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Reliability of spot samples for assessment of urinary excretion of pyridinoline in patients with rheumatoid arthritis

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ABSTRACT

Objective

To determine how well a spot urine sample of patients with active rheumatoid arthritis (RA) can predict 24-hour urinary pyridinoline and deoxypyridinoline excretion.

Methods

Urine samples of 11 hospitalized RA patients taken on 2 consecutive days at 8 a.m. and 4 p.m. were compared with samples from 24-hour collections (gold standard). High-performance liquid chromatography was used to measure the collagen crosslink concentrations.

Results

Sampling time was the only significant factor (repeated measurement ANOVA). Significant differences were found between morning and 24-hour samples and between morning and afternoon samples, but not between afternoon and 24-hour samples.

Conclusions

Samples collected in the afternoon (4 p.m.) give the best approximation of 24-hour urinary pyridinoline excretion in patients with active rheumatoid arthritis. In longitudinal studies the sampling time should be fixed.

Introduction

The pyridinium derivatives pyridinoline (Pyd = hydroxylysylpyridinoline) and deoxypyridinoline (Dpd = lysylpyridinoline) are collagen crosslinks in bone, cartilage and skin. When bone is resorbed, these pyridinium crosslinks are released into the circulation and subsequently excreted in the urine. Dpd is present in type I collagen which is the main protein component of bone and dentine. Most excreted Dpd comes from bone as this tissue has quick remodelling. Therefore, urinary excretion of Dpd is a marker of bone resorption. Pyd is more widely distributed and is present in considerable amounts in cartilage-specific collagens: types II, IX and XI. The excretion of Pyd and Dpd is not influenced by diet or physical exercise. However, a circadian rhythmicity in bone remodelling has been shown for the excretion of pyridinium cross-links in healthy premenopausal females (1). It is as yet unclear to what extent a circadian rhythm is present at higher excretion levels of Pyd and Dpd (2).

Rheumatoid arthritis (RA) is a systemic disease with destruction of joints in hands and feet as a hallmark. This destruction of collagen in bone and cartilage in RA can be accompanied by an increase in Pyd and Dpd excretion, reflecting disease activity (2). To date, 24-hour collection to assess excretion is the standard. For practical reasons, spot sampling (or collection of urine during a part of the day) is desirable (3). The aim of this study was to determine how well a sample of urine of patients with active RA can predict 24-hour pyridinium crosslink excretion.

Methods

Study design

Urinary samples were collected from 17 RA patients [ACR 1987 criteria (4)] hospitalized in the Department of Internal Medicine of the Maastricht University Hospital for exacerbation of disease activity. There were no dietary restrictions. Concentrations of Pyd, Dpd and creatinine (Cr) as well as 24-hour urinary volumes were measured.

Urine collection started at 8 a.m.; a first spot sample was taken at 4 p.m., and a second one the next day at 8 a.m just before completion of the 24-hour collection. Ten milliliter aliquots of each sample were stored at -20°C. A third aliquot was sampled from the 24-hour collection (after stirring). This procedure was carried out in each patient on 2 consecutive days.

For each patient the sex, birth date, RA disease duration (from date of diagnosis), weight, height, erythrocyte sedimentation rate (ESR), medication and serum creatinine were noted. As a criterion for appropriate urine collection, we used the ratio of total creatinine excretion on day 1 and day 2; this ratio had to be between 0.8 and 1.25 (not more than a 20% discrepancy between the 2 collections) (4).

Pyridinoline and deoxypyridinoline chromatographic analysis

The total amount of excreted Pyd and Dpd was measured by high-performance liquid chromatography (HPLC) (5). Urine samples were hydrolysed in

Table I. Patient characteristics and laboratory values (n = 11; 7 females and 4 males).

