Antibodies to human tissue transglutaminase and alterations of vitamin D metabolism in ankylosing spondylitis and psoriatic arthritis

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Objectives: Both in Ankylosing Spondylitis (ASP) and Psoriatic Arthritis (PsA), osteopenia is present in one-third of women and men, whereas osteoporosis mainly affects men, even in their 30s. Subclinical gut inflammation has been described in patients with AS or PsA. Joint involvement also occurs with other gastrointestinal diseases such as celiac disease. We tested the hypothesis, whether elevated serum levels of human anti-tissue-Transglutaminase-IgA (htTG) are associated with changes in disease activity, vitamin D metabolism and bone mineral density (BMD) in patients with ASP and PsA..

Methods: In a cross-sectional study, we evaluated both biochemical markers of bone turnover, BMD and htTG in 76 patients with ASP and 120 patients with PsA..

Results: A reduction of BMD in lumbar spine was determined both in ASP (42.7%) and in PsA (57.3%). Furthermore, a significantly higher prevalence of htTG was detected only in ASP (14/76). ASP-patients with negative htTG status have significant higher 25-vitamin D3 levels. ASP-patients with positive htTG status are younger.

Conclusion: A positive htTG status entails the risk of a bad vitamin D supply, which should be considered in young patients since this constellation favours a reduction in bone density. The coincidence of ASP along with detection of htTG and clinically asymptomatic celiac disease as an accessory source of inflammation with a negative influence on the bone metabolism is also conceivable. Clinicians need to be aware of patients younger than 45 years with ASP, which have important implications for the correct treatment and vitamin D supplementation.

Key words: anti-human-tissue- Transglutaminase IgA, vitamin D metabolites, Ankylosing Spondylitis, Psoriatric Arthritis

INTRODUCTION

Patients with both Ankylosing Spondylitis (ASP) and Psoriatric Arthritis (PsA) have local and systemic bone loss, and may be at an increased risk of suffering from bone fractures [1 – 4]. Various factors such as treatment, hormone disorders, and decreased mobility or physical activity may contribute to development of osteopenia (osteoporosis in ASP and PSA [5, 6]. A prospective study demonstrated a significant loss of bone mass in early ASP with strong association with inflammation activity [7]. Celiac disease is a gluten sensitive enteropathy charactericed by a wide spectrum of clinical manifestations, such as diarrhoea, weight loss, anaemia and herpetiform dermatitis. Most of these are related to malabsorption which may be limited to one or a few nutrients up to a complete malabsorptive syndrome. However, joint involvement e.g. in sacroiliac joints has also been reported related to celiac disease [8].

In this cross sectional study we tested the hypothesis, weather positive htTG status is associated with alterations of vitamin D metabolism, osteoporosis/osteopenia, marker of inflammation, age of patients and HLA B 27 marker in a large and well-characterized cohort of patients with ASP or PsA.

PATIENTS AND METHODS

Seventy six patients with ASP as defined by the New York clinical criteria [9] (age range 19 – 58 years, mean 49.5 years, duration of disease 1 – 34 years, mean 19.3 years) and 116 patients with PsA as defined by the Classification of Psoriatic Arthritis – CASPAR [10] (age range 22 – 61 years, mean 50.7 years, duration of disease 1 – 24 years, mean 17.1 years) were studied from March to November 2005. All the women were premenopausal with a physiologic sexual hormone status. A selective IgA-deficiency was excluded. Serum creatinine levels were normal in the entire study population. Criteria for exclusion were the presence of intestinal disease (terminal ileitis, ulcerative colitis), malnutrition, marasmus and drug intake known to affect bone metabolism and the endocrine system. All study persons had been physically active twice a week during the preceding 12 month.

