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ORIGINAL ARTICLE

Serum Pyridinoline is Associated With Radiographic Joint Erosions in Rheumatoid Arthritis

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ABSTRACT

Objectives: This study aims to compare the serum pyridinoline (Pyd) levels between rheumatoid arthritis (RA) patients and healthy controls and to determine the correlation of serum Pyd levels with radiographic joint erosions.

Patients and methods: Serum samples were obtained from 48 patients with RA (9 males, 39 females; mean age 60.5 years; range 54 to 64 years) and 48 healthy controls (9 males, 39 females; mean age 57.5 years; range, 47 to 65 years). The enzyme-linked immunosorbent assay method was used for quantitative analysis of serum Pyd. Besides, all RA patients were assessed for joint damage based on modified Sharp score, disease activity based on disease activity score in 28 joints and functional capacity based on health assessment questionnaire-disability index.

Results: The median serum Pyd levels were significantly higher among the RA patients (110.20 ng/mL [92.30-120.64]) compared to the controls (98.22 ng/mL [85.54-111.41]); $p<0.05$. RA patients with erosive disease had significantly higher serum Pyd levels ($p=0.024$). There was a significant positive correlation between serum Pyd levels and joint erosion score ($r=0.285$, $p=0.049$). The serum Pyd levels had no demonstrable association with disease activity or functional capacity. Steroid therapy did not appear to influence the levels of serum Pyd.

Conclusion: Rheumatoid arthritis patients had significantly higher levels of serum Pyd compared to healthy controls. The serum Pyd levels had significant correlation with radiographic joint erosions which reflected disease damage.

Keywords: Disease activity, joint erosion, rheumatoid arthritis, serum pyridinoline.

Rheumatoid arthritis (RA) is a disease with symmetrical polyarthritis that is characterized by soft tissue swelling, periarticular erosions and joint space narrowing.[1] RA is associated with secondary osteoporosis due to systemic bone loss. There are multiple factors that lead to bone loss in RA including systemic inflammation, reduced physical activity and the usage of glucocorticoids.[2]

Multiple pro-inflammatory cytokines have been implicated in the pathogenesis of RA such as interleukins 1, 6, 23 and tumor necrosis factor-alpha. The macrophages tend to activate receptor activator of nuclear factor kappa-B ligand (RANKL) promoting osteoclast differentiation leading to the damage of the bones and cartilages.

Bone loss may occur starting from the very early stages of RA, but need not necessarily correlate with inflammatory parameters.[1] In a prospective clinical study by Kaltenhäuser et al.,[3] multivariate analysis of independent contributions of covariates to progression of joint destruction showed no significant association with clinical variables or acute phase reactants. Till today, there is still a profound lack of biochemical predictors of joint erosions in RA.

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Pyridinoline (Pyd) is a 3-hydroxypyridinium derivative which is an intermolecular cross-link compound of type I and II collagen.[4] It is formed during extracellular maturation of fibrillary collagens and bridges several collagen peptides to stabilize the collagen molecules.[5] It is mainly found in the bones and some are present in other tissues such as cartilage and aorta.[6]

During bone resorption, the cross-linked collagens will be broken down and are released into the circulation and excreted in the urine both in free form (40%) and in peptide-bound form (60%).[6] Since bones have a much higher turnover rate compared to the other tissues, the levels of serum and urine Pyd are mainly derived from the bones.[5] It is a marker of bone resorption based on bone biopsy and radioisotope kinetics studies.[7]

In RA, destruction of bones may contribute to increased levels of Pyd. Theoretically, Pyd levels are expected to correlate well with the severity of joint erosions. Several studies have pointed out that urine Pyd levels were markedly elevated in RA patients compared to healthy controls.[8-10] In a double-blinded, randomized study by Garnero et al.,[11] increased baseline levels of urinary Pyd were associated with a higher risk of progression of joint damage over one year, independent of baseline joint damage, treatment or disease activity.

