Sclerostin and Dkk-1 in patients with ankylosing spondylitis

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ABSTRACT

Objective: To determine the serum Dickkopf-related protein 1 (Dkk-1) and sclerostin levels, and their relationship to structural damage and disease activity in patients with ankylosing spondylitis (AS), as well as to compare the serum Dkk-1 and sclerostin levels in patients receiving and not receiving anti-TNF-α treatment.
Materials and Methods: This cross-sectional study included 44 AS patients and 41 healthy age- and gender-matched controls. Demographic data, disease activity parameters, and Bath Ankylosing Spondylitis Radiologic Index (BASRI) scores were recorded. Serum Dkk-1 and sclerostin levels were measured using commercially available ELISA.
Results: Serum Dkk-1 levels were lower ($P > 0.05$) and sclerostin levels were significantly lower ($P < 0.05$) in the AS patients than in the controls. Dkk-1 and sclerostin levels were similar in the patients that did and didn't receive anti-TNF-α treatment, and in the patients with active and inactive disease ($P > 0.05$). There wasn't a correlation between serum Dkk-1 or sclerostin levels, and disease activity indices ($P > 0.05$). BASRI scores did not correlate with serum Dkk-1 or sclerostin levels ($P > 0.05$).
Discussion: Sclerostin expression is impaired in AS, but this is not the case for Dkk-1. The lack of an association between Dkk-1 or sclerostin levels, and anti-TNF-α treatment, disease activity indices, and radiological damage might indicate that neither the Dkk-1 nor sclerostin level induce inflammation and radiological damage in AS patients. Pathologic bone formation in AS might be due to molecular dysfunction of sclerostin and Dkk-1 at the cellular level.

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Keywords: Ankylosing spondylitis; Structural damage; Anti-TNF-α; Dkk-1; Sclerostin.

INTRODUCTION

Ankylosing spondylitis (AS) – a chronic inflammatory disease that predominantly affects axial joints and intervertebral spaces - is characterized by new bone formation and is thereby associated with syndesmophytes and ankylosis[1]. Several biomarkers, including components of the Wnt pathway signaling cascade that regulate bone formation, have been evaluated in order to determine their role in the clinical prognosis of AS and the response to treatment[2-9]. The most commonly studied secreted Wnt inhibitors are sclerostin and Dickkopf-related protein 1 (Dkk-1). Dkk-1 appears to be the most important biologically, as it was recently reported that Dkk-1 is a regulator of joint remodeling in animal models of arthritis, and that elevated Dkk-1 levels were linked to bone resorption and low Dkk-1 levels were associated with new bone formation[10]. Sclerostin, a soluble inhibitor of the Wnt pathway that is closely related to Dkk-1, is a bone-specific molecule produced by osteocytes that prevents binding of Wnt proteins, leading to inhibition of the Wnt pathway[8]. Loss-of-function mutations of the gene encoding sclerostin are linked to diseases characterized by increased bone mass.

Numerous studies have sought to identify and clarify the mechanisms of bone turnover in AS, focusing on exploration of the potential role of Dkk-1 and sclerostin levels, and anti-TNF-α treatment in new bone formation in AS patients[2-9]; however, the findings have been inconsistent. Some studies reported elevated serum sclerostin and Dkk-1 levels in AS patients, as compared to controls, whereas others reported lower levels and others reported similar levels in AS patients and healthy controls[2-9]. Findings regarding the effects of anti-TNF-α

treatment on serum sclerostin and Dkk-1 levels, and the relationship between these Wnt inhibitors, and disease activity status and structural damage, are also inconsistent[2-9]. As such, the present study aimed to add to the limited available data via examination of the relationship between sclerostin and Dkk-1 levels, and structural damage in AS patients.

MATERIALS AND METHODS

This cross-sectional study included 44 AS patients recruited from the physical medicine and rehabilitation clinic of our hospital, and 41 age- and gender-matched healthy controls. All the AS patients included fulfilled the modified New York criteria[11]. The study protocol was approved by the Local Ethics Committee.

Demographic data and disease-specific data were recorded. Disease activity was assessed using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)[12]. Patients with a BASDAI score ≥4 were considered to have active disease. Markers of inflammation (the erythrocyte sedimentation rate [ESR] and C-reactive protein [CRP] level) were measured in all patients and controls. Radiographic assessment was performed using the Bath Ankylosing Spondylitis Radiologic Index (BASRI)[13]. Serum samples were obtained from all participants and were stored in aliquots of 200 µL at –20 °C.

