbohydrates as possible precursors of pentosidine in vivo. *J Biol Chem* **266**: 11654-11660, 1991.
- DYER DG, DUNN JA, THORPE SR, BAILIE KE, LYONS TJ, MCCANCE DR, BAYNES JW: Accumulation of Maillard reaction products in skin collagen in diabetes and aging. *J Clin Invest* **91**: 2463-2469, 1993.
- GRANDHEE SK, MONNIER VM: Mechanism of formation of the Maillard protein cross-link pentosidine. Glucose, fructose, and ascorbate as pentosidine precursors. *J Biol Chem* **266**: 11649-11653, 1991.

HOSHINO H, TAKAHASHI M, KUSHIDA K, OHISHI T, KAWANA K, INOUE T:

Quantitation of the crosslinks, pyridinoline, deoxypyridinoline and pentosidine, in human aorta with dystrophic calcification. *Atherosclerosis* **112**: 39-46, 1995.

JÄGER M, WIRTH CJ: *Praxis der Orthopädie*. Thieme, Stuttgart New York, 1986, 980.

JONES PN: On collagen fibril diameter distributions. *Connect Tissue Res* **26**: 11-21, 1991.

LEQUESNE MG, MERY C, SAMSON M, GERARD P: Indexes of severity for osteoarthritis of the hip and knee. Validation--value in comparison with other assessment tests. *Scand J Rheumatol Suppl* **65**: 85-89, 1987.

NOYES FR, STABLER CL: A system for grading articular cartilage lesions at arthroscopy. *Am J Sports Med* **17**: 505-513, 1989.

OUTERBRIDGE RE: The etiology of chondromalacia patellae. *J Bone Joint Surg Br* **43-B**: 752-757, 1961.

PARRY DA, CRAIG AS, BARNES GR: Tendon and ligament from the horse: an ultrastructural study of collagen fibrils and elastic fibres as a function of age. *Proc R Soc Lond B Biol Sci* **203**: 293-303, 1978.

RODRIGUEZ-GARCIA J, REQUENA JR, RODRIGUEZ-SEGADE S: Increased concentrations of serum pentosidine in rheumatoid arthritis. *Clin Chem* **44**: 250-255, 1998.

SANTANA RB, XU L, CHASE HB, AMAR S, GRAVES DT, TRACKMAN PC: A role for advanced glycation end products in diminished bone healing in type 1 diabetes. *Diabetes* **52**: 1502-1510, 2003.

SELL DR, MONNIER VM: Structure elucidation of a senescence cross-link from human extracellular matrix. Implication of pentoses in the aging process. *J Biol Chem* **264**: 21597-21602, 1989.

SCHLEICHER ED, WAGNER E, NERLICH AG: Increased accumulation of the

- glycoxidation product N(epsilon)-(carboxymethyl)lysine in human tissues in diabetes and aging. *J Clin Invest* **99**: 457-468, 1997.
- TAKAHASHI M, SUZUKI M, KUSHIDA K, MIYAMOTO S, INOUE T: Relationship between pentosidine levels in serum and urine and activity in rheumatoid arthritis. *Br J Rheumatol* **36**: 637-642, 1997.
- TEGNER Y, LYSHOLM J: Rating systems in the evaluation of knee ligament injuries. *Clin Orthop Relat Res* **198**: 43-49, 1985.
- UCHIYAMA A, OHISHI T, TAKAHASHI M, KUSHIDA K, INOUE T, FUJIE M, HORIUCHI K: Fluorophores from aging human articular cartilage. *J Biochem (Tokyo)* **110**: 714-718, 1991.
- VERZIJL N, DEGROOT J, BANK RA, BAYLISS MT, BIJLSMA JW, LAFEBER FP, MAROUDAS A, TEKOPPELE JM: Age-related accumulation of the advanced glycation endproduct pentosidine in human articular cartilage aggrecan: the use of pentosidine levels as a quantitative measure of protein turnover. *Matrix Biol* **20**: 409-417, 2001.
- WELLS-KNECHT MC, THORPE SR, BAYNES JW: Pathways of formation of glycoxidation products during glycation of collagen. *Biochemistry* **34**: 15134-15141, 1995.
- WU JZ, HERZOG W: Elastic anisotropy of articular cartilage is associated with the microstructures of collagen fibers and chondrocytes. *J Biomech* **35**: 931-942, 2002.

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Tables

Table 1. Groups of patients according to age and diagnosis.

Age	ACL	CH	ME	S	OA
15-25	6	7	2		
26-40	19	8	11	1	1
41-55		4	12	5	5
56-70				1	18
71-85					25

Table 2. Correlation between X-ray diagnosis, clinical examination and native cartilage fluorescence.

