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The Relationship of Serum Levels of Potential Biomarkers Affecting Bone Formation with Radiological Progression and Disease Activation in Patients with Ankylosing Spondylitis

Ankilozan Spondilit Hastalarında Kemik Formasyonu Üzerine Etki Eden Potansiyel Belirteçlerin Serum Düzeylerinin Radyolojik Progresyon ve Hastalık Aktivasyonu ile İlişkisi

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ABSTRACT Objective: To determine the levels of Dickkopf-1, sclerostin, bone morphogenetic protein (BMP) -2 and 4, interleukin (IL)-17 and 23, which might contribute to the radiographic progression and disease activity in ankylosing spondylitis (AS). **Material and Methods:** A cross-sectional study was carried out on 238 AS patients and age and sex-matched control group of 102 individuals. The disease activity was assessed through the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). In both groups Dickkopf-1, BMP-2 and 4, sclerostin, IL-17 and 23 levels were measured. Radiographic changes were calculated based on the modified Stokes Ankylosing Spondylitis Spinal Score (mSASSS). **Results:** Dickkopf-1, sclerostin, IL-17 and 23 levels were significantly higher in AS group compared to the controls. There was no difference regarding serum BMP-4 levels, whereas BMP-2 levels were significantly higher in the control group ($p<0.001$). Mean mSASSS was 4.4±6.2 and it was determined that biomarkers alone did not affect this score in the evaluation made by taking all factors under control by variance analysis. However, BMP-2 and 4 values together above the median values affected the mSASSS by 3.3% ($p=0.017$). In the correlation analysis, a weak negative significant correlation was found between BASDAI and BMP-4 ($p<0.05$). **Conclusion:** There are many inflammatory and non-inflammatory pathways that contribute to radiographic progression in ankylosing spondylitis. Both the data in the literature and the results of our study point to the importance of the local level and functionality of markers that may contribute to progression rather than serum levels.

Keywords: Ankylosing spondylitis; Dickkopf-1; sclerostin; bone morphogenetic protein; modified Stokes Ankylosing Spondylitis Spinal Score

ÖZET Amaç: Bu çalışmada, ankilozan spondilit (AS) hastalarında radyografik progresyona ve hastalık aktivitesine katkıda bulunabilecek Dickkopf-1, sclerostin, kemik morfogenetik protein [bone morphogenetic protein (BMP)]-2 ve 4, interlökin (IL)-17 ve 23 düzeylerinin belirlenmesi amaçlanmıştır. **Gereç ve Yöntemler:** Çalışmamız kesitsel tarzda olup, 238 AS hastası ve 102 kişiden oluşan, yaş ve cinsiyet yönünden eşleştirilmiş kontrol grubu üzerinde yapılmıştır. Hastaların çalışmaya dâhil edildiği andaki hastalık aktivitesi, Bath Ankilozan Spondilit Hastalık Aktivite İndeksi [Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)] ile değerlendirildi. Hasta ve kontrol gruplarında Dickkopf-1, BMP-2 ve 4, sclerostin, IL-17 ve 23'ün kandaki seviyeleri ölçüldü. Hastaların radyografik değerlendirmesi, modifiye Stokes Ankilozan Spondilit Spinal Skoru (mSASSS) esas alınarak hesaplandı. **Bulgular:** AS hastalarının serum Dickkopf-1, sclerostin, IL-17 ve 23 seviyeleri kontrol grubuna göre anlamlı derecede yüksekti. Serum BMP-4 düzeyleri açısından fark bulunmazken, BMP-2 düzeyleri kontrol grubunda anlamlı olarak yüksekti ($p<0,001$). mSASSS ortalaması 4,4±6,2 olup, varyans analizi ile tüm faktörler kontrol altına alınarak yapılan değerlendirmede, biyobelirteçlerin tek başına bu skoru etkilemediği belirlendi. Ancak ortanca değerlerin üzerindeki BMP-2 ve 4 değerleri birlikte mSASSS'yi %3,3 etkilemiştir ($p=0,017$). Korelasyon analizinde BASDAI ile BMP-4 arasında zayıf, negatif anlamlı bir korelasyon bulundu ($p<0,05$). **Sonuç:** AS'de radyografik ilerlemeye katkıda bulunan birçok inflamatuar ve inflamatuar olmayan yol vardır. Hem literatürdeki veriler hem de çalışmamızın sonuçları, progresyona katkıda bulunabilecek belirteçlerin serum seviyelerinden ziyade lokal seviyedeki düzey ve işlevselliğinin önemine işaret etmektedir.