SERUM DKK-1 AND SCLEROSTIN MEASUREMENT

Serum Dkk-1 levels were measured using a commercially available ELISA kit (Human Dkk-1 ELISA Kit), according to the manufacturer's instructions (Adipo Bioscience, Santa Clara, USA). Serum sclerostin levels were measured using a commercially available ELISA kit (Human Soluble Sclerostin [SOST] ELISA Kit), according to the manufacturer's instructions (Adipo Bioscience, Santa Clara, USA). All measurements were performed in triplicate for each sample, and a mean value was calculated.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS v.13.0 for Windows (SPSS, Inc., Chicago, IL). Variables were tested for normality via the Shapiro-Wilk test. Correlations between Dkk-1, sclerostin, and other variables were analyzed via Pearson's or Spearman's test, as appropriate. The Mann-Whitney U test was used for group comparisons. The level of statistical significance was set at $P < 0.05$.

RESULTS

The study included 34 (77%) male and 10 (23%) female patients. In all, 19 (43%) patients were using an anti-TNFα agent for a mean 2.74 ± 1.52 years and 25 (57%) were receiving sulfasalazine - a disease-modifying anti-rheumatic drug. Among the patients, 11 (25%) had a BASDAI score ≥4, 14 (32%) had an ESR >20 mm h^{-1}, and 20 (44%) had a CRP >0.8 mg dL^{-1}. BASDAI scores were significantly lower in the patients that were using an anti-TNF-α agent than in those that were not (1.97 ± 2.38 vs. 3.16 ± 1.57, P = 0.012). Patient demographic and clinical data are presented in Table I.

Serum sclerostin levels were significantly lower in the patients with AS than in the healthy controls (P = 0.037) (Table II). Although numerically lower, the difference between the Dkk-1 level in the AS patients and controls was not significant (314.96 pg mL^{-1} vs. 613.34 pg mL^{-1}, P = 0.062). Serum sclerostin and Dkk-1 levels were similar in the patients that did (n = 19) and did not (n = 25) use an anti-TNF-α agent (P > 0.05) (Figures 1 and 2, and Table II). Serum sclerostin and Dkk-1 levels were similar in the patients with active disease and inactive disease (P > 0.05) (Figures 1 and

TABLE I. DEMOGRAPHIC AND CLINICAL DATA OF THE PATIENTS

Demographics	Mean±SD
Age (years)	40.06±9.51
Disease duration (years)	10.34±6.19
BASDAI (range 0-10)	2.65±2.02
CRP (mg/dl)	1.06±1.30
ESR (mm/hour)	19.90±19.87
BASRI (range 2-12)	7.22±1.95
	n (%)
Male	34 (77.27)
Female	10 (22.73)
Patients receiving anti-TNF agent (n)	19 (43.18)

BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C-reactive Protein; ESR, erythrocyte sedimentation rate; BASRI, Bath Ankylosing Spondylitis Radiologic Index

TABLE II. SERUM SCLEROSTIN AND DKK-1 LEVELS OF THE PATIENTS AND HEALTHY SUBJECTS

	n	Sclerostin ± SD (pg/ml)	P	Dkk-1 ± SD (pg/ml)	P
Patients with AS	44	427.69 ±368.10	0.037*	314.96±196.73	0.062
Healthy subjects	41	656.32±643.51		613.34±861.86	
Receiving anti-TNF-α agent	19	393.21±305.80	0.507	275.07±120.42	0.586
Not eceiving anti-TNF-α agent	25	453.90±413.50		345.28±237.17	
Patients with active disease	11	449.15±327.94	0.574	331.85±267.65	0.504
Patients with inactive disease	33	420.54±385.05		309.33±385.05	

*p<0.05.
AS, ankylosing spondylitis; anti-TNF-α, anti–tumor necrosis factor-α.

2, Table II). There wasn't an association between the serum sclerostin or Dkk-1 level, and disease activity indices (BASDAI, ESR, and CRP) (P > 0.05). Structural damage assessed via BASRI did not correlate with serum sclerostin or Dkk-1 levels (P > 0.05).

DISCUSSION

In the present cross-sectional study it was observed that the serum sclerostin level was significantly higher in the AS patients than in the healthy controls. Although numerically lower, the difference between the Dkk-1 level in the AS patients and controls was not significant. Additionally, the sclerostin and Dkk-1 levels were similar in the patients with active disease and inactive disease. Moreover, sclerostin and Dkk-1 levels were similar in the patients that were using an anti-TNF-α treatment and those that were not, and structural damage, as assessed via BASRI, did not correlate with serum Dkk-1 or sclerostin levels.

