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Factors Modifying Collagen Catabolism in Patients with Ankylosing Spondylitis

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Abstract
Objective: We quantified the total excretion of the collagen crosslinks (CL) pyridinoline (PYD) and deoxypyridinoline (DPD) in 108 ankylosing spondylitis (AS) patients (29 f, 79 m) in correlation to different characteristics of disease to evaluate different mechanism contributing to development of osteoporosis in AS.
Methods: PYD and DPD were measured by HPLC.
Results: AS patients show a highly significant positive correlation between PYD and inflammatory activity. In cases involving peripheral joints, significantly higher CL levels in urine were found. Patients with syndesmophytes excreted significantly more CL vs. those without. In the more advanced stages of sacroiliitis (stage III and IV), CL levels tended to be higher. Among those patients treated with NSAIDs, a tendency to decreased levels of DPD and consecutive raised levels of the quotient PYD/DPD were observed.

No significant correlation was found between restricted spine mobility or duration of disease and amount of excreted CL.
Conclusions: Our investigations show that the inflammatory process, the involvement of the peripheral joints, the presence of syndesmophytes and the stage of sacroiliitis all have an influence on the extent of collagen degradation in AS patients. NSAIDs do not increase but appear to reduce collagen I catabolism.

Key words: ankylosing spondylitis, osteoporosis, collagen crosslinks, inflammation, NSAIDs

List of abbreviations: collagen crosslinks = CL, pyridinoline = PYD, deoxypyridinoline = DPD, ankylosing spondylitis = AS, normal controls = NC

Introduction

Ankylosing spondylitis (AS) is a chronic rheumatic inflammatory disease included in the group of spondylarthropathies that primarily affects the sacroiliac joints and spine [1]. The prevalence of this disease is estimated at 0.2-0.8% [2]. AS is characterized by sacroiliitis, spondylitis, sometimes pauciarticular synovitis and occasionally also enthesitis. AS patients mainly suffer from back pain, increasing stiffness and therefore loss of motion. Spine ossifications and syndesmophytes are considered to be a hallmark of the disease, reflecting processes of abnormal bone formation. However, AS is also characterized by reduction of vertebral spongious bone resulting in osteoporosis. There is some evidence that osteopenia/osteoporosis in AS may be mediated by substances regulating both the inflammatory process and bone turnover [3, 4]. In conditions of chronic inflammation, proinflammatory cytokines such as TNF α, IL-1 and IL-6 may enhance the process of bone resorption [4, 5] and reduce osteoblast activity in the remodelling process. Contributory factors could be spine immobility secondary to ankylosis as well as to the substantial pain. The use of glucocorticoids and the deficit in sex hormone secretion [6, 7] may also have a role in resulting osteoporosis. The precise influence of prolonged use of nonsteroidal anti-inflammatory drugs (NSAID) on bone metabolism remains unclear.

Although TNF α blocking substances have been recently introduced with excellent effects in treatment, today the standard therapy for AS in addition to physical therapy remains the use of NSAIDs for controlling the pain and stiffness.

Among the markers of bone metabolism, the pyridinium crosslinks of collagen are particularly useful for measuring bone resorption activity [8]. Pyridinoline (PYD), a trifunctional 3-hydroxypyridinium crosslink and its analogue, deoxypyridinoline (DPD), are two non-reducible collagen crosslinks. PYD occurs in type I and II collagen and, with the exception of skin, sclerae, and cornea, in most connective tissues, i.e. bone and cartilage. DPD originates mainly from bone [9]. The excretion of these crosslinks largely reflects the process of collagen degradation.

The aim of this study was to investigate different factors thought to be able to influence collagen catabolism in patients with AS to quantify the total excretion of the pyridinium crosslinks PYD and DPD in 108 AS patients; and to uncover for possible correlations between these bone degradation markers and factors like disease activity, mobility, grading of sacroiliitis, proof of syndesmophytes, inflammatory involvement of peripheral joints and the use of NSAIDs.