A medical history was obtained, 61 of 76 ASP patients and 107 of 116 PsA patients received intermittent nonsteroidal antirheumatic (NSAID) therapy in the previous 12 months. No patient was receiving glucocorticoid medication at the time of the study, only 7 patients had received such medication for a short time in the past (10 mg /day for 2 – 3 months during the disease duration) 32 PsA patients got a disease modifying medication at the time of the study (methotrexate 15 mg weekly in 24 cases and sulfasalazine in 8 cases).

Disease status in terms of disease activity, disease progression and prognosis, is difficult to define in ASP and PsA. We used C-reactive protein, and the Westergren erythrocyte sedimentation rate (ESR).

BIOCHEMICAL MEASUREMENTS

Blood examples were taken from all participants every morning at the same fixed time. The collected serum samples were immediately stored in aliquots at -20°C in our serum bank before testing. Informed consent was obtained before blood sampling. The specific serum parameters of this study were calcitriol ("1,25 (OH)2 Vitamin D"-kit from Biermann Bad Nauheim, Germany); and calcefediol ("25 (OH) Vitamin D"-kit from Biermann Bad Nauheim, Germany)mun Diagnostik, Bensheim, Germany; competitive protein-binding-assay). The Microtiter ELISA was intended for the determination of human anti-tissue-Transglutaminase-IgA (htTG) antibodies in serum (Euroimmun Medizinische Labordiagnostik AG, Lübeck, Germany). (Expected value: htTG IgA Serum cut off > 22 AU/ml positive. htTG IgA .Serum cut off < 16 AU/ml negative. Results between 16 - 22 AU/ml are in the grey range). C reactive protein (CRP; normal range: < 5 ng/l, immunonephelometry using Behring Nephelometer Systems, N Latex CRP mono, Behring Diagnostics, Marburg, Germany).

ASSESSMENT OF BONE MINERAL DENSITY

BMD was measured at the lumbar spine and the left femoral neck by dual-energy X-ray absorptiometry (DEXA) using Prodigny Lunar (Milwaukee, WI). The coefficient of variation of repeated measurements was 0.9% for the lumbar spine and 1.6% for the femoral neck. Osteopenia (T-score between – 1.0 and – 2.5) and osteoporosis (T-score below – 2.5) were defined according to the criteria of the World Health Organization.

STATISTICAL ANALYSIS

Results are presented by mean values and standard deviation. The following methods were applied for statistical analysis: a single factor variance analysis, the exact Fisher's –Test and the Mann-Whitney-U-Test. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows [11].

RESULTS

In comparison with the PsA group (n = 4), ASP patients (n = 14) showed significantly higher prevalence of htTG antibodies in serum. In 18 of 196 (9.81) patients we found positive htTG antibodies in serum (Table 1). However, we found no statistically significant association of positive htTG antibodies with the biochemical markers of inflammation: CRP and ESR (Table 2). Measurement of BMD of ASP and PsA patients revealed osteoporose in 35/76 patients (42.7%) and 47/120 (57.3%). No associations were found between ASP or PsA and osteoporose (p value of exact Fisher's Test p= 0.374) or positive htTG antibodies in ASP or PsA and osteoporose (p value of exact Fisher's Test p= 0.130). Finally, in ASP patients HLA B 27 marker is not associated with positive htTG antibodies.

Serum levels of both 25 (OH) D3 and 1,25 (OH)2 D3 in the combined cohort of female and male patients with PsA were higher compared with the ASP group. (Table3). The 25 (OH) D3 levels in PsA were significant highered compared to those of ASP ($p < 0.0005$). Pooled cohort of patients both with ASP and PsA showed a statistically significant negative correlation between CRP and 25(OH) D3 levels ($p < 0.0005$); (Table 4).

In patients with positive htTG antibodies the mean of 25 (OH) D3 levels was 17.4 ng/ml whereas patients with negative htTG antibodies presented 41.5 ng/ml. (Table 5). This difference was statistically significant ($p < 0.005$). There was a similar trend in 1,25 (OH)2 Vitamin D3 but without any statistical significance ($p = 0.274$).

