Retinol (Vitamin A) and Retinol-Binding Protein Levels Are Decreased in Ankylosing Spondylitis: Clinical and Genetic Analysis

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ABSTRACT. **_Objective._** Retinol (vitamin A) plays an important role in bone structure and function. Treatment with retinoids has been associated with bone abnormalities mimicking spondyloarthropathy and diffuse idiopathic skeletal hyperostosis. To determine whether retinol concentrations are altered in patients with ankylosing spondylitis (AS), we examined serum retinol levels in patients with AS and healthy controls.
Methods. Retinol was assessed using mass spectrometry, and retinol-binding protein levels were assessed by ELISA. Retinol levels were correlated with clinical disease activity indices. The CYP26 gene, which plays a key role in retinol metabolism, was examined to define any single-nucleotide polymorphisms (SNP) associations with AS.
Results. Retinol levels were significantly lower in the AS cohort than in controls (mean 2.39 ± 0.88 μmol/l for AS, 3.34 ± 1.01 μmol/l for controls; $p < 0.0001$). Retinol-binding protein levels were also lower in AS than controls (AS 4.65 ± 2.10 μg/l; controls 7.48 ± 4.87 μg/l; $p < 0.001$). Serum retinol levels did not correlate with indices of disease activity defined serologically (C-reactive protein, erythrocyte sedimentation rate) or clinically (Bath AS Disease Activity Index, Bath AS Functional Index). Genetic analysis showed that an exonic CYP26C1 SNP (rs11187265) is not associated with AS.
Conclusion. The hallmark of AS is neo-ossification. AS is associated with abnormal serum levels of retinol, a biochemical factor linked to pathological hyperostosis. Further genetic studies are warranted into the genetic basis of the retinol-AS relationship. (First Release Nov 15 2007; J Rheumatol 2007;34:2457–9)

Key Indexing Terms:
ANKYLOSING SPONDYLITIS   DISEASE ACTIVITY   GENETIC STUDIES
RETINOL   RETINOL BINDING PROTEIN

Retinol (vitamin A) is known to play an important role in bone structure and function. Retinoids, synthetic derivatives of vitamin A, are administered primarily for dermatological conditions such as acne, psoriasis, and disorders of keratinization. Toxicity has proven to be a significant problem with longterm administration of retinoids. Bone lesions mimicking spondyloarthropathy (SpA) and diffuse idiopathic skeletal hyperostosis (DISH) as well as arthritis, myopathy, and vasculitis have been described[1]. Hyperostotic changes and calcification of tendons and ligaments closely resembling DISH are the most frequently seen changes associated with systemic retinoid therapy[2]. Chronic hypervitaminosis A, the clinical features of which have been described extensively, might be regarded as a prototype to predict the abnormalities occurring with newer retinoid therapy. The role that retinol may be playing in various rheumatic diseases in general, and SpA in particular, has received little attention in the literature.

Retinol-binding protein (RBP) is synthesized primarily in the liver, where it requires the binding of retinol to trigger its secretion. The common precursor of the active retinoids, all-*trans*-retinol, is transported in the serum in a 1:1 complex with RBP[3]. Following uptake by target cells, retinol is converted through several enzymatic steps into its active derivatives. Cytochrome p450 26 (CYP26) metabolizes retinol into these hydroxylated and oxidized derivatives. The CYP26 gene family has 3 genes in distinct subfamilies: A1, B1, and C1. All 3 have a role in metabolism of retinol or its derivatives[4]. Deficiency of CYP26A1 in mice produces lethal morphogenetic phenotypes that mimic those generated by excess retinol administration[5].

Since abnormalities in retinol or retinoid metabolism are associated with hyperostosis, the question arises whether

abnormalities in retinol pathways may be associated with ankylosing spondylitis (AS), a disease whose hallmark is pathological new bone formation. The goals of our study were (1) to identify the levels of retinol and RBP in a well defined cohort of patients with AS in comparison to a group of healthy controls; (2) to correlate retinol levels with other disease activity indices (clinical and serological); and (3) to identify any association between the CYP26 gene and AS.