Material and Methods

AS Patients

108 patients (29f., 79 m.) satisfying the modified New York criteria for AS (1) and 82 NC (43 f., 39 m.) were

studied.

No patients were receiving glucocorticoid medication. 21 patients of the 33 in the subgroup with involved peripheral joints were receiving disease-modifying drugs at the time of study (methotrexate in 11 cases and sulfasalazine in 10 cases). The patients were assessed by two rheumatologists for documentation of clinical findings. The study groups were analyzed for grading of sacroiliitis [10] on the basis of pelvic X-ray findings as well as for the absence or presence of syndesmophytes by dorsolumbar X-ray features. Peripheral disease was defined by involvement of clinically significant and/or radiologically visible evidence for arthritis.

Inflammatory activity was assessed by the Westergren erythrocyte sedimentation rate (ESR) and the C-reactive protein (CRP) by laser nephelometry.

Sample Collection and Storage

Fasting second void urine samples were taken in the morning without any predetermined diet and centrifugated (3000 x g) for 10 min, and aliquots for the different assays were stored at -20 °C. All the assays were performed within 12 months of sample collection.

Urinary Assays of the Pyridinium Crosslinks by HPLC

HPLC assays of PYD and DPD were performed using hydrolyzed urinary samples as described previously [11, 12] and expressed as nmol/mmol creatinine. The introduction of the internal standard isodesmosine determined by UV-detection (280 nm) under the same conditions increases the accuracy and the reproducibility of this method. The participation in international trials and the use of pooled urines as controls improves also the inter-assay and intra-assay variation. The detection limit of the assay was 25 fmol for PYD and 56 fmol for DPD. Interassay variation was assessed using pooled urine samples. The mean ± S.D. recovery for urine sample I was 525.7 nmol/l ± 9.5 % for PYD and 124.1 nmol/l ± 16.4% for DPD; for urine sample II 609.9 nmol/l ± 10.4% for PYD and 160.5 nmol/l ± 17.6 % for DPD. The interassay variation with pooled urine samples for PYD was 3.8 % and 2.8 % and for DPD 7.2 % and 5.3 % in urine samples I and II.

Statistical Analysis

The Mann-Whitney U-test for two independent samples, the Kruskal-Wallis H test for several independent samples and the bivariate correlation analysis (Spearman's rho) were used for statistical evaluation performed by SPSS (version 10.0) software. A normal distribution was excluded using the Kolmogorov-Smirnov-test.

Results

Significant differences between AS patients and the NC are listed in Table 1.

In order to determine whether CL excretion was enhanced in AS patients with active inflammatory disease, the relationship between PYD and DPD and the markers of disease activity ESR and CRP was studied.

A positive correlation was found between PYD and CRP ($r = 0.318$, $p = 0.001$) as well as between PYD and ESR ($r = 0.346$, $p < 0.0001$). A tendency for positive correlation could be shown also between DPD and these markers.

Significantly elevated urinary levels of PYD were observed in the group of patients with raised CRP vs. normal or low CRP levels < 10 mg/l (49.5 ± 21.9 vs. 42.4 ± 19.8, $p = 0.042$) and for the quotient PYD/DPD (5.76 ± 2.54 vs. 4.84 ± 1.90, $p = 0.019$) as well as for raised ESR (≥ 30 mm/h) vs. the group with normal or low ESR levels regarding PYD (58.0 ± 23.7 vs. 43.8 ± 19.8, $p = 0.01$).

In addition we examined the different subsets with regard to (non)presence of syndesmophytes, stage of sacroiliitis, and movement function (Schober's Test and finger-to-bottom distance), in relation to the excretion level of pyridinium crosslinks (Table 2).