Patients with positive htTG antibodies were younger than those of a negative antibody titre. More than 61 % of the ASP and PsA patients with positive htTG antibodies were younger than 45 years (Table 6).

DISCUSSION

The aim of our study was to find unidentified or neglected cases of positive htTG antibodies by using serological screening in a large and well-characterized cohort of patients with ASP or PsA. In 14 / 76 ASP patients, positive htTG antibodies in serum were detected. In the consecutive endoscopy none of these patients presents the typical villous atrophy with crypt hyperplasia in small bowel biopsy specimen, which confirmed the diagnosis of celiac disease according to Marsh criteria [12]. Therefore we had to discuss possibly the elevated prevalence of positive htTG antibodies in ASP and PsA patients as a part of an autoimmune overlap syndrome.

There are conflicting data about autoimmune disorders and both htTG antibodies and celiac disease. Kallikorm et al. found an association of seronegative spondylarthropathy with celiac disease in one patient out of 74 [13] Altogether, 74 patients (28 females and 46 males, from15 to 72 years of age, mean age 40.4 + 1.6 years) with seronegative spondylarthropathy were examined. Of these, 23 patients had reactive arthritis, 12 Reiter's syndrome, 11 PsA, 18 ASP and 10 undifferentiated spondylarthropathy. From this reason our results may differ, too. The study of Riente et al. failed to show an increased prevalence of autoantibodies associated with celiac disease in patients with ASP or PsA [14]. The prevalence of previously undiagnosed celiac disease, tested serologically with endomysial antibody, in rheumatoid arthritis is 0 in 160 patients [15].

Given the conflicting results of our study and Riente's and Kallikorm's group, the estimation of htTG antibodies in serum of patients with seronegative spondylarthropathy including ASP or PsA differed possibly by the used ELISA or other laboratory methods. In Kallikorm's study IgA-type endomysium-antibodies were measured by an immunfixation method, using cryostat sections from human umbilical cord as the antigen substrate [13]. The available ELISA based on the commercial guinea pig tTG antigen may possibly detect antibodies that are non specific for celiac disease, and hence a test based on pure human tTG may yield increased specificity.

What were the clinical consequences of positive htTG antibodies in patients with ASP or PsA? We were able to present both a serological observation and a functional link between positive htTG antibodies and alterations of the vitamin D metabolism. Our ASP patients have been shown expressed both a higher prevalence of positive htTG antibodies and lowered levels of 25 (OH) D3. Compared to recent studies, the prevalence of osteopenia and osteoporosis was highest in newly diagnosed celiac patients and in patients with disease not in remission. A low 25 (OH)D3 vitamin concentration was a typical biochemical abnormality in celiac patients (64% of men and 71% of women) [16].

Vitamin D metabolites have important functions in regulating inflammatory processes and act on both types of bone cells: osteoblasts and osteoclasts. Furthermore, decreased levels of 1,25 (OH)2 Vitamin D3 in ASP patients may induce a reduction in intestinal calcium absorption and periods of disease activity may contribute to a further acceleration of a pre-existing negative calcium balance. Lowered levels of 1,25 (OH)2 Vitamin D3 as an endogeneous immune modulator suppressing activated T cells and the proliferation of cells may accelerate the inflammatory process. [17]. However, 25 (OH) D3 have been shown to be within normal range in ASP patients in the Lange study [17]. The additional positive htTG antibodies in ASP patients of our study were directly related to lowered 25 (OH) D3. The cohort of PsA patients with a small number of positive htTG antibodies presented markedly elevated levels of 25 (OH) D3. The immune response is more severe expressed in younger

ASP patients with additional positive htTG antibodies. However, the younger patients with ASP were well characterized with higher physical activity and longer sun light exposure. Therefore, these patients were able to compensate for lower 25 (OH) D3 and presented 1,25 (OH)2 Vitamin D3 within the normal range.