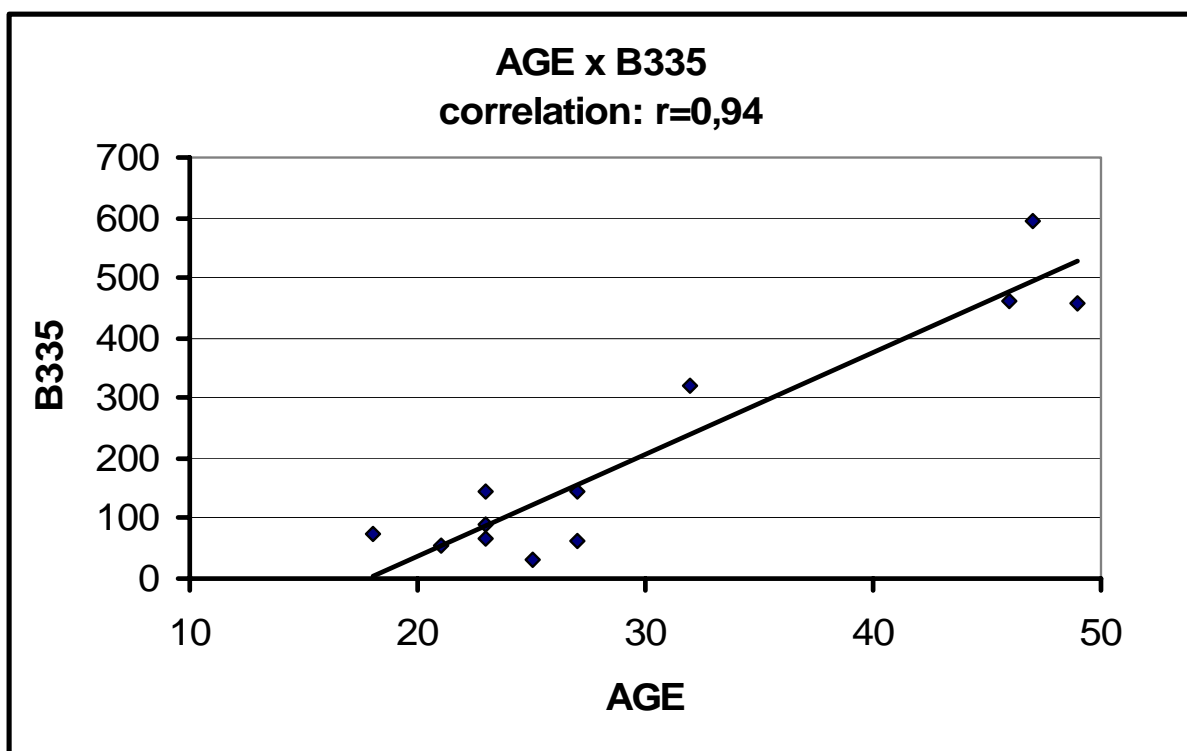
	X-ray	Outerbridge	Tegner	Lequense	335/400 fluorescence	370/440 fluorescence
X-ray	1	0.877 (<0.0001)	-0.826 (<0.0001)	0.626 (<0.0001)	0.270 (0.0029)	0.480 (<0.0001)
Outerbridge	0.877 (<0.0001)	1	-0.743 (<0.0001)	0.613 (<0.0001)	0.245 (0.0072)	0.502 (<0.0001)
Tegner	-0.826 (<0.0001)	-0.743 (<0.0001)	1	-0.677 (<0.0001)	-0.245 (0.0073)	-0.527 (<0.0001)
Lequense	0.626 (<0.0001)	0.613 (<0.0001)	-0.677 (<0.0001)	1	0.089 (0.3394)	0.38 (<0.0001)