MATERIALS AND METHODS

Patients were selected from the Spondylitis Clinic at the Toronto Western Hospital. All satisfied the modified New York criteria for AS[6]. Patients were seen according to a standard protocol in which all clinical data was recorded. Serum samples corresponding to the clinic visit were stored at –70°C. All patients gave written consent for the study.

Sample analysis. Retinol was assessed using liquid chromatography-mass spectrometry. The high performance liquid chromatography system utilized an Agilent 1100 instrument (Agilent Technologies, Palo Alto, CA, USA) and an API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) was used.

Retinol-binding protein assay. RBP was assayed using an ELISA kit (Alpco Diagnostics, Salem, NH, USA). Serum samples were diluted 1:5000 before use as per the manufacturer's instructions.

Genotyping. AS patients and healthy controls were genotyped for an exonic variant of CYP26C1 (*rs11187265*). The hypothesis was that a gain of function might lead to lower retinol levels. Selection of this single-nucleotide polymorphism (SNP) was based on the frequency of the minor allele (~13% in Caucasians), a likelihood that the resultant amino acid change has functional relevance, and its availability from Applied Biosystems. DNA was prepared from peripheral blood lymphocytes using standard techniques. Genotyping was performed using a CYP26C1 exonic variant, *rs11187265* [*A/G*]. Optimized allele discrimination assays for this SNP were from Applied Biosystems. The plates were read using the IQ5 multi-color PCR detection system (BioRad).

Statistical analysis. Levels in patients and controls were compared using Student's t test, and correlations were analyzed using a Pearson product coefficient.

RESULTS

Forty patients with AS were studied (mean age 38.2 yrs, range 19–59 yrs); clinical features are listed in Table 1. Twenty-three of the 40 AS patients had active disease [Bath AS Disease Activity Index (BASDAI) > 4.0] at the time of the assessment. Forty-six healthy controls were studied for comparison (mean age 53 yrs, range 17–86 yrs); 45% of the controls were male.

Mean retinol level for the AS cohort was 2.39 ± 0.88 μmol/l and mean retinol level for controls was 3.34 ± 1.01 μmol/l ($p < 0.0001$) (Figure 1). For patients with active disease, the mean retinol level was 2.22 ± 0.78 μmol/l, and for the inactive disease subgroup, 2.61 ± 0.97 μmol/l (p = nonsignificant).

Retinol levels showed no correlation with the clinical indices of disease activity, the BASDAI ($r = –0.06$) and the Bath AS Functional Index ($r = –0.26$); or the serological indices C-reactive protein ($r = 0.08$), erythrocyte sedimentation rate ($r = 0.4$), and alkaline phosphatase ($r = 0.054$). Retinol levels were also not correlated with the modified Stoke AS Spine Score radiographic score ($r = –0.03$).

The mean RBP in AS was 4.65 ± 2.10 μg/l, and in controls 7.48 ± 4.87 μg/l ($p = 0.001$). The mean RBP for the active-disease AS subgroup was 4.27 μg/l, and for the inactive AS subgroup 5.34 μg/l (p = nonsignificant).

To test whether there was any association of a CYP26C1 exonic variant, *rs11187265* [*A/G*], we genotyped a larger population of 123 AS patients and 131 healthy controls. The minor allele frequency was 9% (24/262) for controls and 11.4% (28/246) for AS patients (p = nonsignificant).

DISCUSSION

Serum retinol levels differ in patients with AS in comparison with healthy controls, and unexpectedly the levels in AS were decreased. We observed that retinol does not function as an acute-phase reactant and is not associated with disease activity. These findings contrast with results of 2 studies with small

Table 1. Characteristics of the AS cohort.