The major aim of this study was to define whether serum measurement of htTG antibodies, which can easily be performed in daily clinical practice, had a diagnostic or predictive value regarding vitamin D metabolism in patients suffering from ASP or PsA. Based on our results, we conclude that the assessment of htTG in ASP may be of interest from a pathophysiological point of view; however its role in clinical routine as a biochemical marker remains questionable [18].

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TABLES

Table 1: htTG status in 196 patients with ASP or PsA

			ASP	PsA	total
Antibody	Neg.	number	62	116	178
htTG		%	34,8%	65,2%	100%
	Pos.	number	14	4	18
		%	77,8%	22,2%	100%
Total		number	76	120	196
		%	38,8%	61,2%	100%

p-value of exact Fisher's Test: p=0,001.

Table 2: Association of positive htTG antibodies with the biochemical markers of inflammation: CRP and ESR

CRP	Mean Value	Std. Dev.	N	Min	Max	MV ranks
htTG neg.	3.81	8.20	178	0	76,3	96.29
htTG pos.	6.36	9.58	18	0	38,0	120.36

ESR	Mean Value	Std. Dev.	N	Min	Max	MV ranks
htTG neg.	16.66	13.51	178	1	100	98.44
htTG pos.	17.50	14.16	18	2	47	99.08

p-value of Mann-Whitney-U-Tests concerning differences in means of ranks:
CRP: p=0,085; ESR: p=0,963.

Table 3: Serum levels of both 25 (OH) D3 and 1,25 (OH)2 D3 in the combined cohort of female and male patients with PsA and ASP. (The numbers of estimated Vitamine D differ from total number of patients because of haemolytic probes).

1,25 (OH)2 D3 [pg/ml]	**Means**	**Std. Dev.**	**N**	**Min**	**Max**	**MV ranks**
PsA	41.523	38.099	115	1	350	**66.73**
ASP	42.520	53.7	15	2	220	**56.10**

25 (OH) D3 [ng/ml]	**Means**	**Std. Dev.**	**N**	**Min**	**Max**	**MV ranks**
PsA	48.403	46.359	119	0	219	**114.10**
ASP	24.935	32.114	76	1	250	**72.80**

Table 4: Inverse correlation (Spearman's Rank- correlation Test) between ESR/CRP and 25 (OH) D3 in the combined cohort of female and male patients with PsA and ASP.

	1,25 (OH)2 D3 [pg/ml]	**25 (OH) D3** [ng/ml]
ESR	R=-0.061 p=0.490 N=130	R=-0.061 p=0.398 N=195
CRP	R=-0.072 p=0.417 N=130	**R=-0.350** **p<0.0005** **N=195**

Table 5: Association of positive htTG antibodies with 25 (OH) D3 and 1,25 (OH)2 D3 in the combined cohort of female and male patients with PsA and ASP. (The number of estimated Vitamine D metabolites differed from total number of patients because of haemolytic probes).

1,25 (OH)2 D3 [pg/ml]	**Means**	**Std. Dev.**	**N**	**Min**	**Max**	**MV Ranks**
htTG neg.	42.198	40.442	124	1	350	66.29
htTG pos.	30.067	27.716	6	2.3	68.4	49.08

25 (OH) D3 [ng/ml]	**Means**	**Std. Dev.**	**N**	**Min**	**Max**	**MV Ranks**
htTG neg.	41.478	44.215	177	0	250	101.88
htTG pos.	17.411	13.193	18	4.2	46.4	59.89

Table 6: Association of patient's age with positive htTG antibodies

			Age		
			< 45 yrs.	**>= 45 yrs.**	**total**
	htTG neg.	n	52	126	178
		%	29.2%	70.8%	100%
	htTG neg.	n	11	7	18
		%	61.1%	38.9%	100%
total		**n**	**63**	**133**	**196**
		% of sex	**32.1%**	**67.9%**	**100%**