J Oral Pathol Med (2004) 33: 218–23

www.blackwellmunksgaard.com/jopm

Utility of urinary pyridinoline and deoxypyridinoline ratio for diagnosis of osteoarthritis at temporomandibular joint

K. Tanimoto[1,*], S. Ohno[1,*], M. Imada[1], K. Honda[1], M. Ohno-Nakahara[1], S. Kapila[2], K. Tanne[1]

[1]*Department of Orthodontics & Craniofacial Developmental Biology, Division of Cervico-Gnathostomatology, Hiroshima University Graduate School of Biomedical Sciences, 1-2-3 Kasumi, Minami-ku, 734-8553 Hiroshima, Japan, and* [2]*Department of Growth and Development, University of California San Francisco, San Francisco, CA, USA*

BACKGROUND: Pyridinoline (Pyr) and deoxypyridinoline (Dpyr) collagen cross-links are known markers of bone and cartilage turnover that are found in urine in various diseases. The present study was designed to quantify Pyr and Dpyr levels in urine of patients with osteoarthritis (OA) of the temporomandibular joint (TMJ), and to evaluate whether their concentrations are related to specific pathologic findings in the TMJ.

METHODS: Urine samples were obtained from 12 patients with OA of the TMJ and 16 asymptomatic controls, and following appropriate preparation, analyzed by high-performance liquid chromatography (HPLC) and fluorescence spectroscopy for Pyr and Dpyr.

RESULTS: The urinary concentration of Pyr and the Pyr to Dpyr (Pyr/Dpyr) ratio were significantly higher ($P < 0.05$) in OA patients than in the controls (182.2 ± 86.5 pmol/ml vs. 115.6 ± 27.9 pmol/ml and 4.00 ± 1.53 pmol/ml vs. 2.86 ± 0.97 pmol/ml, respectively). However, the Pyr/Dpyr ratio was not associated with any specific clinical or radiographic findings.

CONCLUSION: These findings suggest that the level of Pyr and the Pyr/Dpyr ratio in urine may be a useful diagnostic indicator for intra-articular pathologic changes during TMJ OA.

J Oral Pathol Med (2004) **33**: 218–23

Keywords: deoxypyridinoline; high-performance liquid chromatography; osteoarthritis (OA); pyridinoline; temporomandibular joint disorders

Introduction

Temporomandibular joint (TMJ) disorders include osteoarthritis (OA), internal derangement, primary arthritides, and rheumatoid arthritis (1). Among TMJ disorders, OA is an important topic in the field of clinical dentistry. OA is chronic and progressive in nature, and usually regarded as a monoarticular disorder accompanied by the degradation of articular cartilage in its early phase (2, 3).

For the differential diagnosis of OA, usually, subchondral bone resorption is evaluated on radiographs. However, morphologic changes of the bony structures are first detected only in the later stages of a sequence of pathologic alterations. It is not possible to detect cartilage loss by the use of radiographic imaging alone. Furthermore, it is impossible by a single image analysis to determine whether bony changes are progressing or not. Consequently, biochemical indicators, which are directly associated with the metabolism of bone and cartilage, would be highly desirable in the early differential diagnosis of OA.

Serum tartrate-resistant acid phosphatase (4), urinary galactosyl hydroxylysine (5), and urinary hydroxyproline (6) have all been used as markers of bone degradation and destruction. Matrix metalloproteinases (MMPs) (7) and type II procollagen carboxypeptide (C-II propeptide) (8) in synovial fluid, and keratan sulfate in blood (9) have been used for the detection of damaged cartilage. However, the sampling of synovial fluid or biopsy tissues for the examination involves invasive procedures and is difficult to perform in routine clinical practice. A less invasive and easy method, such as using serum for the detection of markers of TMJ OA, would therefore be highly practical and is more likely to be utilized.