Anahtar Kelimeler: Ankilozan spondilit; Dickkopf-1; sclerostin; kemik morfogenetik protein; modifiye Stokes Ankilozan Spondilit Spinal Skoru

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Ankylosing spondylitis (AS) is a progressive chronic inflammatory disease primarily involving the sacroiliac joint and spine and characterized by enthesitis. If AS is not treated, radiographic damage may lead to functional limitation. The main reason for this limitation is syndesmophytes that develop in the spine, and syndesmophytes can be characterized as new bone formation as a result of the repair process following inflammation.[1,2]

Pathogenic processes underlying radiographic progression in AS have always attracted attention. The absence of a clear effect on the slowdown of radiographic progression in relatively short-term use (<2 years), despite the suppression of inflammation with anti-tumor necrosis factor-α (anti-TNF-α) treatment, has caused the development of different hypotheses. Consequently, the importance of local pathogenic factors (altered gene expression, biomechanical factors, etc.) has been revealed. It is still unclear how the new bone formation that develops after inflammation gains autonomy and how it is triggered.[3,4]

It is quite important to stop or slow down radiographic progression and control the regulation of new bone formation at the molecular level in AS. Various inflammatory and non-inflammatory processes play a role in the new bone formation process, among which Wnt pathway, bone morphogenetic protein (BMPs) and interleukin (IL)-23/IL-17 axis can be counted as the most prominent ones. Wnt proteins are known to be potent inducers of new bone formation, has been observed to play a role in the development of syndesmophytes. Natural inhibitors of Wnt, such as Dickkopf-1 (DKK-1) and sclerostin (SOST), have been found to neutralize Wnt activation and prevent new bone formation.[5] Although the effects of DKK-1 and SOST on bone formation are theoretically clear, studies examining DKK-1 and SOST levels in AS patients in the literature give conflicting results.[6-8]

BMPs are the members of the transforming growth factor-β family and play a critical role in osteoblast differentiation. BMPs are subclassified based on phylogenetic analysis of nucleotide similarity such as BMP2/4, BMP5/6/7/8, BMP9/10, BMP12/13/14 and BMP2/4 is the most emphasized in inflammatory arthritis.[9] In a study by Chen et al., it was observed that AS patients with high BMP-2, BMP-4 and BMP-7 levels were more prone to radiographic progression.[10] In a study by Bleil et al., on the contrary, it was shown that BMP-2 and BMP-7 in facet biopsies were low in patients with AS, which make us think to what extent local factors may contribute the bone formation.[11]

The IL-23/IL-17 axis plays an important role in the pathogenesis of AS and its levels are increased in AS patients.[12] Inhibition of these cytokines effectively suppresses inflammation in AS patients. It has been reported that IL-23 is overexpressed in the entheses of AS patients and induces the production of IL-17 and IL-22. IL-22 has been shown to induce osteoblast differentiation and bone formation in entheses via STAT-3.[13] On the other hand, in vitro studies have revealed that IL-23 and IL-17 induce osteoclastogenesis through RANK expression.[14] Therefore, the IL-23/IL-17 axis appears to have pleiotropic effects on bone formation in AS patients.

In this study, we primarily aimed to investigate the relationship of both non-inflammatory (Wnt, BMP) and inflammatory (IL-23/IL-17 axis) pathways with radiographic damage and disease activity. To the best of our knowledge, our study is the first in the literature to examine all three pathways simultaneously.

MATERIAL AND METHODS

DESIGN AND PARTICIPANTS

A cross-sectional single-center study was planned on AS, rheumatoid arthritis patients and healthy controls, who applied to rheumatology outpatient clinic at Ankara University Medical School, Department of Physical Medicine and Rehabilitation, Division of Rheumatology between December 2014 and March 2017. Ankara University Faculty of Medicine Clinical Research Ethics Committee approval (date: March 11, 2013, no: 2013-04-161-13) and written informed consent of participants were obtained prior to the performance of any study procedures, which was in accordance with the Helsinki Declaration.