There are many RA studies in the literature on urine Pyd and most of them focused more on disease activity rather than the degree of joint damage.[12-14] There is still a paucity of data on serum Pyd in RA and its association with radiographic joint erosions. Hence, in this study, we aimed to compare the serum Pyd levels between RA patients and healthy controls and to determine the correlation of serum Pyd levels with radiographic joint erosions.

PATIENTS AND METHODS

This was a monocentric, cross sectional, case-control study which was conducted between June 2016 and February 2017 at Department of Internal Medicine, Universiti Kebangsaan Malaysia. A total of 48 patients with RA (9 males, 39 females; mean age 60.5 years; range 54 to 64 years) were recruited from the Rheumatology Clinic of UKMMC. *The inclusion criteria for the RA patients were: (i)* patients with confirmed RA based on the American College of Rheumatology 2010 criteria;[15] *(ii)* patients aged 18 years and above; and *(iii)* patients who were able to provide written or verbal consent. *The exclusion criteria were: (i)* pregnant patients; *(ii)* patients with underlying renal impairment; *(iii)* patients who underwent parathyroidectomy; *(iv)* patients on antiresorptive agents such as bisphosphonates and denosumab; and *(v)* patients with malignancy. The 48 control subjects (9 males, 39 females; mean age 57.5 years; range, 47 to 65 years) were age- and sex-matched healthy individuals and the above mentioned exclusion criteria were applied to the controls. Individuals with poor command of English or Bahasa Malaysia were briefed with the assistance of an interpreter. The study protocol was approved by the Universiti Kebangsaan Malaysia Ethics Committee (Study Code: IP-2014-053). A written informed consent was obtained from each participant. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Sample size was calculated by using the Power Sample Size software (NCSS Statistical Software, Utah, USA) and based on values from a similar study by Kim et al.[16] The calculated number of patients needed in each arm was 43. To provide a slight margin of error given the possibility of 10% drop out, a total of 48 subjects were recruited for each arm. The power of study was set at 80% with a 5% level of significance.

The demographic data such as age, sex, race, body mass index (BMI) and menopausal status (for females) were recorded. Data on disease duration, seropositivity and medications for the RA subjects were collected by reviewing the medical records. RA subjects who tested positive for either rheumatoid factor and or anti-citrullinated cyclic peptide were labelled as having seropositive disease. All subjects were tested for serum Pyd levels. Besides, subjects with RA were assessed for their disease activity based on the Disease Activity Score in 28 joints (DAS28) and interviewed to determine the health assessment questionnaire-disability index (HAQ-DI) scores by a single interviewer. Their hand radiographs were scored using the modified Sharp score (MSS) by a single radiologist who was blinded to the subjects.

Disease Activity Score in 28 joints is a quantitative measure of disease activity in RA. It has a validated formula which is used worldwide particularly in clinical trials.[17] The four parameters used to calculate DAS28 are a 28 swollen joint count (range 0-28), a 28 tender joint count (range 0-28), C-reactive protein or erythrocyte sedimentation rate (ESR), and the patient's global assessment (range 0-100).[18] We used the ESR to calculate DAS28 in all subjects to be more uniform. The disease activity can be divided into four main categories as high disease activity (a value of more than 5.1), moderate disease activity (between 3.2 and 5.1), low disease activity (between 2.6 and below 3.2) and remission (less than 2.6). Patients with moderate to high disease activity are considered to have active disease.[17]

The radiographic scoring system in RA has been established and modified since 1971.[19] The most popular scoring method used in numerous landmark trials was the van der Heijde modification of the Sharp scoring system.[20] The MSS looks at the joint erosions and joint space narrowing in the hands, wrists and feet. In this study, the feet were excluded. The joints were examined for erosions at 16 sites with scores of 0 (normal) to 5 (complete collapse of joint). There were 15 sites in each hand and wrist which were examined for joint space narrowing on a scale of 0 (no narrowing) to 4 (complete loss of joint space). The maximum total erosion and narrowing/subluxation scores of the hands are 160 and 120 units, respectively.[20]