Pyridinoline (Pyr) and deoxypyridinoline (Dpyr), the cross-links between collagens, are present in various tissues. Pyr is abundant in both bone and cartilage, and the proportion of Dpyr to Pyr is much lower in cartilage than in bone. Neither Pyr nor Dpyr is metabolized intravitally and both are excreted into urine. It has been demonstrated that the Pyr/Dpyr ratio in urine increases in patients with knee or hip joint arthritis (10, 11). Therefore, it is presumed that the Pyr/Dpyr ratio in urine increases following cartilage destruction in the TMJ. Although the applicability of urinary Pyr and Dpyr has been demonstrated in detecting arthritis of the knee or hip joints, the association between these cross-links and TMJ arthritis has not yet been evaluated. Therefore, the purpose of this study was to determine whether urinary Pyr and Dpyr concentrations are elevated in patients with TMJ OA relative to those in healthy controls. We also investigated if there is

Correspondence: Dr Shigeru Ohno, Department of Orthodontics & Craniofacial Developmental Biology, Division of Cervico-Gnathostomatology, Hiroshima University Graduate School of Biomedical Sciences, 1-2-3 Kasumi, Minami-ku, 734-8553 Hiroshima, Japan. Tel.: +81 82 257 5686. Fax: +81 82 257 5687. E-mail: shigebon@hiroshima-u.ac.jp
*These authors contributed equally to this work.
Accepted for publication June 11, 2003

an association between specified clinical and radiographic findings and the Pyr/Dpyr ratio in symptomatic subjects.

Materials and methods

Asymptomatic and symptomatic subjects were recruited by announcements posted in the Department of Orthodontics, Hiroshima University Faculty of Dentistry or from patients in Oral Surgery, Hiroshima University Dental Hospital. Subjects included in the study were limited to 20–49-year-old healthy males or females who satisfied the TMJ diagnosis of OA as proposed by the American Academy of Orofacial Pain (12) (AAOP; Table 1). The exclusion criteria included systemic OA or rheumatoid arthritis or any bone metabolic disease as determined from the health history questionnaire. OA was defined as proposed by AAOP where a finding of pain together with condylar flattening, erosion or osteophyte was present in at least one TMJ. Two males and 10 females aged 20–49 years (mean 33.7 years) with OA following the criteria served as symptomatic subjects in this study. As a control group, seven males and nine females aged 24–49 years (mean 31.7 years) who had never experienced any signs or symptoms of TMD and who had no aberrations on the X-ray images were selected from the staff at the Department of Orthodontics, Hiroshima University Faculty of Dentistry. Informed consent for the urinalysis was obtained from patients prior to the series experiments.

Table 1 Criteria for TMJ OA (OA patients were diagnosed following the criteria established by the AAOP 1996

TMJ articular disorders
Congentital or developmental disorders
Aplasia
Hypoplasia
Hyperplasia
Neoplasia
Disc derangement disorders
Disc displacement with reduction
Disc displacement without reduction
TMJ dislocation
Inflarrimatory disorders
Capsulitis/Synovitis
Polyarthritides
Osteoarthritis (noninflammatory disorders)
Osteoarthritis (primary)
Osteoarthritis (secondary)
Ankylosis

In order to evaluate pain and jaw dysfunction, the patients were requested to fill in a self-questionnaire (13). Pain was categorized as spontaneous, functioning and oppressive pain, and the intensity was estimated by the aid of a visual analog scale (VAS). Radiographic changes were determined by transcranial radiographic imaging of TMJ (14) and defined by two clinicians (K.T. and S.O.). The radiographic findings that were considered abnormal included flattening, erosion and osteophyte on the mandibular condyle. Flattening was defined as a flat bony contour deviating from the convex shape, erosion was defined as an area of decreased density of the cortical bone and the adjacent subcortical bone, and osteophyte was defined as a marginal bony outgrowth on the condyle (Fig. 1). TMJ sounds were also examined by palpation and auscultation. Urinary samples were collected, adjusted to pH 5, and stored at -30°C. There is diurnal variation in the content of urinary Pyr and Dpyr, and these cross-links are detected most readily at night or early in the morning (15). The first urine samples of the morning were therefore collected for our study. Aliquots (0.5 ml) were hydrolyzed with an equal volume of 12 M HCl