A total of 238 AS patients and 102 age and gender-matched healthy controls were included in the study. Patients, who fulfilled the modified New York

criteria and/or the Assessment of SpondyloArthritis International Society classification criteria for AS were enrolled in the study.[15,16] AS patients with a diagnosis period of less than 10 years were included in our study. The initial demographic data of the patients, such as age, sex, disease duration, and drugs used concomitantly, were recorded. Patients, who had had already a diagnosis and had been followed up in the outpatient clinic, were included in this study during their routine control and to investigate the effect of different treatment modalities [non-steroidal anti-inflammatory drugs (NSAID) vs anti-TNF-α] on biomarkers newly diagnosed patients (treatment-naive patients) during the study period, were excluded. The patients included in our study were divided into 2 groups as those who had been receiving TNF-α blocker (with NSAID use if necessary) or NSAID (regular or as needed) treatment for at least 6 months. Patients who previously used different TNF-α blockers were not excluded from the study, while patients who received anti-cytokine therapy other than TNF-α blocker were not included in the study. Additionally, patients who had a history of bone fracture within the last 2 years and received medical treatment for osteoporosis, were under 18 and over 55 years of age, were pregnant, and had malignancy, acute infection, secondary amyloidosis, severe hepatic, renal, or cardiac disease, concomitantly with any other rheumatic disease, were excluded from the study.

DISEASE ACTIVITY

Disease activity was assessed through Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) for AS patients and patients with a BASDAI score ≥4 were considered to have active disease and <4 were considered to have inactive disease activity.[17]

RADIOGRAPHIC EVALUATION AND ENTHISITIS SCORE

Radiographic evaluation of the patients will be calculated based on the modified Stokes Ankylosing Spondylitis Spinal Score (mSASSS).[18] Lateral radiographs of the lumbar and cervical spine will be taken according to this system. According to mSASSS, normal vertebrae are scored as 0, erosion/sclerosis/squaring: 1, presence of syndesmophytes: 2, and ankylosis as 3. A total of 24 regions from the lower corner of the C2 vertebra to the upper corner of the T1 vertebra, from the lower corner of the T12 vertebra to the upper corner of the sacrum are included in the examination, and the total score ranges from 0 to 72. All vertebral regions that need to be examined during radiographic evaluation may not be displayed properly (low or high dose exposure, intestinal gases, etc.), so that for the mSASSS, only scores of radiographs with ≤3 missing vertebral corners per segment (cervical or lumbar) were used.[19] The radiographs were independently scored by 1 trained readers blinded to chronologic order, clinical characteristics, and other imaging data at 2 different times and then scores were averaged.

Maastricht Ankylosing Spondylitis Enthesitis Score (MASES) was used to calculate the patients' enthesitis score.[20] MASES analyses 13 sites: the bilateral first and seventh costochondral joints, the anterior and posterior superior iliac spines, the iliac crests, the fifth lumbar spinous process, and the proximal insertion of Achilles tendon (overall score range 0-13).

LABORATORY MEASUREMENTS

All assays were carried out in the same biochemical laboratory. The patients' erythrocyte sedimentation rates and C-reactive protein values, which are among the acute phase reactants, were checked during the last visit. HLA-B27 for AS patients were recorded from patient files. Venous blood samples were also obtained after a minimum of 8 hours of fasting to determine plasma DKK-1, SOST, IL-17, IL-23, BMP-2, and BMP-4 levels. Samples for these biomarkers were collected in sterile containers and centrifuged within a maximum of 120 minutes at 4,000 rpm for 10 minutes and then stored at-80° until examination. The serum concentrations of SOST were assessed using the commercial kit ELISA (Aviscera Bioscience, Santa Clara, USA). The serum concentrations of IL-17, DKK-1, BMP-2, and BMP-4 were assessed using the commercial kit ELISA (Boster Biological Technology, Fremont, USA). The serum concentrations of IL-23 were assessed using the commercial kit ELISA (eBioscience, Vienna, Austria). The levels of the markers mentioned above were

measured following the manufacturer's instructions. The sensitivity of the DKK-1 kit was <15.6 pg/mL, 0.39 pg/mL for the SOST kit, <2 pg/mL for the BMP-2 and BMP-4 kits, <1 pg/mL for the IL-17 and IL-23 kits.