The HAQ-DI is a validated and patient-orientated assessment tool to determine the functional ability of RA patients. The assessment includes questions on fine movements of the upper extremity, locomotor activities of the lower extremity, and activities that involve both upper and lower extremities.[21] There are 20 questions in eight categories that represent a comprehensive set of functional activities including dressing, rising, eating, walking, hygiene, reach, grip, and usual activities. The patient's responses are scored from 0 (no disability) to 3 (completely disabled).[22]

Approximately 3-5 mL morning blood samples were collected from the subjects and the serum concentration of Pyd was measured using an enzyme-linked immunosorbent assay. The kit (Elabscience, Wuhan, China) was pre-coated with antibody specific to Pyd.[23] The samples were stored at 4°C and then centrifuged at approximately 3000 rpm for 15 minutes. Trained laboratory technicians were appointed from the Department of Medical Microbiology and Immunology to perform the test. The medical laboratory technologists were blinded to the cases. The value of serum Pyd was measured in ng/mL.

Table 1. Sociodemographic data of study subjects

	Patients (n=48)				Controls (n=48)				
	n	%	Median	Range	n	%	Median	Range	*p*
Age (year)*			60.50	54.00-64.00			57.50	47.00-64.75	0.460
Menopausal age (year)*			50.00	48.00-53.00			50.00	49.00-53.50	0.593
Body mass index (kg/m^2)*			25.00	21.63-29.68			25.67	22.41-28.09	0.657
Sex									
Male	9	18.8			9	18.8			
Female	39	81.3			39	81.3			
Race									0.531
Malay	28	58.3			31	64.6			
Chinese	9	18.8			10	20.8			
Indian	10	20.8			5	10.4			
Others	1	2.1			2	4.2			
Postmenopause	35	87.5			28	70.0			0.160
Serum pyridinoline (ng/mL)*			110.20	92.30-120.64			98.22	85.54-111.41	**0.023**

* Analysis was performed with Mann-Whitney U test.

Statistical analysis

All data were analyzed using the IBM SPSS version 23.0 software (IBM Corp., Armonk, NY, USA). The continuous variables were tested for normality using Kolmogorov-Smirnov test. As all the variables were not normally distributed, the data were analyzed using the Mann-Whitney U test and expressed as median (range). The categorical variables were analyzed using the Fisher's exact test. The strength of the above association was identified using the Spearman's rank-order correlation test. A p value of less than 0.05 was considered significant. A receiver operating characteristic (ROC) curve analysis was performed to determine the sensitivity and specificity of serum Pyd at different cut-off values.

RESULTS

The RA patients and the controls were matched in terms of age, sex, ethnicity and BMI. Although the proportion of post-menopausal females was higher in the RA group (87.5%), the difference did not reach statistical significance. The sociodemographic characteristics of the study subjects were shown in Table 1.

The median disease duration was 7.00 years (range, 3.25-16.00 years) and a majority of the patients were seropositive (84%). A significant proportion (62.5%) of the patients had active disease (moderate to severe disease activity). Erosive disease was present in 26 patients (54.2%) with median score of 2.00 (range, 0-10.00) while joint space narrowing (JSN) was detected in 40 patients (83.3%) with a median score of 33.00 (range, 6.00-54.75).

In terms of functional ability, 16 patients (33.3%) had significant disability with a HAQ-DI of ≥1. More than half of the patients were on double or triple therapy of disease-modifying antirheumatic drugs (DMARDs) (53.2%). The conventional DMARDs received by the subjects included methotrexate, sulfasalazine, leflunomide and hydroxychloroquine. Four patients (8.5%) were on biologic therapy (etanercept, adalimumab