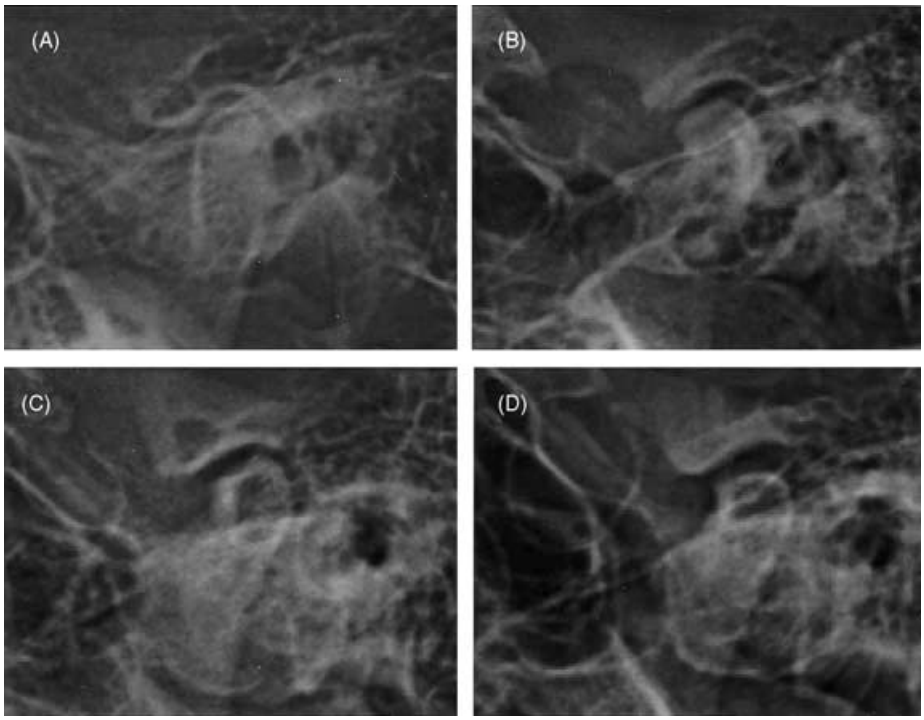

Figure 1 Classification of radiographic hard tissue changes in patients with OA in TMJ. The hard tissue changes were evaluated as: (A) normal, (B) flattening, (C) erosion, and (D) osteophyte.

Table 2 Radiographic, clinical findings and Pyr/Dpyr ratio in patients with TMJ OA

	Right				*Left*					
Subject	*Pain*	*Click*	*Crepitus*	*Radiographic findings*	*Pain*	*Click*	*Crepitus*	*Radiographic findings*	*VAS of TMJ pain*	*Pyr/Dpyr ratio*
1	+	+	−	Flattening	−	−	−	Normal	2.1	3.18
2	+	−	+	Flattening	−	−	−	Normal	1.9	5.31
3	−	−	−	Normal	+	+	−	Flattening	4.9	2.75
4	−	−	−	Normal	+	+	−	Flattening	1.2	5.19
5	+	+	−	Flattening	+	−	+	Flattening	2.4	4.21
6	−	−	−	Flattening	+	+	−	Erosion	3.0	5.53
7	+	−	+	Erosion	−	−	−	Normal	5.2	4.45
8	−	−	−	Normal	+	+	−	Erosion	9.1	4.44
9	+	+	−	Erosion	+	−	−	Erosion	6.2	4.30
10	−	−	−	Normal	+	−	+	Osteophyte	3.5	2.65
11	+	+	−	Osteophyte	−	−	−	Normal	1.0	1.98
12	−	−	−	Osteophyte	+	−	+	Osteophyte	8.7	1.83

Pain was assessed by a visual analog scale (VAS). Condylar deformity was assessed and divided into three types (erosion, flattening and osteophyte). TMJ sounds were registered as click and crepitus.

with polytetrafluoroethylene lined for 18 h at 107°C. The hydrolyses were centrifuged, and the supernatants were applied to a minicolumn filled with CC31 microgranular cellulose powder of 100 mg (Whatman Biosystems, Maidstone, UK). The dilutor reservoir was filled with mobile phase (butanol/acetic acid/water, 4/1/1, v/v/v) and placed in the solvent rack containing 90% aqueous acetic acid. Purified fractions of 0.5 ml in a mixture (butanol/acetic acid, 4/1) of 2.5 ml were applied to the minicolumn and washed with 8 ml of mobile phase and 0.5 ml of tetrahydrofuran. Pyr and Dpyr were eluted by 0.63 ml heptafluorobutyric acid (HFBA) (50 mM) and filtered through a 0.45-mm millipore filter (Gelman Sciences, Ann Arbor, MI, USA).