STATISTICAL ANALYSIS

Statistical analysis of the data was done with SPSS 21.0 (SPSS Inc., Chicago, IL, USA) package program. Data were analyzed with the Shapiro Wilk test in terms of normal distribution. Median (minimum-maximum) was used for continuous data, and number and percentage were used for categorical data. Mann-Whitney U test was used to compare 2 independent groups, Kruskal Wallis test was used for comparison of 3 or more independent groups, and Mann-Whitney U test with Bonferroni correction was used to evaluate the parameters with significant difference. Chi-square test was used to compare categorical data. Correlation of markers with disease activation markers was evaluated with Spearman correlation test. $p<0.05$ was considered statistically significant.

In order to evaluate the effect of DKK, SOST, BMP-2, BMP-4, IL-17 and IL-23 levels on the mSASSS score, these biomarkers were divided into 2 groups as high and low according to their median values, and 2-way analysis of variance was applied by controlling other factors that may affect the mSASSS score.

RESULTS

Two hundred and thirty eight AS patients and 102 control groups were included in the study. One hundred eighty two (53.5%) of the participants included in the study were male, 158 (46.5%) were female, and their median age was 39. In the examination, no difference was observed between the groups in terms of age and gender (p=0.167 and p=1.000, respectively). The mean disease duration of AS patients was 7.22±4.43 years, and 66.8% of them were HLA-B27 positive. In the examination, no difference was observed between the groups in terms of age and gender (p=0.167 and p=1.000, respectively). Mean mSASSS was 4.4±6.2 and mean MASES was 2.8±3.9. Demographic, clinical, radiographic and laboratory features of the patients and controls are given in Table 1.

TABLE 1: Demographic, clinical, radiographic and laboratory features of AS patients and healthy controls.

	AS (n=238)	Control (n=102)	p value
Age	39 (18-66)	38 (18-55)	0.167
Sex			1.000
Male (n, %)	127, 53.4	55, 53.9	
Female (n, %)	111, 46.6	47, 46.1	
ESR	12.0 (1-95)	-	
CRP	3.6 (1-126.8)	-	
Disease duration (years) (mean±SD)	7.22±4.43	-	
BASDAI (range 0-10)	3.4±2.17	-	
Anti-TNF-α (n, %)	140, 58.8	-	
Average use of anti-TNF-α (mean±SD) (years)	2.7±3.6		
NSAID treatment (n, %)	90, 41.2	-	
HLA-B27 (n, %)	159, 66.8	-	
mSASSS (mean±SD)	4.4±6.2	-	
MASES (mean±SD)	2.8±3.9	-	

Data are presented as mean±standard deviation unless otherwise specified; $p<0.05$ was considered statistically significant; AS: Ankylosing spondylitis; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; HLA: Human leukocyte antigen; NSAID: Non-steroidal anti-inflammatory drug; MASES: Maastricht Ankylosing Spondylitis Enthesitis Score; mSASSS: The modified Stoke Ankylosing Spondylitis Spinal Score.

DKK-1, SOST, IL-17 and IL-23 levels were significantly higher in the AS group compared to the control group, and BMP-2 values were significantly lower in the AS group ($p<0.001$). There was no difference between the groups in terms of BMP-4 values (p=0.585) (Table 2).

TABLE 2: Distribution of examined biomarkers in AS patients and control group.

	AS (n=238)	Control (n=102)	p value
DKK-1	6257.0 (2031-27201.0)	4192.1 (928.3-9494.4)	<0.001
SOST	175.3 (45.3-1247.6)	93.7 (15-730.4)	<0.001
BMP-2	29.5 (2-1776.2)	195.3 (2-1145.9)	<0.001
BMP-4	459.4 (4-6180.7)	511.9 (163.8-2624.4)	0.585
IL-17	549.4 (1-2000)	312.4 (35.7-2848)	<0.001
IL-23	16.4 (0-389.9)	6.7 (4-110.4)	<0.001

$p<0.05$ was considered statistically significant; AS: Ankylosing spondylitis; DKK-1: Dickkopf-1; SOST: Sclerostin; BMP-2: Bone morphogenetic protein-2; BMP-4: Bone morphogenetic protein-4; IL-17: Interleukin-1; IL-23: Interleukin-23.

TABLE 3: Correlation between biomarkers and BASDAI.