Table 2. Clinical characteristics of rheumatoid arthritis patients

Parameters	n	%	Median	Range
Duration of Illness (year)			7.00	3.25-16.00
Seropositive rheumatoid arthritis#	42	84.0		
Rheumatoid factor positive	35	72.9		
Anti citrullinated cyclic peptide positive	40	83.3		
Erythrocyte sedimentation rate (IU/mL)			49.00	29.00-69.00
C-reactive protein (mg/dL)			0.70	0.05-9.83
Visual analog scale			30.00	20.00-40.00
Swollen joints			2.00	0-4.00
Tender joints			0	0-0.75
Disease Activity Score in 28 joints			3.50	2.94-4.22
≥3.2	30	62.5		
<3.2	18	37.5		
Erosive disease	26	54.2		
Median erosion score			2.00	0-10.00
Median joint space narrowing score			33.00	6.00-54.75
Total modified Sharp score				
Health Assessment Questionnaire-Disability Index			0.63	0.41-1.13
>1	16	33.3		
Medications				
Monotherapy	18	38.3		
Double/triple therapy	25	53.2		
Biologics	4	8.5		
Prednisolone	17	35.4		

Sum of rheumatoid factor and anti-citrullinated cyclic peptide positive was higher than total number of rheumatoid arthritis subjects as there were subjects who tested positive for both antibodies.

Table 3. Serum pyridinoline levels in various subcategories of rheumatoid arthritis patients

		Pyridinoline		
	n	Median	Range	*p*
Disease Activity Score in 28 joints				
Remission to mild disease activity	18	109.51	85.84-119.84	0.886
Moderate to high disease activity	30	110.20	93.79-122.15	
Non erosive disease	22	106.78	78.55-115.67	**0.024**
Erosive disease	26	110.20	102.98-126.86	
Health Assessment Questionnaire-Disability Index				
<1	32	110.91	92.29-124.38	0.246
≥1	16	105.93	85.64-112.89	
Menopause				
Pre-menopause	4	76.60	64.95-129.86	**0.043**
Post-menopause	35	110.81	96.21-123.40	
On prednisolone	17	111.01	101.65-124.66	0.336
Not on prednisolone	31	106.48	86.85-118.66	

and tocilizumab) and 17 (35.4%) were on steroid therapy. All results were summarized in Table 2.

The median serum Pyd levels were higher in the RA group (110.20 [92.30-120.64] ng/mL) compared to the control group (98.22 [85.54-111.41] ng/mL), with a statistically significant difference (p=0.023) (Table 1). In both groups, postmenopausal subjects had higher serum Pyd levels, although a statistical significance was only reached in the RA group (p=0.043). Among RA patients with erosive disease, the median serum Pyd levels did not differ significantly between the elderly RA patients (108.20 [104.67-138.40] ng/mL) and the young RA patients (108.99 [64.30-148.16] ng/mL). Steroid therapy (p=0.336) did not significantly influence the levels of serum Pyd (Table 3).

Table 4 summarizes the correlation analysis. There was a significant positive correlation between serum Pyd level and the erosion score (p=0.049). On linear regression analysis, serum Pyd level had a significant positive relationship with erosion score (p=0.043). In keeping with the above mentioned finding, the serum Pyd level was significantly higher in patients with erosive disease compared to those with non-erosive disease (p=0.024) (Table 4) despite no significant difference in the proportion of postmenopausal females among patients with erosive disease or non-erosive disease. However, the strength of the relationship between serum Pyd level and erosion score was considered weak as the r value was 0.285. The cut-off serum Pyd level which predicts erosive disease based on the ROC curve analysis was 107.28 ng/mL with a sensitivity of 61.5%

Table 4. Correlation between serum pyridinoline levels and clinical variables

		Serum pyridinoline	
	R	r^2	*p*
Disease Activity Score in 28 joints	- 0.010	7.431	0.945
Joint erosion score	0.285	0.086	**0.049**
Joint space narrowing score	-0.076	7.560	0.605
Total modified Sharp score	-0.009	0.005	0.952
Health Assessment Questionnaire-Disability Index	- 0.186	0.051	0.205
C-reactive protein	-0.013	0.003	0.905
Erythrocyte sedimentation rate	-0.019	0.003	0.932

Test was performed with Spearman's rank correlation test.

and specificity of 62.3% (area under the ROC curve: 0.688, p=0.005, 95% confidence interval of 0.569 to 0.808).