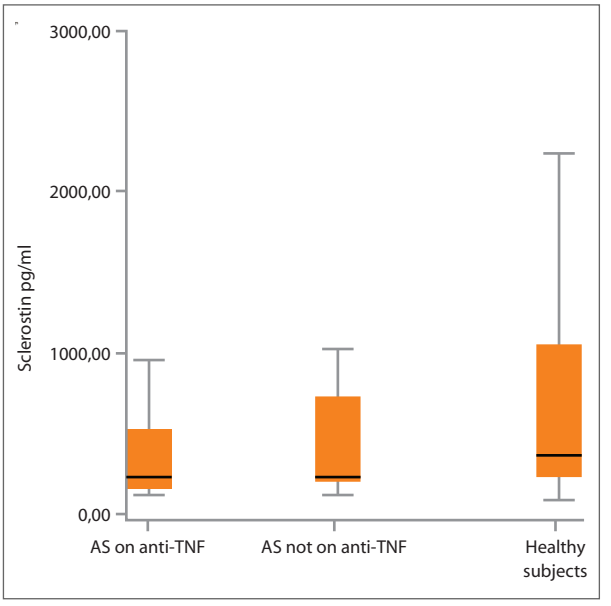

FIGURE 1. Serum sclerostin levels of the subjects
Patients with AS had significantly lower sclerostin levels compared with controls (p<0.05). Patients receiving anti-TNF-α agents and who were not receiving anti-TNF-α agents had similar sclerostin levels (p>0.05).
Values are presented as mean and SD.
AS, ankylosing spondylitis; anti-TNFα, anti–tumor necrosis factor α

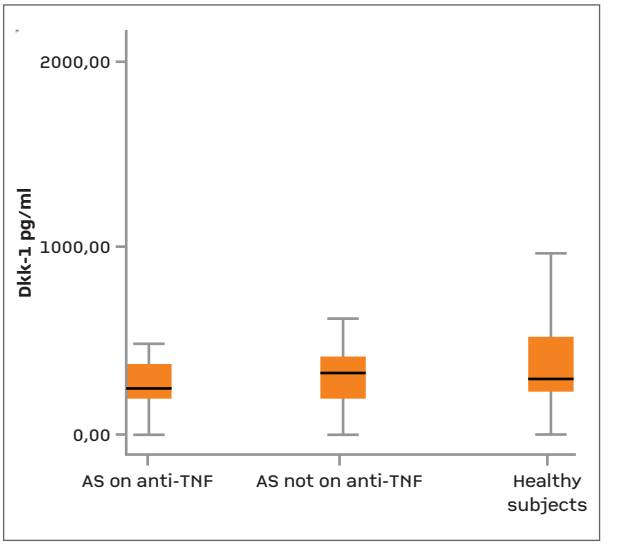

FIGURE 2. Serum Dkk-1 levels of the subjects
Although numerically lower, the difference between level of Dkk-1 in AS patients and healthy subjects did not reach statistical significance (314.96 vs. 613.34 pg/ml, P=0.062). Patients receiving anti-TNF-α agents and who were not receiving anti-TNF-α agents had similar Dkk-1 levels (p>0.05).
Values are presented as mean and SD.
AS, ankylosing spondylitis; anti-TNF-α, anti–tumor necrosis factor α

The present findings regarding sclerostin levels are in accordance with those of Appel *et al.*[6] and Saad *et al.*[3]. Appel *et al.* were among the first researchers to evaluate serum sclerostin levels in AS patients. They compared 46 AS patients and 50 healthy controls, and reported that sclerostin levels were lower in the AS patients. Moreover, they reported correlation with formation of new syndesmophytes[6]; however, in the present study an association between structural damage and the sclerostin level was not observed. This difference in findings might be due to differences in the indices used to evaluate the radiological status of AS patients; whereas Appel *et al*. used the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS), the present study used BASRI for structural damage assessment. These 2 indices use completely different parameters for scoring. Saad *et al*. also reported lower sclerostin levels in AS patients than in controls; however, they reported a significant increase in the sclerostin level after 12 months of anti-TNF-α treatment in their cohort[3], whereas in the present study an association between the sclerostin level and anti-TNF-α treatment was not noted. This difference in findings might be due to the differences in the 2 study's methodologies.