Measurement of the cross-links was performed by high-performance liquid chromatography (HPLC; Waters 600E Multisolvent Delivery System; Waters, Milford, USA) using the method described previously (16). A 4.6×150 mm column pre-packed with Radial-Pak C18 (Symmetry 3.5 mm; Waters, Milford, USA) was used. Acetonitrile (AcCN)/10 mM of HFBA (25/75, v/v) was used for a mobile phase. The flow rate was 1.0 ml/min. Each prepared sample of 20 µl was injected into the HPLC column for the measurement of Pyr and Dpyr. The column effluent was monitored by fluorescence spectroscopy at excitation and emission wavelengths of 295 and 400 nm, respectively. Standard curves were constructed using pure Pyr and Dpyr (Wako, Osaka, Japan). Pyr adjusted to 43, 107.5, 215 pmol/ml, and Dpyr adjusted to 44.6, 223, and 446 pmol/ml were subjected to HPLC three times, and from the mean value of the peak area, the corrected standard curves were drawn. The concentration of Pyr or Dpyr was evaluated using these standard curves, and the Pyr/Dpyr ratio was obtained from the results.

Significant differences in the measured values of the urinary Pyr and Dpyr concentrations and the ratio of these cross-links between patient and control groups were determined by Student's *t*-test with significance set at $P < 0.05$. The correlation of clinical and radiographic variables with the Pyr/Dpyr ratio was determined by Mann–Whitney test.

Results

Two patients had bilateral TMJ pain and 10 unilateral TMJ pain. Eight and four patients exhibited clicking sounds and crepitation, respectively. Seven condyles (six patients) showed flattening, five condyles (four patients) showed erosion, and four condyles (three patients) showed osteophytes (Table 2).

Representative chromatographic patterns of desalted urine from controls and patients are shown in Fig. 2. The retention time of Pyr and Dpyr was 14.3 and 14.8 min, respectively. Although the peaks of Pyr and Dpyr approximated each other, they were distinct. Figure 3 shows the actual concentrations of each cross-link in urine of controls and patients. The distribution of the values for Pyr concentration was significantly higher ($P < 0.05$) in the OA patients than in the controls. The distribution of the values of the concentration of Dpyr was also higher in the patient group than the control group (Fig. 3; Table 3).

The mean values of the concentration of Pyr were significantly higher ($P < 0.05$) in the patient group than the control group. The mean Dpyr levels were slightly higher in the patient than in the control group but this difference was not statistically significant. Consequently these findings resulted in the Pyr/Dpyr ratio being significantly higher ($P < 0.05$) in the patient vs. the control group (Table 3). The relationships of Pyr level, Dpyr level, and Pyr/Dpyr ratio with clinical or radiographic findings were tested statistically, but no significant correlation was found between these variables (Table 2).

Discussion

Pyridinoline and Dpyr are collagenous cross-links that play a role in maintaining the structure of fully processed collagen molecules (17). Cartilage-specific collagens such as type II, IX and XI collagens have been shown to contain abundant Pyr (18). Articular cartilage of the TMJ, which is composed of fibrous cartilage, differs histologically and in the overall matrix composition from cartilage in the knee and hip joints. The surface of the condylar cartilage contains much type I collagen; type II collagen is abundant in the