		DKK-1	SOST	BMP-2	BMP-4	IL-17	IL-23
BASDAI	Rho	0.079	0.101	0.069	-0.142	0.030	-0.058
	p	0.223	0.120	0.292	0.028	0.644	0.371

p<0.05 was considered statistically significant; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; DKK-1: Dickkopf-1; SOST: Sclerostin; BMP-2: Bone morphogenetic protein-2; BMP-4: Bone morphogenetic protein-4; IL-17: Interleukin-1; IL-23: Interleukin-23; Rho: Spearman correlation coefficient.

TABLE 4: Distribution of examined biomarkers in active and inactive AS patients according to the BASDAI score.

	Inactive (n=170)	Active (n=68)	p value
DDK-1	6220 (2031-27201)	6324.5 (2815-12968)	0.357
SOST	171.9 (45.3-1247.6)	175.6 (63.7-771.3)	0.327
BMP-2	14.2 (2-1776.2)	108.7 (2-1676.5)	0.046
BMP-4	509.6 (4-6180.7)	376.1 (4-2778.9)	0.033
IL-17	551.9 (1-2000)	541.9 (1-1254.3)	0.988
IL-23	4 (0-128.1)	4 (4-389.9)	0.609

p<0.05 was considered statistically significant; DKK-1: Dickkopf-1; SOST: Sclerostin; BMP-2: Bone morphogenetic protein-2; BMP-4: Bone morphogenetic protein-4; IL-17: Interleukin-1; IL-23: Interleukin-23.

TABLE 5: Levels of biomarkers according to treatment type in AS patients.

	anti-TNF-α (n=140)	NSAID (n=98)	p value
DKK-1	6452 (2031-27201)	5991 (2553-17490)	0.286
SOST	173.8 (63.7-1247.6)	175.7 (45.3-771.3)	0.579
BMP-2	29.5 (2-1776.2)	29.5 (2-1264.8)	0.932
BMP-4	559.4 (4-6180.7)	462.4 (4-4150.7)	0.568
IL-17	566.4 (1-1368.9)	527.2 (1-2000)	0.922
IL-23	4 (4-190.8)	4 (0-389.9)	0.373

p<0.05 was considered statistically significant; DKK-1: Dickkopf-1; SOST: Sclerostin; BMP-2: Bone morphogenetic protein-2; BMP-4: Bone morphogenetic protein-4; IL-17: Interleukin-1; IL-23: Interleukin-23; TNF-α: Tumor necrosis factor alpha; NSAID: Non-steroidal anti-inflammatory drug.

In the correlation analysis, a weak negative significant correlation was found between BASDAI and BMP-4 (p<0.05) (Table 3). After that, we divided the patients into 2 groups as active (score ≥4) and inactive (<4) according to their BASDAI scores and examined the level of biomarkers. As a result, BMP-2 values were significantly higher in active disease, while BMP-4 values were significantly lower (p=0.046, p=0.033, respectively) (Table 4).

In the analysis made according to the type of treatment, no difference was found in any of the markers in the groups receiving anti-TNF-α and NSAID treatment in AS patients (p>0.05) (Table 5).

In another examination performed by dividing the patients into 2 groups as receiving anti-TNF-α and NSAID treatment, the MASES score was found to be significantly higher in NSAID users than in anti-TNF users (p=0.005). No difference was found in terms of mSASSS between 2 groups (p>0.05) (Table 6).

TABLE 6: mSASSS and MASES scores according to treatment type in AS patients.

	anti-TNF-α (n=140)	NSAID (n=98)	p value
mSASSS	4.8±7.3	4.1±5.9	0.110
MASES	1.1±2.6	3.2±3.3	0.005

p<0.05 was considered statistically significant; TNF-α: Tumor necrosis factor alpha, NSAID: Non-steroidal anti-inflammatory drug; MASES: Maastricht Ankylosing Spondylitis Enthesitis Score; mSASSS: The modified Stoke Ankylosing Spondylitis Spinal Score.

It was determined that biomarkers alone did not affect the mSASSS score in the evaluation made by taking all factors under control by variance analysis. However, BMP-2 and BMP-4 values together above the median values affected the mSASSS score by 3.3% (p=0.017), but this effect was found to be at a very low level, although it was significant (p=0.029) (Table 7).