For the group with syndesmophytes we found higher levels of PYD (57.0 ± 21.6 vs. 44.7 ± 20.8, $p = 0.01$) and DPD (13.0 ± 5.6 vs. 10.4 ± 5.7, $p = 0.026$) compared to those without (Fig. 1). A tendency to higher mean levels of PYD was observed in patients with more pronounced sacroiliitis (stage III and IV). In patients with involved peripheral joints, significantly higher levels of PYD and PYD/DPD were found (PYD, 57.8 ± 23.6 vs. 42.9 ± 18.0, $p = 0.002$; PYD/DPD, 5.9 ± 2.15 vs. 5.0 ± 2.2, $p = 0.015$) vs. without. For patients on NSAID therapy we found

Table. 1. Pyridinoline and Deoxypyridinoline in free and total form for AS and NC.

Diagnosis	PYD[1)]	DPD[1)]	PYD/DPD	PYD free[1)]	DPD free[1)]	PYD/DPD free
AS (n = 108)	46.7 ± 21.0 (12.3-91.0)	10.7 ± 5.5 (2.3-20.0)	4.5 ± 1.7 (1.5-7.9)	19.6 ± 8.5 (5.9-36.2)	7.6 ± 5.7 (1.7-20.1)	3.3 ± 1.7 (0.8-6.2)
NC (n = 82)	32.0 ± 17.0 (11.8-71.4)	8.9 ± 5.1 (2.9-17.3)	4.1 ± 1.9 (2.0-8.4)	15.7 ± 6.4 (8.8-31.3)	6.2 ± 4.8 (1.4-20.3)	3.8 ± 4.0 (1.2-13.0)
p =	0.0001	0.009	0.003	0.001	0.021	0.581

1) [nmol/mmol creatinine]
The values in brackets are the 95 % confidence intervals.

Table 2. Differentiation of the AS patients with regard to collagen crosslinks.

	variable	number	PYD[1)]	DPD[1)]	PYD/DPD
Disease duration	< 5 y. ≥ 5 y.	33 75	48.3 ± 24.5 46.1 ± 19.6	12.2 ± 7.2 10.1 ± 4.6	4.9 ± 2.5 5.5 ± 2.1
ESR (20.2 ± 18.8) 1.5 – 62.0 [2)]	< 30 mm/h ≥ 30 mm/h	85 23	43.8 ± 19.8 58.0 ± 23.7	10.4 ± 5.5 12.4 ± 5.9	5.3 ± 2.4 5.1 ± 1.5
CRP (23.3 ± 30.5) 2.3 - 101 [2)]	< 10 mg/l ≥ 10 mg/l	56 52	42.4 ± 19.8 49.5 ± 21.9	10.5 ± 5.4 11.0 ± 5.9	4.8 ± 1.9 5.8 ± 2.5
Finger-to-bottom distance	< 10 cm ≥ 10 cm	51 57	45.2 ± 20.4 48.1 ± 22.7	11.1 ± 5.6 10.8 ± 5.8	5.2 ± 2.7 5.5 ± 2.0
Schober's Test	< 5 cm ≥ 5 cm	58 50	42.9 ± 19.6 48.6 ± 22.5	9.5 ± 4.7 12.1 ± 6.1	5.3 ± 2.1 5.3 ± 2.6
NSAID intake	yes no	80 28	45.8 ± 21.0 48.1 ± 21.3	10.2 ± 5.5 12.0 ± 5.8	4.8 ± 1.4 6.7 ± 3.4
Sacroiliitis stage	1-2 3-4	40 68	40.7 ± 18.1 49.2 ± 19.7	9.8 ± 5.0 11.1 ± 5.1	4.8 ± 1.6 5.7 ± 2.6
Syndesmophytes	yes no	58 50	57.0 ± 21.6 44.7 ± 20.8	13.0 ± 5.6 10.4 ± 5.7	6.1 ± 3.2 4.7 ± 1.4
Peripheral joint involving	yes no	33 75	57.8 ± 23.6 42.9 ± 18.0	12.0 ± 6.1 10.5 ± 5.4	5.9 ± 2.2 5.0 ± 2.2

[1)][nmol/mmol creatinine]
[2)] mean value and the 95 % confidence intervals
A significance between two groups was determined by the Mann-Whitney U test; significances are reported in the text.