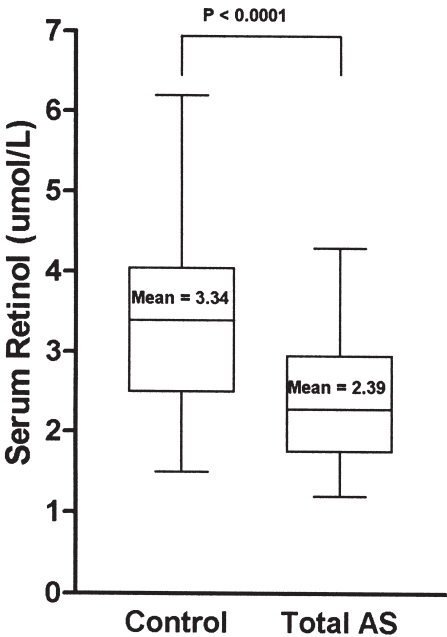| Characteristic	No. (%)
Total AS patients	40
Male/female	34 (85)/6 (15)
HLA-B27-positive, %	89
Active AS (BASDAI > 4.0), n (%)	23 (57.5)
Inactive AS (BASDAI < 4.0), n (%)	17 (42.5)
History of inflammatory bowel disease, n (%)	8 (20); 4 in each of the active and inactive disease groups

BASDAI: Bath AS Disease Activity Index.

Figure 1. Serum retinol concentrations (μmol/l, range and mean) for patients with AS and controls.

sample sizes that had inferred that such changes may just reflect the degree of inflammatory burden of disease[7,8]. Using a combination of BASDAI, ESR, and CRP results we were able to more accurately define active and inactive AS disease compared to the earlier studies. The statistically different values compared to controls point to an intrinsic difference, likely genetically determined.

The exact effect of retinol on bone is unresolved. Hyperostosis and extra bone formation are well defined consequences of excess vitamin A or its synthetic derivatives[1]. Paradoxically, there is growing evidence that excess vitamin A is also associated with low bone mineral density[9]. Recently, several human studies have suggested an association between chronic high intake of preformed vitamin A and bone loss that potentially leads to osteoporosis[10-12], but the studies were observational and raise new questions about the complexity of retinol and bone interaction. Although an increased risk of hip fracture is associated with increased retinol intake, it has not been possible to determine specific levels of retinol above which bone health is compromised.

Recently it has been demonstrated that RBP is linked to glucose uptake in adipocytes and systemic insulin sensitivity. It has been shown that serum levels of RBP4, the only specific transport protein for retinol in the circulation, are increased in insulin-resistant states[13]. Serum RBP4 levels correlated with the magnitude of insulin resistance in subjects with obesity, impaired glucose tolerance, or type 2 diabetes, and in non-obese, nondiabetic subjects with a strong family history of type 2 diabetes.

Cytochrome p450 genes (such as CYP2D6) have been implicated in genome-wide scans in AS, but to date the biological basis of such an association has not been resolved. It has been suggested that altered metabolism of a natural toxin or antigen by the CYP2D6 gene may mediate altered susceptibility to AS. Recurrence-risk modeling in AS suggests that between 3 and 9 genes operating in addition to HLA-B27 fit the observed pattern of recurrence risk in relatives of patients with AS[14]. In addition to the gene for CYP2D6 on chromosome 22q, 6 regions lying on chromosomes 2p, 2q, 3p, 10q, 11p, and 16q achieved logarithmic odds scores > 1.0. The same region on chromosome 10q has been shown to be associated with the development of diabetes[15]. The gene for RBP4, implicated in the pathogenesis of diabetes, as noted, is also located on chromosome 10q. The genes for CYP26 (A1 and C1) are also located in the vicinity of this chromosomal region.

We postulated that the lower retinol levels in our AS cohort are due to a polymorphism in the enzymes involved in inactivation of all-*trans* retinoic acid. Rare CYP26A1 polymorphisms (< 2.5%) are detected in normal Caucasians and a rare CYP26A1 mutation (deletion of nt 3116 in the coding region, resulting in the generation of a premature stop codon) was found in one patient with spina bifida (1.3% of these patients had this mutation)[16]. As it is generally assumed that complex diseases such as AS are associated with gene polymorphisms with higher frequencies (> 5%), we chose to examine an exonic SNP of CYP26C1 based on this criterion (minor allele frequency is 13% in normal Caucasians). Although the exact functional consequences of this variant remain unclear, it is likely that the resultant change in amino acid (R to Q) has some functional relevance. Our analysis of CYP26 examined a relatively small cohort and thus further analysis of the genetic determinants of retinol metabolism in AS are warranted.

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