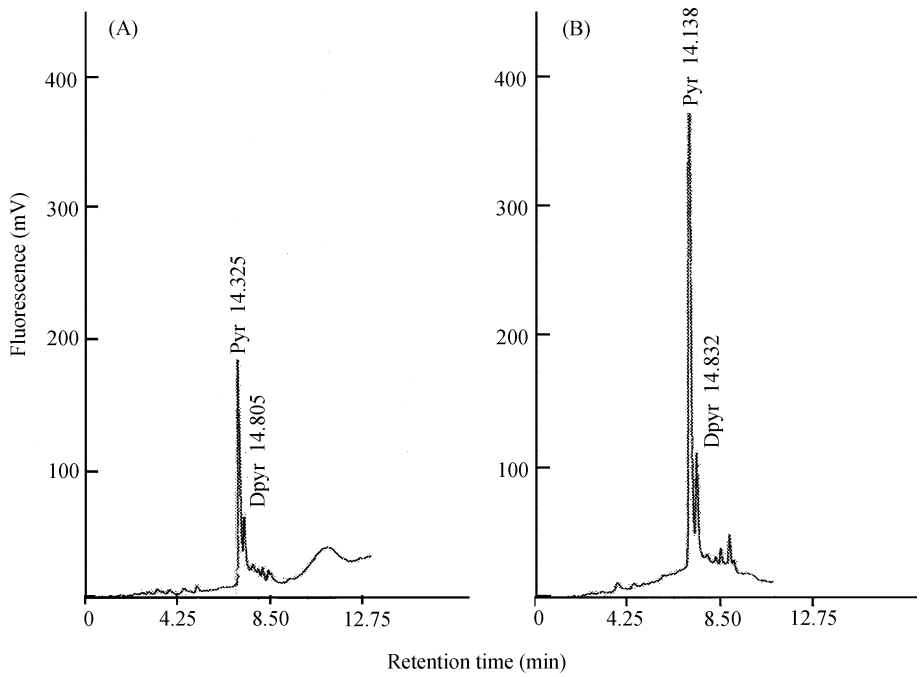

Figure 2 Typical chromatograms of a fractionated hydrolysate of urine from a control patient (A) and patient with TMJ OA (B).

mature cartilage layer beneath the fibrous layer (19). An immunohistologic study has recently shown that both type IX and XI collagens are present in neonatal mammalian condylar cartilage (20). These findings together suggest that TMJ condylar cartilage is rich in Pyr. Although Dpyr is also detected in cartilage, its proportion in relation to Pyr is much lower in all tissues, including cartilage except in bone (21). Neither Pyr nor Dpyr is metabolized intravitally and both are

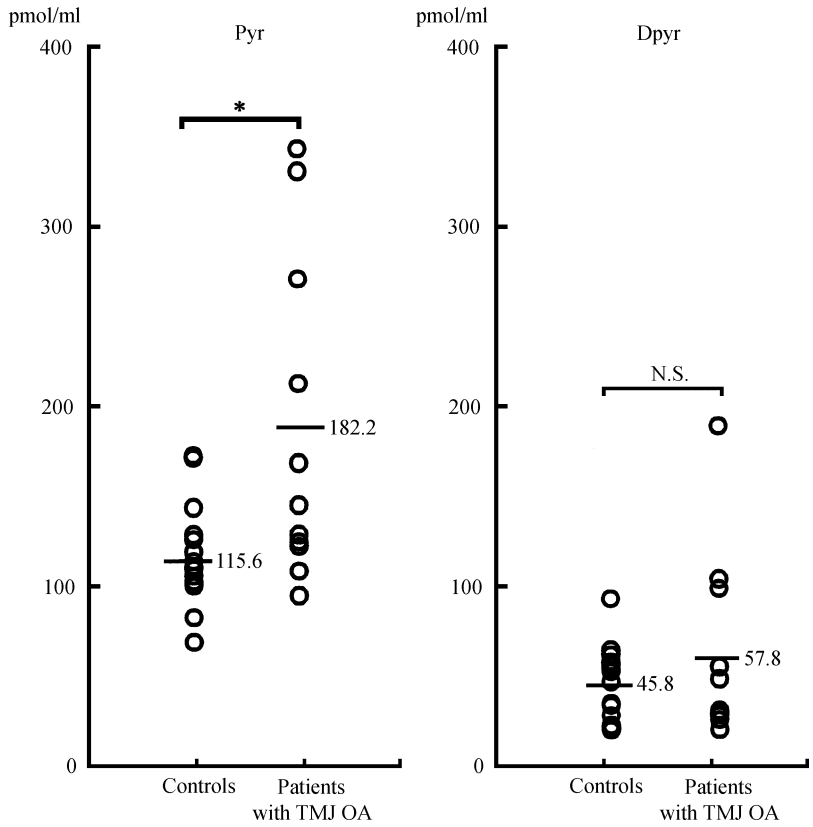

Figure 3 Distribution of concentration of the cross-links in control and patient groups. Bars indicate the median of the group. *Significantly different from controls at $P < 0.05$. N.S.: not significant.

Table 3 Pyridinium cross-link values (median and SD) in patients with TMJ pain and controls

	Controls (N = 16)	*Patients with TMJ OA (N = 12)*
Pyr (pmol/ml)	115.6 ± 27.9	$182.2 \pm 86.5^*$
Dpyr (pmol/ml)	45.8 ± 20.0	57.8 ± 49.5
Pyr/Dpyr ratio	2.86 ± 0.97	$4.00 \pm 1.53^*$

*Significantly different from controls at $P < 0.05$.

excreted into urine. Therefore, these cross-links would be released and detectable in urine in the presence of active condylar resorption in joints composed of bone, cartilage, and synovial membrane that are a rich source of these cross-links. Based on the very low levels of Dpyr in cartilage, it would be reasonable to assume that the release of Pyr, but not Dpyr, is enhanced by cartilage degradation.