Although in the present study 19 patients received anti-TNF-α treatment for a mean 2.74 years, their pretreatment sclerostin levels were not known. Moreover, the sclerostin levels in the patients in Saad *et al.* study after 2 years are not known. Resistance or insensitivity always occurs with anti-TNF-α treatment, which can decrease the sclerostin level to the pretreatment level, which makes direct comparison between studies difficult. Nonetheless, Taylan *et al*. reported that sclerostin levels were similar in AS patients and healthy controls[4], whereas, sclerostin and Dkk-1 levels did not vary according to disease activity. In contrast, Korkosz[2] reported that sclerostin levels were significantly higher and Dkk-1 levels lower in patients with high disease activity; this contradiction might indicate that the interaction between disease activity and inhibitors of bone formation in AS is complex.

Daoussis *et al.*[9] reported higher Dkk-1 levels in AS patients, Kwon *et al.*[7] reported lower Dkk-1 levels in AS patients, and Taylan *et al.*[4] reported similar Dkk-1 levels in AS patients, as compared to healthy controls. The present findings are in accordance those of Taylan *et al.* Daoussis *et al.* reported that patients receiving anti-TNF-α treatment had higher Dkk-1 levels than those that were not[9]; whereas, both Kwan *et al.* and Taylan *et al.* reported that Dkk-1 levels did not vary according to anti-TNF-α treatment[4,7], which is in agreement with the present findings. The inconsistency of findings might indirectly indicate that neither TNF-α per se, nor inflammation in general, are the primary inducers of Dkk-1.

In an animal model of chronic inflammatory arthritis TNF-α was shown to induce skeletal expression of Dkk-1, which in turn triggered sclerostin production[14] - both molecules being potent inhibitors of new bone formation[15-17]; however, the present findings show that serum levels of sclerostin and Dkk-1 did not differ according to disease activity or use of anti-TNF-α treatment, which might indicate that there are molecular mechanisms other than those related to acute-phase response and anti-TNF-α treatment that are responsible for modulation of serum Dkk-1 and sclerostin levels in AS patients[10]. The link between TNF-α, and sclerostin and Dkk-1 warrants additional research, as it could have pathogenic and clinical implications in AS.

TNF-α blockers have been successfully used to suppress inflammation in AS[18-20]. In the present study the patients that were using an anti-TNF-α agent had significantly lower disease activity. The hypothesis that ankylosis is invariably preceded by inflammation in AS[21] is yet to be definitively proven by prospective studies, but might be due to either the presence of underlying inflammation that is not detectable via MRI[22,23] or the role of a non-inflammatory pathway. The development of new bone in the spine in the form of syndesmophytes and ankylosis is still evaluated via plain radiography[24]. A minimum of 2 years is required before radiographic changes can be reliably detected[25]. The hypothesis that anti-TNF-α agents might not prevent the development of spinal ankylosis in AS[26,27] also remains to be definitively proven via prospective research, which indicates that even in AS patients inflammation and effective suppression of inflammation could be more relevant than a minor increase in osteoproliferation. The AS patients in the present study that were using anti-TNF-α agents used them for about 3 years (on average), which is not considered to be long-term use, and the present study employed a cross-sectional - not prospective - design.

Lastly, in the present study an association between structural damage and the serum Dkk-1 level was not observed, which is in agreement with Taylan *et al.*[4] and Korkozs *et al.*[2]. Moreover, Dkk-1 and sclerostin levels in the present study did not correlate with radiological damage. Although Dkk-1 and sclerostin have been shown to be potent inhibitors of bone remodeling, the

lack of association between structural damage, and sclerostin and Dkk-1 in the present study and earlier studies might indicate that serum sclerostin and Dkk-1 levels are not the primary predictors of structural damage in AS patients, but might be indicative of a global bone metabolism. More comprehensive cellular mechanisms and receptorial dysfunction of sclerostin and Dkk-1 might be factors that play a more important role in structural damage in AS patients.

The present study has some limitations; primarily, the moderate sample size and the lack data on pretreatment sclerostin and Dkk-1 levels in the patients that were receiving anti-TNF-α agents, as well use of a cross-sectional rather than prospective design, and lack of examination of additional Wnt pathway inhibitors, bone mineral density values, and bone turnover markers. The remaining issue is whether or not serum sclerostin and Dkk-1 levels are stable enough to warrant the conclusion that structural damage is not associated with these molecules. Finally, the lack of data on patient HLA-B27 positivity or negativity is another limitation. Nevertheless, we think the present findings are clinically important.

CONCLUSION

Sclerostin expression is impaired in AS, but this is not the case for Dkk-1. Lack of an association between Dkk-1 or sclerostin levels, and anti-TNF-α treatment, disease activity indices, and radiological damage might indicate that neither Dkk-1 nor sclerostin level are factors associated with inflammation and radiological damage in AS. Lastly, pathologic bone formation in AS might be due to molecular dysfunction of sclerostin and Dkk-1 at the cellular level.

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