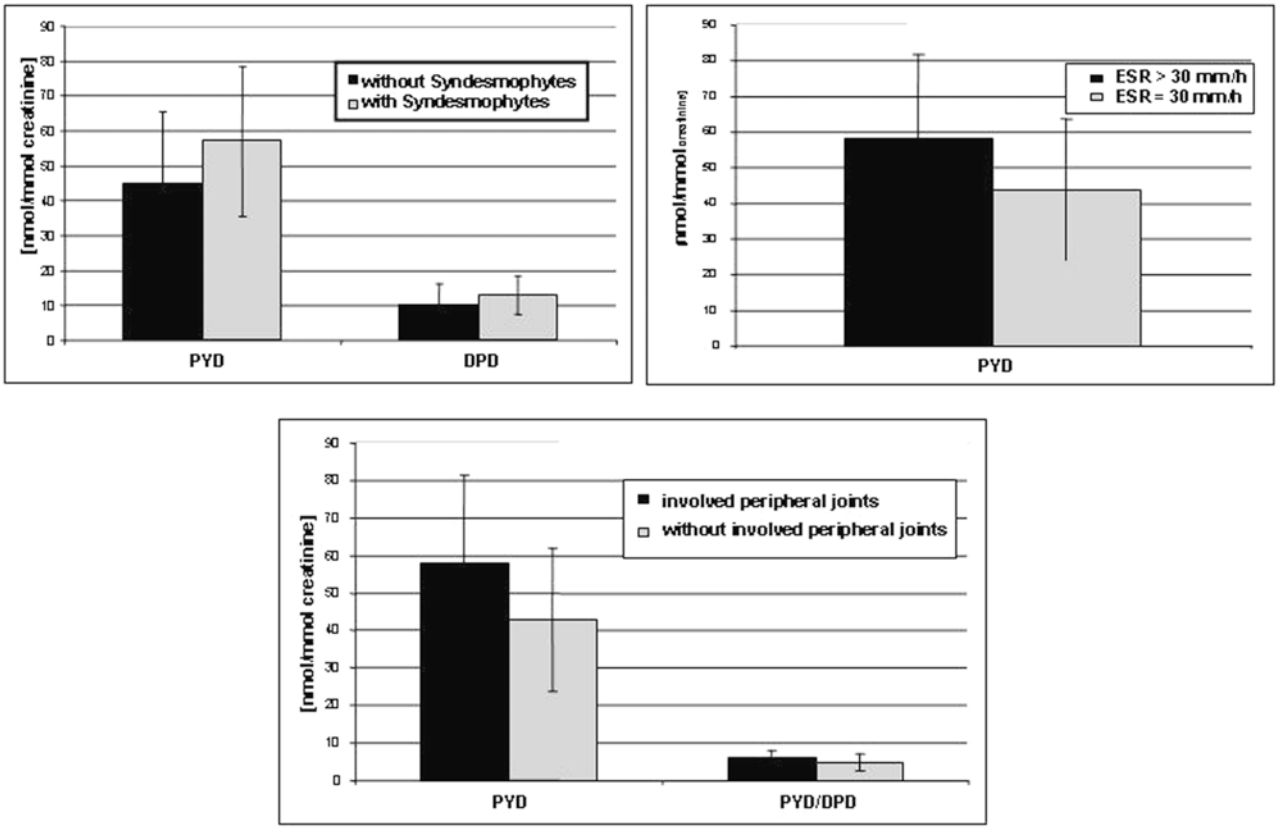

Fig. 1. Significant differences of collagen crosslink levels in relation to some relevant parameters in AS patients.

lower levels of DPD (10.2 ± 5.5 vs. 12.0 ± 5.8, p = 0.207) and higher levels of PYD/DPD (4.8 ± 1.4 vs. 6.7 ± 3.4, p = 0.023) compared to patients without NSAID therapy but the difference was not significant for DPD. In patients with good spine movement (Schober ≥ 5) we could demonstrate significantly higher levels of DPD (p = 0.021) compared to patients with reduced spine mobility. The duration of disease did not show a significant influence on the amount of CL-excretion excluding the quotient PYD/DPD (p = 0.022; r = 0.223).