In this study, the HPLC method employed for the assay of Pyr and Dpyr was that established by Black et al. (22). This method is more accurate and more widely used than the enzyme immunoassay. It has been reported that there is no association between age and the urinary concentration of cross-links in cartilage or urine in adults (23, 24), although the content of Pyr and Dpyr in urine has been found to be higher in growing children and the elderly as compared to that in grown-up and middle-aged adults (25). No differences in the concentrations of urinary Pyr and Dpyr have been noted between the sexes in subjects ranging from 20 to 40 years old (26), but high levels of Pyr and Dpyr are found in post-menopausal women (27–30). Based on these findings, we used the urine samples collected in the morning from men and women aged from 20 to 49 years.

As the absolute quantity of Pyr and Dpyr in urinary samples can be readily altered during treatment of various metabolic diseases, the ratio of these cross-links remains fairly invariable. Therefore, besides quantifying Pyr and Dpyr, we also determined the Pyr/Dpyr ratio in this investigation. It is documented that the level of urinary Pyr is higher in patients with knee or hip arthritis than in healthy controls (10, 31, 32). As the ratio of Pyr and Dpyr is approximately 3 : 1, 50 : 1, and 25 : 1 in bone, cartilage, and synovial membrane, respectively (21), it is plausible that the release of Pyr is predominantly enhanced, resulting in an increased Pyr/Dpyr ratio during the early or developing stages of arthritis when cartilage is undergoing degeneration.

In this study, the Pyr/Dpyr ratio in urine was significantly higher in the patients with TMJ OA than in the controls. As the presence of systemic metabolic diseases, which could potentially affect our results, was an exclusion criterion, we believe that the possible influences of other joint diseases on the findings of the present study were minimized or negated. Our findings also show that the types of radiographic condylar deformity (flattening, erosion, and osteophyte) were not associated significantly with the increase of Pyr/Dpyr ratio in urine. This is in agreement with the results by Graverand et al. (33) who reported no association between the severity of knee or hip joint OA determined by X-ray imaging and Pyr/Dpyr ratio in urine. This lack of an association between radiographic observations and Pyr/Dpyr ratio may be because of either the difficulty in detecting early radiographic bony changes or the time lag between changes on the images and the progress in the intra-articular pathologic status. No significant correlation was found between Pyr/Dpyr ratio in urine and VAS score of the pain. This might be because of that the patients had TMJ pain caused by other reasons than the degenerative changes.

The examination of urinary Pyr and Dpyr is standard for the evaluation of knee or hip joint damage and is a reliable method for the differential diagnosis of arthritis in these joints (10, 11). As the TMJ is a small joint, it may be difficult to detect the intra-articular pathologic changes in the TMJ by means of urinalysis. However, it has been reported that a significant increase of Pyr was observed in urine of beagle dogs with aggressive local periodontitis induced by ligature of the gingival sulcus (34). Our previous studies have shown that the urinary Pyr/Dpyr ratio increases in collagenase-induced experimental TMJ OA in the rat (35). Furthermore, a recent study demonstrated that urinary Pyr and Dpyr levels exhibited metabolic changes in mandibular growth (36). Together these results indicate that it is possible to use urinary cross-links to detect local degenerative or developmental changes in the TMJ. However, further investigations with larger number of patients are required to further clarify how the Pyr/Dpyr ratio in urine directly pertains to destruction and remodeling of the condyle.

In conclusion, we found that the Pyr/Dpyr ratio in urine is significantly higher in patients with TMJ OA than in control subjects. Our findings suggest the potential applicability of this assay in conjunction with conventional image analysis for the differential diagnosis of TMJ OA.