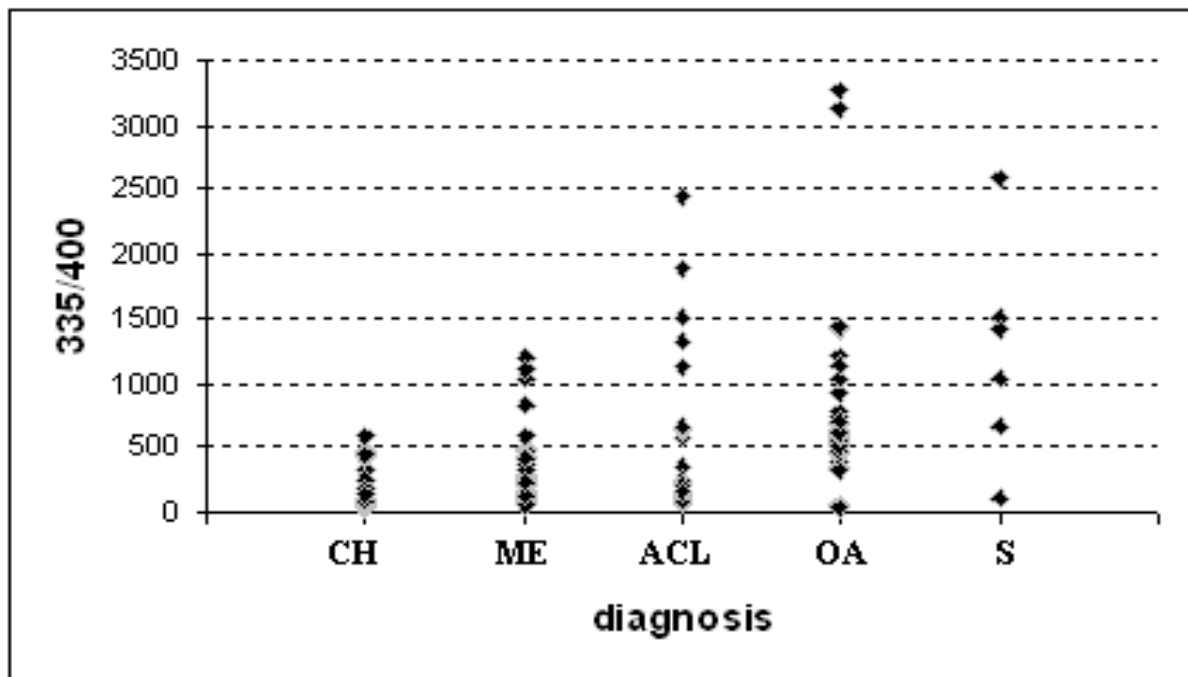
Correlation coefficients and appropriate *p* values (in parentheses) for X-ray findings and clinical examinations and native cartilage fluorescence. 370/440 fluorescence examination appeared to be significant, correlation for both methods, i.e. 335/400 fluorescence and 370/440 fluorescence, is evident.

Legends to figures

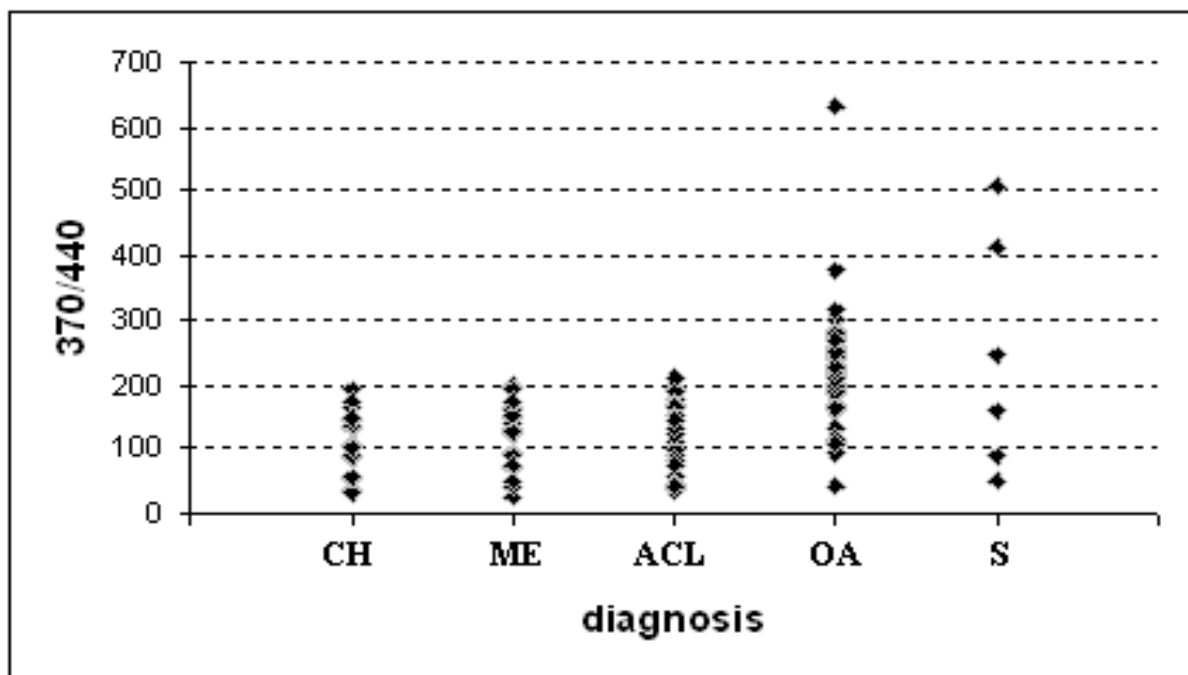
Fig. 1. Age-dependent fluorescence of pentosidine.