TABLE 7: Variance analysis results of biomarkers that may affect the mSASSS score.*

	F	p value	Effect size
DKK-1	0.139	0.709	0.001
SOST	0.787	0.376	0.004
BMP-2	0.382	0.537	0.002
BMP-4	0.007	0.933	0.000
IL-17	0.477	0.491	0.003
IL-23	0.002	0.962	0.000
DKK-1, SOST	3.239	0.074	0.018
Il-17, IL-23	3.186	0.076	0.017
BMP-2, BMP-4	6.192	0.014	0.033

*All parameters are divided into high and low levels by median value; p<0.05 was considered statistically significant; DKK-1: Dickkopf-1; SOST: Sclerostin; BMP-2: Bone morphogenetic protein-2; BMP-4: Bone morphogenetic protein-4; IL-17: Interleukin-1; IL-23: Interleukin-23.

DISCUSSION

The DKK-1, SOST, IL-17, and IL-23 levels of the AS patients included in our study were significantly higher compared to the control group, whereas BMP-2 levels were significantly lower, and BMP-4 levels were similar to those of the control group. In terms of the treatments used in AS patients (anti-TNF-α vs NSAID), there was no difference between the levels in the examination. Biomarkers alone did not affect the mSASSS, however BMP-2 and BMP-4 together (above the median levels) found to affect mSASSS at a very low level, although it was significant. Disease activity is examined and a weak negative significant correlation was found between BASDAI and BMP-4. When BASDAI scores were divided into 2 groups as active (score ≥4) and inactive (<4), BMP-2 values were significantly higher in active disease, while BMP-4 values were significantly lower.

In AS patients, inflammation beginning in the vertebral entheses (led by TNF-α in particular) leads to erosion in the cartilage and bone. Then, fibrous and adipose tissue infiltration occurs in these lesions, and finally, ossification takes place, resulting in abnormal bone formation (syndesmophytes) which is associated with radiographic progression.[21] There was no significant difference in radiographic progression after the use of anti-TNF-α in AS patients for 2 years, but starting treatment in the early period of the disease and receiving anti-TNF-α treatment for >4 years were observed to slow down radiographic progression.[3,5] This situation can be explained as follows: TNF-α activates osteoclasts together with IL-17 at the beginning of inflammation and leads to erosion. Right afterward, the differentiation of TNF-α preadipocytes into adipocytes is accelerated, forming a basis for the development of syndesmophytes. However, the rapid administration of anti-TNF-α treatment blocks this transformation and prevents the development of fat infiltration after inflammation. This can be explained by the cumulative effect of the suppression of the regions, where new inflammation starts, without adipose transformation in different vertebrae except for the vertebral corners, where fat infiltration develops with the same logic in long-term use (since anti-TNF-α treatment does not stop the progression in regions where fat infiltration develops).[3,5] Briefly, the more effectively the early inflammation (erosion development phase) stage is suppressed at the vertebral corner, the more the radiographic progression slows down. Continuous NSAID use is known to reduce radiographic progression.[21] In our study, the mean mSASSS of the patients who received anti-TNF-α and NASID treatment were similar. Both the short-term disease duration of our patients and the mean anti-TNF-α use of 2.7±3.6 years (<4 years) make this result understandable. On the other hand, when examined in terms of treatment modalities, the lack of difference between all biomarkers indicates that the pathways involved in the pathogenesis of AS are independent processes.

The Wnt pathway and natural inhibitors of this pathway, DKK-1 and SOST (generally affect synergistically), have been researched extensively in terms of syndesmophyte development in AS patients. Syndesmophyte development has been shown to be higher in AS patients who have low levels of DKK-1 and SOST.[22] Despite contradictory publications on serum levels of DKK-1, a meta-analysis stated that DKK-1 levels were generally higher in AS patients compared to the control group, as in our study.[23] However, less development of syndesmophytes would be expected in AS patients in this case, which is explained by the concept of functional DKK-1. Accordingly, total serum DKK-1 and functional DKK-

1 levels are not correlated, and functional DKK-1 levels, i.e., levels in entheses are lower in AS patients.[23] Another study reported that low-density lipoprotein receptor-associated protein LRP-5/LRP-6 binding of DKK-1 was reduced, resulting in the accelerated development of syndesmophytes due to the decrease in Wnt inhibition, and this decrease in binding was not influenced by the serum DKK-1 level.[24] All these findings indicate the importance of local factors in AS. In our study, the non-measurement of the functional level can be considered a limitation.