The correlation between serum Pyd levels and JSN as well as total MSS were insignificant (p=0.605 and p=0.952, respectively). There was no significant relationship between serum Pyd levels and disease activity (p=0.886) or functional ability (p=0.246).

DISCUSSION

This study demonstrated a significantly higher level of serum Pyd in RA patients compared to healthy controls (p<0.05). This finding was consistent with a previous study in 2003 by Müller et al.,[24] which showed that Pyd levels were more elevated in RA patients compared to not only normal population but also osteoarthritis and psoriatic arthritis patients. Along these lines, there were many studies in the past that have pointed out that urinary excretion of Pyd was increased in arthritis patients.[8,25] The use of serum Pyd as an investigational tool in RA is certainly less cumbersome than urine Pyd. Urine samples for urine Pyd ideally have to be second-void fasting urine given between 8 to 10 am.[26,27]

The novel finding of this study was the significant positive correlation between serum Pyd level and joint erosion score (r=0.285, p=0.049). Our RA subjects with erosive disease had significantly higher levels of serum Pyd than those without erosions (p=0.024). Higher levels of Pyd indicate higher degree of bone destruction. Krabben et al.[28] reported similar findings. Moreover, Gineyts et al.[29] found that urinary Pyd level was elevated in RA patients with erosive disease and the levels correlated with the number of affected joints. Bone resorption in the periarticular bone gives rise to joint erosions in RA. Joint erosions in RA are due to increased osteoclastic activity when the ratio between osteoprotegerin and RANKL is decreased resulting in collagen fragments in the form of free Pyd getting released into the blood circulation.[30,31] High baseline levels of urine Pyd were associated with increased risk of progression of joint destruction over one year in early RA.[11]

We found no significant relationship between the level of serum Pyd and disease activity (p=0.945). Kaufmann et al.[12] in 2003 had parallel findings. They found that unlike serum Pyd, urine and synovial fluid Pyd levels correlated with disease activity in RA. Many studies have highlighted that urine Pyd levels were related to disease activity.[13,14,32] Hence, urine Pyd levels may not necessarily correspond to the serum levels, probably owing to metabolic or elimination processes.

We also demonstrated that the level of serum Pyd was higher in postmenopausal females compared to premenopausal females in both study groups. However, the results reached statistical significance only in the RA group (p=0.043). Higher levels in postmenopausal females are expected as Pyd is a marker of bone resorption. There is accelerated osteoclastic activity after menopause due to the lack of estrogen.[33] Hassager et al.[34] in 1992 showed that the level of urine Pyd started to increase six months after the last menstrual period and its changes were related to the hormonal levels.

The degree of functional disability among RA patients is a direct consequence of both disease activity and structural joint damage.[35] In most patients, the declining functional capacity is associated with disease activity in early RA and mainly affected by joint damage in the later course of the disease.[36] This study showed no significant correlation (p=0.205) between the level of Pyd and HAQ-DI. This corresponds with the study by Gough et al.,[37] which showed no significant relationship between urine Pyd and functional ability. However, HAQ-DI is a subjective method of assessment of functional capacity as it relies upon responses provided by the subjects rather than the physicians' objective assessment.

This study has several drawbacks. The sample size was relatively small for both the RA patients and the healthy controls, since we were unable to recruit more patients due to budget constraints. Furthermore, the scoring of JSN, joint erosions and total MSS were only calculated based on radiographs of both hands and not involving the feet. The full total MSS is a combination score between hands and feet.[38] Although radiographs provide optimal documentation of joint destruction, ultrasound is a more sensitive tool in this regard, which may detect early erosions.[38,39] Ideally, each subject should have

been tested for Pyd levels on two-three separate occasions as the level might fluctuate with time or during the day. The completion of the HAQ-DI questionnaire was interviewer-assisted. Self-completed questionnaires are less prone to investigators' bias.

In conclusion, this study highlights that serum Pyd is a useful biomarker of joint erosions in RA. It appears to correlate well with disease damage but not with disease activity or functional capacity of the patients. Serum Pyd is a potential clinical tool to prognosticate patients which may influence the choice of DMARDs therapy. The rheumatologist may consider advanced therapies early in RA patients with high levels of serum Pyd to prevent joint damage.