References

1. de Bont LG, Stegenga B. Pathology of temporomandibular joint internal derangement and osteoarthrosis. *Int J Oral Maxillofac Surg* 1993; **22**: 71–4.
2. Mankin HJ. Clinical features of osteoarthritis. In: Kelley WN, Harris ED, Ruddy S, Sledge CB, eds. *Textbook of Rheumatology.* Philadelphia: Saunders Company, 1989; 1480.
3. Hough AJ, Sokoloff L. Pathology of osteoarthritis. In: Mccarty DJ, ed. *Arthritis and Allied Conditions. Textbook of Rheumatology.* Philadelphia: Lea & Febiger 1989; 1571.
4. Scarnecchia L, Minisola S, Pacitti MT, et al. Clinical usefulness of serum tartrate-resistant acid phosphatase activity determination to evaluate bone turnover. *Scand J Clin Lab Invest* 1991; **51**: 517–24.
5. Krane SM, Kantrowitz FG, Byrne M, Pinnell SR, Singer FR. Urinary excretion of hydroxylysine and its glycosides as an index of collagen degradation. *J Clin Invest* 1977; **59**: 819–27.
6. Bettica P, Moro L, Robins SP, et al. Bone-resorption markers galactosyl hydroxylysine, pyridinium crosslinks, and hydroxyproline compared. *Clin Chem* 1992; **38**: 2313–8.
7. Okada Y, Shinmei M, Tanaka O, et al. Localization of matrix metalloproteinase 3 (stromelysin) in osteoarthritic cartilage and synovium. *Lab Invest* 1992; **66**: 680–90.
8. Kobayashi T, Yoshihara Y, Samura A, et al. Synovial fluid concentrations of the C-propeptide of type II collagen correlate with body mass index in primary knee osteoarthritis. *Ann Rheum Dis* 1997; **56**: 500–3.
9. Thonar EJ, Schnitzer TJ, Kuettner KE. Quantification of keratan sulfate in blood as a marker of cartilage catabolism. *J Rheumatol* 1987; **14**: 23–4.