Discussion

Patients with AS often develop osteoporosis. A number of factors are considered to contribute to the development of AS and its secondary diseases.

Our study was undertaken to determine the urinary levels of the pyridinium crosslinks as markers of collagen degradation (in particular collagen I degradation of bone) in relation to markers of disease activity, different subsets of AS and intake of NSAIDs, and to examine the value of these factors on the process of collagen degradation and on the likelihood of developing osteoporosis.

Recently, a prospective study showed a clear relationship between loss of bone mass and inflammatory activity in early AS [13]. We found a significant difference between the AS group and the NC for all evaluated crosslink compounds. This result is consistent with the results of Marhoffer et al. [14] and contradicts the findings of Toussirot et al. [15]. Other studies have found pyridinium crosslinks levels in the normal range [16, 17]. This may perhaps be explained by differences in, for example, the varying stages of disease, different disease activity, and other clinical features.

To the best of our knowledge, our results regarding different factors and their influence on collagen catabolism in AS patients, including effects of NSAID therapy on levels of PYD and DPD, are the first to be published.

The most important cause of accelerated collagen degradation in AS seems to be the inflammatory process. The higher the inflammatory activity as measured by ESR and CRP, the higher the urine levels of collagen crosslinks. This is evident especially in particular for PYD, a crosslink molecule for collagen I as well as collagen II.

DPD is more specific for collagen I degradation (primarily originating from bone), but bone collagen contains much more PYD. We assume that inflammatory factors substantially accelerate bone catabolism. The slight increase of the PYD/DPD ratio may also hint at an increased breakdown of cartilage under these conditions.

The function of movement (Schober's test and finger-to-bottom distance) and levels of excreted pyridinium crosslinks showed no significant negative correlations.

The higher levels of PYD and DPD for the group with syndesmophytes suggest that the disease in these patients is characterized by more pronounced remodelling activity resulting in a new apposition of bone (syndesmophytes) as well as in significantly heightened bone resorptive processes especially of the trabecular bone.

The slightly lower DPD levels in patients treated with NSAIDs are remarkable, even when considering that inflammation and pain are the reasons for this therapy. Our observations do not support the suggestion that NSAIDs are a cause for consequent development of osteoporosis as assumed by Will et al. [3]. NSAID intake decreases the levels of prostaglandins resulting not only in reduction of pain and increased mobility of AS patients [18] but also in reduced osteoclast formation and activation [19]. Thereby the risk of osteopenia/osteoporosis seems not to be increased with NSAID therapy.

In contrast: It may be suggested that early intervention with anti-inflammatory therapy is able to minimize the inflammation process and should be highly beneficial against osteoporosis, and possibly also against development of syndesmophytes (downregulation of remodelling and modelling processes). Further on a strategy of continuous use of NSAIDs reduces radiographic progression in symptomatic patients with AS, without increasing toxicity substantially [20].

That suppression of inflammation results in an improvement of osteopenia/osteoporosis in AS has also been shown by the influence of TNF α blockers on BMD in AS patients. The powerful inhibition of inflammation by these substances was followed by a significant increase of BMD both at the spine and total hip after 6 months [21].

In conclusion, the results show that there are different mechanisms contributing to collagen catabolism and resulting in osteoporosis in AS. The most significant factor appears to be chronic inflammation. Therapy against inflammation has a prophylactic effect on the development of these complications.

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