Declaration of conflicting interests

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REFERENCES

1. Fardellone P, Séjourné A, Paccou J, Goëb V. Bone remodelling markers in rheumatoid arthritis. Mediators Inflamm 2014;2014:484280.
2. Goldring SR, Gravallese EM. Mechanisms of bone loss in inflammatory arthritis: diagnosis and therapeutic implications. Arthritis Res 2000;2:33-7.
3. Kaltenhäuser S, Wagner U, Schuster E, Wassmuth R, Arnold S, Seidel W, et al. Immunogenetic markers and seropositivity predict radiological progression in early rheumatoid arthritis independent of disease activity. J Rheumatol 2001;28:735-44.
4. Nemoto R, Nakamura I, Nishijima Y, Shiobara K, Shimizu M, Takehara T, et al. Serum pyridinoline crosslinks as markers of tumour-induced bone resorption. Br J Urol 1997;80:274-80.
5. Seibel MJ. Biochemical markers of bone turnover: part I: biochemistry and variability. Clin Biochem Rev 2005;26:97-122.
6. Niwa T, Shiobara K, Hamada T, Miyazaki T, Tsukushi S, Uema K, et al. Serum pyridinolines as specific markers of bone resorption in hemodialyzed patients. Clin Chim Acta 1995;235:33-40.
7. Hata K, Miura M, Fukumoto S, Matsumoto T. Assay of serum pyridinoline: a potential marker for bone resorption. Clin Chim Acta 1995;235:221-7.
8. Robins SP, Stewart P, Astbury C, Bird HA. Measurement of the cross linking compound, pyridinoline, in urine as an index of collagen degradation in joint disease. Ann Rheum Dis 1986;45:969-73.
9. Molenaar ET, Lems WF, Dijkmans BA, de Koning MH, van de Stadt RJ, Voskuyl AE. Levels of markers of bone resorption are moderately increased in patients with inactive rheumatoid arthritis. Rheumatology (Oxford) 2000;39:742-4.
10. Otsuka M, Kurosaka R, Kim JS, Kawai S, Arakawa N. Evaluation of urinary pyridinoline in healthy adults and patients with rheumatoid arthritis by an improved high-performance liquid chromatographic assay. J Nutr Sci Vitaminol (Tokyo) 1996;42:485-90.
11. Garnero P, Gineyts E, Christgau S, Finck B, Delmas PD. Association of baseline levels of urinary glucosyl-galactosyl-pyridinoline and type II collagen C-telopeptide with progression of joint destruction in patients with early rheumatoid arthritis. Arthritis Rheum 2002;46:21-30.
12. Kaufmann J, Mueller A, Voigt A, Carl HD, Gursche A, Zacher J, et al. Hydroxypyridinium collagen crosslinks in serum, urine, synovial fluid and synovial tissue in patients with rheumatoid arthritis compared with osteoarthritis. Rheumatology (Oxford) 2003;42:314-20.
13. Hein G, Franke S, Müller A, Bräunig E, Eidner T, Stein G. The determination of pyridinium crosslinks in urine and serum as a possible marker of cartilage degradation in rheumatoid arthritis. Clin Rheumatol 1997;16:167-72.
14. Ostanek L, Pawlik A, Brzosko I, Brzosko M, Sterna R, Drozdzik M, et al. The urinary excretion of pyridinoline and deoxypyridinoline during rheumatoid arthritis therapy with infliximab. Clin Rheumatol 2004;23:214-7.
15. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 2010;62:2569-81.
16. Kim HR, Cho ML, Kim KW, Juhn JY, Hwang SY, Yoon CH, et al. Up-regulation of IL-23p19 expression in rheumatoid arthritis synovial fibroblasts by IL-17 through PI3-kinase-, NF-kappaB- and p38 MAPK-dependent signalling pathways. Rheumatology (Oxford) 2007;46:57-64.
17. Fransen J, van Riel PL. The Disease Activity Score and the EULAR response criteria. Rheum Dis Clin North Am 2009;35:745-57.
18. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44-8.