10. Takahashi M, Kushida K, Inoue T. Receiver operating characteristic analysis of urinary pyridinium cross-links in arthritis. *Br J Rheumatol* 1995; **34**: 792–3.
11. Astbury C, Bird HA, McLaren AM, Robins SP. Urinary excretion of pyridinium crosslinks of collagen correlated with joint damage in arthritis. *Br J Rheumatol* 1994; **33**: 11–5.
12. Okeson JP. Orofacial pain: guidelines for assessment, diagnosis, and management. In: Okeson JP, ed. *Temporomandibular Disorders*. Chicago: Quintessence, 1996.
13. Clark GT, Moody DG, Sanders B. Analysis of arthroscopically treated TMJ derangement and locking. In: Sanders B, Murakami K, Clark GT, eds. *Diagnostic and Surgical Arthroscopy of the Temporomandibular Joint.* Philadelphia: Saunders Company, 1989; 115–36.
14. de Leeuw R, Boering G, Stegenga B, de Bont LG. Radiographic signs of temporomandibular joint osteoarthrosis and internal derangement 30 years after nonsurgical treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1995; **79**: 382–92.
15. Schlemmer A, Hassager C, Jensen SB, Christiansen C. Marked diurnal variation in urinary excretion of pyridinium cross-links in premenopausal women. *J Clin Endocrinol Metab* 1992; **74**: 476–80.
16. Pratt DA, Daniloff Y, Duncan A, Robins SP. Automated analysis of the pyridinium crosslinks of collagen in tissue and urine using solid-phase extraction and reversed-phase high-performance liquid chromatography. *Anal Biochem* 1992; **207**: 168–75.
17. Uchiyama A, Inoue T, Fujimoto D. Synthesis of pyridinoline during in vitro aging of bone collagen. *J Biochem (Tokyo)* 1981; **90**: 1795–8.
18. Eyre DR. Collagen cross-linking amino acid. In: Cunninghum LW, ed. *Methods in Enzymology*, Orland: Academic Press, 1987; 115–39.
19. Hirschmann PN, Shuttleworth CA. The collagen composition of the mandibular joint of the foetal calf. *Arch Oral Biol* 1976; **21**: 771–3.
20. Silbermann M, von der Mark K. An immunohistochemical study of the distribution of matrical proteins in the mandibular condyle of neonatal mice. Part I. Collagens. *J Anat* 1990; **170**: 11–22.
21. Takahashi M, Kushida K, Hoshino H, et al. Concentrations of pyridinoline and deoxypyridinoline in joint tissues from patients with osteoarthritis or rheumatoid arthritis [see comments]. *Ann Rheum Dis* 1996; **55**: 324–7.
22. Black D, Duncan A, Robins SP. Quantitative analysis of the pyridinium crosslinks of collagen in urine using ion-paired reversed-phase high-performance liquid chromatography. *Anal Biochem* 1988; **169**: 197–203.
23. Uchiyama A, Ohishi T, Takahashi M, et al. Fluorophores from aging human articular cartilage. *J Biochem (Tokyo)* 1991; **110**: 714–8.
24. Black D, Marabani M, Sturrock RD, Robins SP. Urinary excretion of the hydroxypyridinium cross links of collagen in patients with rheumatoid arthritis. *Ann Rheum Dis* 1989; **48**: 641–4.
25. Fujimoto D, Suzuki M, Uchiyama A, Miyamoto S, Inoue T. Analysis of pyridinoline, a cross-linking compound of collagen fibers, in human urine. *J Biochem (Tokyo)* 1983; **94**: 1133–6.
26. Ohishi T, Takahashi M, Kawana K, et al. Age-related changes of urinary pyridinoline and deoxypyridinoline in Japanese subjects. *Clin Invest Med* 1993; **16**: 319–25.
27. Uebelhart D, Gineyts E, Chapuy MC, Delmas PD. Urinary excretion of pyridinium crosslinks: a new marker of bone resorption in metabolic bone disease. *Bone Miner* 1990; **8**: 87–96.
28. Hassager C, Colwell A, Assiri AM, Eastell R, Russell RG, Christiansen C. Effect of menopause and hormone replacement therapy on urinary excretion of pyridinium cross-links: a longitudinal and cross-sectional study. *Clin Endocrinol (Oxf)* 1992; **37**: 45–50.
29. Kawana K, Kushida K, Takahashi M, et al. The effect of menopause on biochemical markers and ultrasound densitometry in healthy females. *Calcif Tissue Int* 1994; **55**: 420–5.
30. Uebelhart D, Schlemmer A, Johansen JS, Gineyts E, Christiansen C, Delmas PD. Effect of menopause and hormone replacement therapy on the urinary excretion of pyridinium cross-links. *J Clin Endocrinol Metab* 1991; **72**: 367–73.
31. Thompson PW, Spector TD, James IT, Henderson E, Hart DJ. Urinary collagen crosslinks reflect the radiographic severity of knee osteoarthritis. *Br J Rheumatol* 1992; **31**: 759–61.
32. Seibel MJ, Woitge H, Scheidt-Nave C, et al. Urinary hydroxypyridinium crosslinks of collagen in population-based screening for overt vertebral osteoporosis: results of a pilot study. *J Bone Miner Res* 1994; **9**: 1433–40.
33. Graverand MP, Tron AM, Ichou M, et al. Assessment of urinary hydroxypyridinium cross-links measurement in osteoarthritis. *Br J Rheumatol* 1996; **35**: 1091–5.
34. Shibutani T, Murahashi Y, Tsukada E, Iwayama Y, Heersche JN. Experimentally induced periodontitis in beagle dogs causes rapid increases in osteoclastic resorption of alveolar bone. *J Periodontol* 1997; **68**: 385–91.
35. Imada M, Tanimoto K, Ohno S, Sasaki A, Sugiyama H, Tanne K. Changes in urinary bone resorption markers (pyridinoline, deoxypyridinoline) resulting from experimentally-induced osteoarthritis in the temporomandibular joint of rats. *Cranio* 2002; **21**: 38–45.
36. Tanimoto K, Imada M, Ohno S, Sasaki A, Honda K, Tanne K. Association between craniofacial growth and urinary bone metabolic markers (pyridinoline, deoxypyridinoline) in growing rats. *J Dent Res* 2003; **82**: 28–32.

Acknowledgements

This work was supported by the Research Center for Molecular Medicine, Faculty of Medicine, Hiroshima University. We are grateful to Dr Koyano (Department of Orthopedic Surgery, Jikei University School of Medicine) for his encouragement and advice. We also thank Dr Sugiyama (Department of Second Oral Surgery, Hiroshima University Faculty of Dentistry) and Dr Shigehira (Department of Second Oral Surgery, Hiroshima University Faculty of Dentistry) for their help with obtaining urinary samples from the patients with temporomandibular disease.