	Median	(Range)
Age (yrs)	64	(42 - 75)
Disease duration (yrs)	4	(0.3 - 34)
Body weight (kg)	67	(59 - 98)
Serum creatinine (mmol/l)	67	(57 - 152)
Erythrocyte sedimentation rate (mm/ 1st h)	65	(9 - 95)
Pyridinoline excretion (nmol/ 24h)	321	(140 - 1015)
Deoxypyridinoline excretion (nmol/ 24h)	74	(32 - 191)
Creatinine excretion (mmol/ 24h)	7.7	(5.3 - 11.0)
Pyridinoline / creatinine excretion (nmol/ mmol)	50	(23 - 126)
Dezoxypyridinoline / creatinine excretion (nmol/ mmol)	9.5	(3.9 - 18.9)

6M HCl, dried and reconstituted in 50% acetic acid and injected onto a HPLC system with on-line purification on CC31 cellulose using a Prospekt solid-phase extractor (Separations, The Netherlands). The retained crosslinks were eluted from the CC31 material and chromatographed on-line on a cation exchange column (Whatman Partisil SCX). Eluted crosslinks were detected by a Jasco fluorometer (Model FP-920, Separations, The Netherlands). The Pyd/Dpd HPLC Calibrator (Metra, Palo Alto, CA) was used as the standard. Intra- and interassay coefficients of variation were < 3% and < 5%, respectively, for the Pyd and Dpd measurements. Urinary excretion rates are expressed as nmol/mmol creatinine. Creatinine was measured by the Kodak Ektachem Clinical Chemistry Slide (CREA; Eastman Kodak Company, Rochester, NY, USA).

Statistical analysis

The Pyd/Cr or Dpd/Cr ratios in the sample taken from the 24-hour urinary collection ("day sample") were taken as the gold standard. Results are expressed as means with standard deviations. Agreement between each spot sample and the 24-hour collection was expressed as an intra-class correlation (ICC). Pyd/Cr and Dpd/Cr were analyzed separately. A full repeated measurement ANOVA model quantified the variance associated with differences between individuals, between sampling days (day 1 or 2; df = 1), between sampling time each day (8 a.m., 4 p.m. or 24-hour collection; df = 2), as well as the interaction between day and sampling time (df = 2) with correction according to Bonferroni-Dunn for repeated testing. Post-hoc contrasts between each spot and 24-hour sample, and between the two samples were calculated.

Results

Data from 3 patients was excluded because the urinary collection was not accurate (see Patients and Methods). Data from 3 other patients was excluded because Pyd/Cr measurements were not available for all 6 points in time. Therefore, data from 11 RA patients was analyzed; 7 women and 4 men. Their mean age was 61 years (range 42-75). They were of normal stature and weight (mean body mass index 26 kg/m^2), the mean disease duration was 8 years (median 4) and the ESR was 54 mm/hr (median 65). Three patients were on oral corticosteroids (maximum 12.5 mg prednisone/day). Excretion of Pyd and Dpd was increased both in absolute terms and when expressed as the ratio between crosslinks and creatinine (Table I). The Pyd/Dpd ratio was similar in the spot and 24-hour samples (mean 5.9, median 5.4).

In the full ANOVA model, sampling time was a significant factor: Pyd/Cr (mean ± SD) was 71 nmol/mmol ± 45 in samples taken at 8 a.m., 55 ± 35 in samples taken at 4 p.m., and 57 ± 33 in the 24-hour collections (P = 0.01). There was no evidence for an effect of the measurement day (day 1 or day 2; P = 0.93), or for an interaction between the day and sampling time (P = 0.72). The same applied to Dpd/Cr: 14.6 ± 7.5 in samples taken at 8 a.m., 10.9 ± 5.9 in samples taken at 4 p.m., and 11.5 ± 5.6 in the 24-hour collections (P = 0.02). Here again there was no evidence for an effect of the measurement day (P = 0.93) or an interaction between the day and sampling time (P = 0.26).

Post-hoc contrasts revealed significant differences between morning and 24-hour samples (Pyd/Cr; P = 0.02, Dpd/Cr; P = 0.03), and between morning and afternoon samples (Pyd/Cr; P = 0.007, Dpd/Cr; P = 0.012), but not between afternoon and 24-hour samples (Pyd/Cr; P = 0.69, Dpd/Cr; P = 0.67). Agreement analysis confirmed these results: Pyd/Cr in samples taken at 4 p.m. agreed well with values from the 24-hour collection samples; the mean difference with the day sample ratio was 14%, with an intra-class correlation of 0.96. For Dpd/Cr the mean difference was 16%, intra-class correlation 0.93. Samples taken at 8 a.m. agreed less well: for Pyd/Cr the mean difference with the day sample ratio was 34% and the intra-class correlation was 0.81; for Dpd/Cr the mean difference was 37% and the intra-class correlation was 0.79. Figure 1 shows the 4 p.m. samples close to the line of identity with the day samples (slight underestimation); this contrasts with the overestimation in the 8 a.m. samples.