19. Sharp JT, Lidsky MD, Collins LC, Moreland J. Methods of scoring the progression of radiologic changes in rheumatoid arthritis. Correlation of radiologic, clinical and laboratory abnormalities. Arthritis Rheum 1971;14:706-20.
20. Landewé R, van der Heijde D. Radiographic progression in rheumatoid arthritis. Clin Exp Rheumatol 2005;23(5 Suppl 39):S63-8.
21. Bruce B, Fries JF. The Stanford Health Assessment Questionnaire: dimensions and practical applications. Health Qual Life Outcomes 2003;1:20.
22. Bruce B, Fries JF. The Health Assessment Questionnaire (HAQ). Clin Exp Rheumatol 2005;23:14-8.
23. An enzyme immunoassay for the quantitation of Pyridinoline Crosslinks in serum. Microvue Bone Health 2009.
24. Müller A, Jakob K, Hein GE. Evaluation of free and peptide bound collagen crosslink excretion in different skeletal diseases. Ann Rheum Dis 2003;62:65-7.
25. Seibel MJ, Duncan A, Robins SP. Urinary hydroxypyridinium crosslinks provide indices of cartilage and bone involvement in arthritic diseases. J Rheumatol 1989;16:964-70.
26. Husain SM, Mughal Z, Williams G, Ward K, Smith CS, Dutton J, et al. Urinary excretion of pyridinium crosslinks in healthy 4-10 year olds. Arch Dis Child 1999;80:370-3.
27. Shaw NJ, Dutton J, Fraser WD, Smith CS. Urinary pyridinoline and deoxypyridinoline excretion in children. Clin Endocrinol (Oxf) 1995;42:607-12.
28. Krabben A, Knevel R, Huizinga TW, Cavet G, van der Helm-van Mil AH. Serum pyridinoline levels and prediction of severity of joint destruction in rheumatoid arthritis. J Rheumatol 2013;40:1303-6.
29. Gineyts E, Garnero P, Delmas PD. Urinary excretion of glucosyl-galactosyl pyridinoline: a specific biochemical marker of synovium degradation. Rheumatology (Oxford) 2001;40:315-23.
30. Gravallese EM. Bone destruction in arthritis. Ann Rheum Dis 2002;61 Suppl 2:ii84-6.
31. Rosen HN, Rosen CJ, Mulder JE. Bone physiology and biochemical markers of bone turnover. UpToDate 2015.
32. Oelzner P, Müller A, Deschner F, Hüller M, Abendroth K, Hein G, et al. Relationship between disease activity and serum levels of vitamin D metabolites and PTH in rheumatoid arthritis. Calcif Tissue Int 1998;62:193-8.
33. Demontiero O, Vidal C, Duque G. Aging and bone loss: new insights for the clinician. Ther Adv Musculoskelet Dis 2012;4:61-76.
34. 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.
35. Eberhardt KB, Fex E. Functional impairment and disability in early rheumatoid arthritis--development over 5 years. J Rheumatol 1995;22:1037-42.
36. Welsing PM, van Gestel AM, Swinkels HL, Kiemeney LA, van Riel PL. The relationship between disease activity, joint destruction, and functional capacity over the course of rheumatoid arthritis. Arthritis Rheum 2001;44:2009-17.
37. Gough AK, Peel NF, Eastell R, Holder RL, Lilley J, Emery P. Excretion of pyridinium crosslinks correlates with disease activity and appendicular bone loss in early rheumatoid arthritis. Ann Rheum Dis 1994;53:14-7.
38. Sokka T. Radiographic scoring in rheumatoid arthritis: a short introduction to the methods. Bull NYU Hosp Jt Dis 2008;66:166-8.
39. Kang T, Lanni S, Nam J, Emery P, Wakefield RJ. The evolution of ultrasound in rheumatology. Ther Adv Musculoskelet Dis 2012;4:399-411.