A significant (eleven-fold) increase with age was found in the group with CH ($y = 16.945 \times \text{age} - 301.4$, $r = 0.943$, $p = 0.01276$).





A



B

Fig. 2. Native fluorescence intensities from cartilages of patients with different diagnosis.

A) 335/400 fluorescence from cartilages of patients with CH (20 patients; $I_f =$

213+/-176 a.u.); ME (25 patients; $I_f = 400 \pm 316$ a.u.); ACL (24 patients; $I_f = 537 \pm 610$ a.u.); OA (49 patients; $I_f = 724 \pm 224$ a.u.); S (7 patients; $I_f = 1225 \pm 849$ a.u.).

B) 370/440 fluorescence from cartilages of patients with CH (20 patients; $I_f = 102 \pm 53$ a.u.); ME (25 patients; $I_f = 137 \pm 68$ a.u.); ACL (24 patients; $I_f = 136 \pm 76$ a.u.); OA (49 patients; $I_f = 224 \pm 89$ a.u.); S (7 patients; $I_f = 244 \pm 183$ a.u.).

According to the T-test, patients with OA significantly differ ($p < 0.0001$) from those with CH.

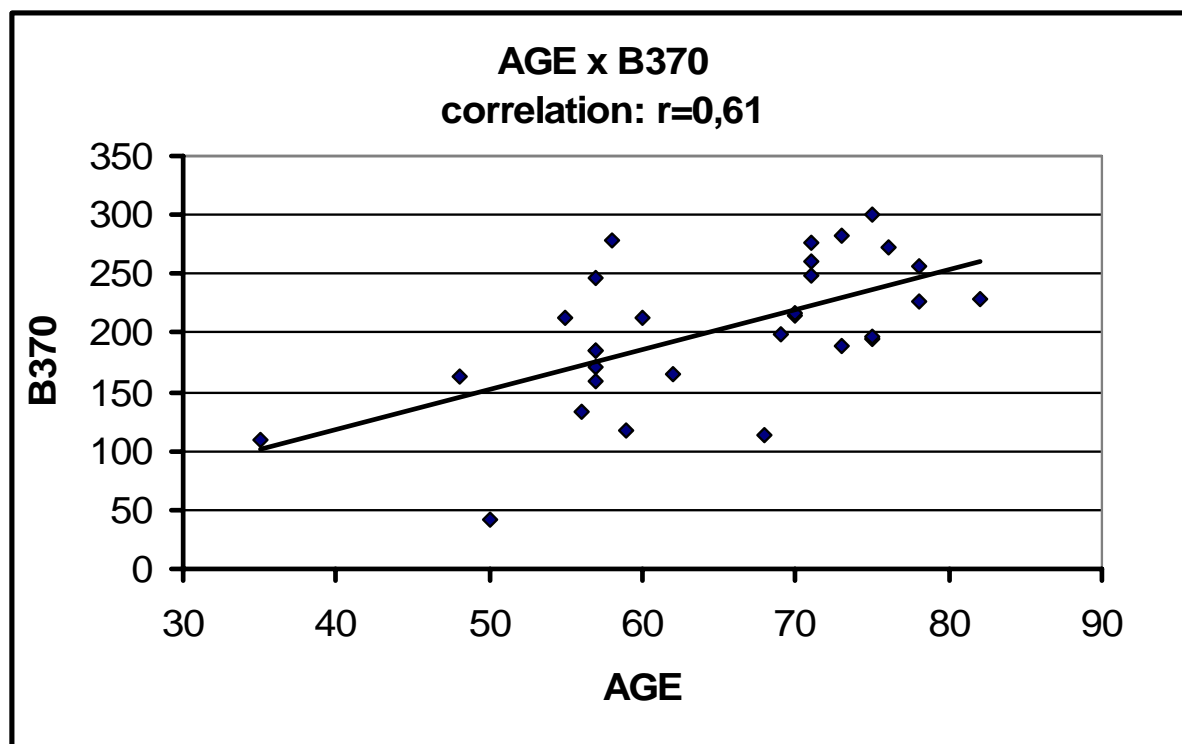


Fig. 3. Age-dependent fluorescence of nonspecific glycation product.

Patients with OA showed a significant correlation of pentosidine fluorescence intensity with age ($y = 3.3742 \times \text{age} - 16.67$, $r = 0.606$, $p = 0.00018$).