Discussion

In rheumatoid arthritis patients with active disease, we observed a circadian rhythm in the levels of crosslink excretion. Morning sample values reflected elevated excretion during the night, as has already been observed in individuals without RA. Therefore morning samples may overestimate 24-hour excretion, whereas afternoon samples are more accurate. Day-to-day variation is small.

Previous studies on circadian rhythms in crosslink excretion were performed in healthy volunteers using 24-hour collection or morning samples taken before breakfast as the gold standard. Consistently, none of these studies found Pyd/Cr and Dpd/Cr ratios higher than 40 and 8 nmol/mmol, respectively (6,7). The hospitalized arthritis patients in this study had modestly raised levels of Pyd and Dpd excretion. The specific

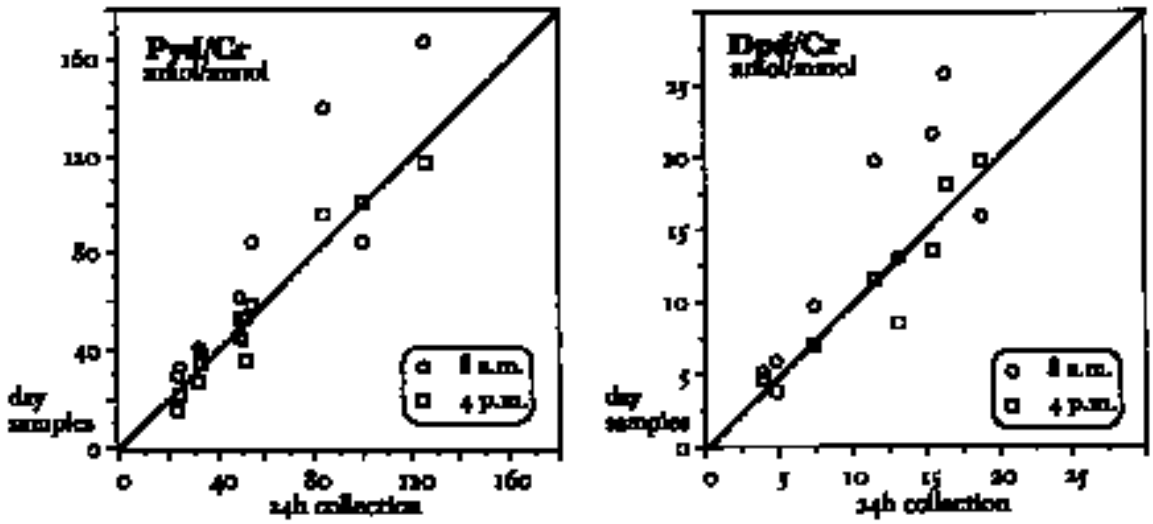

Fig. 1. Pyridinoline to creatinine ratios and deoxypyridinoline to creatinine ratios in 24-hour urinary collections compared to ratios measured in spot samples taken at 8 a.m. and 4 p.m. All results represent the mean of 2 samples per patient taken on 2 consecutive days.

laboratory methods used may have influenced our results, but the similar Pyd/Dpd ratios in the spot samples and the 24-hour excretion samples suggest the reliability of the measurement techniques used. Differences in urinary frequency and subsequently in the amount of urine in the bladder at the moments of sampling may have increased the variance. We applied correction for this by calculating the ratios of creatinine excretion but in the experimental setting the timed sampling of portions is preferred (with fasting before the morning samples). This sampling schedule is hardly feasible in the routine follow-up of patients, and as our findings were meant to be generalized to the follow-up of ambulant as well as hospitalized patients, we chose a relatively simple sampling method. Despite the small number of patients studied, significant differences were revealed.

In conclusion, the collection of spot samples for the assessment of pyridinoline excretion is best performed in the afternoon (4 p.m.), when accurate approximation of the average daily excretion rate is the goal. In longitudinal studies, at the very least the sampling time should be fixed.

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