In our study, AS patients' serum SOST levels were higher than those of the control group. Theoretically, a high level of SOST is against radiographic progression, but the importance of local factors is visible in SOST, as in DKK-1. It has been reported that there is almost no SOST expression in AS patients' periarticular bones, and this can even be a condition specific to AS patients, which will reduce Wnt inhibition, resulting in the development of syndesmophytes.[24] This result shows that the serum level of SOST and the local level are not correlated. In a study investigating whether the SOST level was affected by anti-TNF-α in AS patients, patients who received and did not receive this treatment were evaluated cross-sectionally, but the SOST level did not differ which can indicate that non-inflammatory pathways are not influenced by inflammation.[7] Similarly, in our study, it has been shown that not only the SOST level but also the other biomarkers we examined were not affected by the treatment received when we divided the patients into 2 groups as those receiving anti-TNF-α and NSAIDs.

Similar to the literature, serum IL-17 and IL-23 levels were found to be higher than the control group in our study.[26,27] The IL-17/23 axis has dual effects on AS patients' radiographic progression. Generally, IL-17 stimulates osteoclastogenesis, whereas IL-23 induces osteoblastogenesis through IL-22. However, IL-23 indirectly contributes to osteoclastogenesis by inducing IL-17, and both IL-23 and IL-17 can contribute to osteoblastogenesis by elevating prostaglandin E2.[27] As can be seen, the IL-23/17 axis has quite pleiotropic effects on bone formation, and it is not clear yet which aspects outweigh at which stages. As in our study, other studies have elucidated that this axis is not affected by anti-TNF-α treatment.[12]

BMPs are the important pathway contributing to radiographic progression in AS patients. In a mouse model, BMP-2/6/7 has been shown to be immunohistochemically overexpressed in enthuses.[28] In a study by Bleil et al., BMP-2 and BMP-7 were contrarily shown to be at low levels in facet biopsies in AS patients.[11] In a study conducted by Chen et al., serum BMP-2, BMP-4, and BMP-7 levels were increased and associated with radiographic progression.[10] In our study, serum BMP-4 levels were similar to those of control group, but BMP-2 levels were lower. In another study on AS patients, fibroblasts cultured with BMP-2 exhibited more osteoblastic effects than osteoarthritis.[29] Thus, in pathogenic understanding, a low serum BMP-2 level may not mean less osteo-inductive effect in terms of possible local effects.

It was demonstrated that inflammatory cytokines had an inducing effect on BMP-2 and BMP-6 in the early period of the disease, which possibly indicate the beginning of the bone formation process that started after inflammation and gained autonomy.[21] These results show that the levels of markers that contribute to bone turnover can change over time (early vs late). Although our study included patients with relatively early disease duration, studies comparing patients with very early (<3 years) and >10 years disease duration, about the levels of biomarkers, will provide more accurate information. In our study, although there was no correlation between any biomarker and mSASSS in general, patients with higher median values of BMP-2 and 4 were found to be slightly associated with radiographic progression. However, cross-sectional examination of radiographic progression, as in our study, prevents us from obtaining a clear idea.

In a study of mouse model with early peripheral spondyloarthritis, it was shown that the BMP-2/BMP-4 ratio increased 6.5 times in the peripheral joints as a result of cytokine induction.[30] In our study, we divided the patients into 2 groups as active and inactive according to their BASDAI scores and examined the level of biomarkers. As a result, BMP-2 values were significantly higher in active disease,

while BMP-4 values were significantly lower. Although the 2 studies are not comparable with each other, similar results indicated that BMP-2/BMP-4 pathway may have a more important role on disease activation and on radiographic progression (albeit slightly, as we mentioned above) than other pathways. Publications examining the disease activity with investigated biomarkers (especially with non-inflammatory pathways) are very few in the literature and conflicting results have been reported. Additonally, most of them are not prospective and also not included homogeneous treatment groups.[31,32] As a result, we found that, BMP-2 values were significantly higher in active disease, while BMP-4 values were significantly lower. The relatively short duration of illness of our patients, may explain this relationship. Contrary to our result, in the light of current knowledge, our opinion is that these pathways, including BMPs, will not be directly related to disease activation, except probably in the early stages of AS, that is, after the non-inflammatory pathways in the pathogenesis of the diseases gain autonomy. However, cohorts with randomized treatment arms, beginning at the early stage of disease with long-term follow-up duration, and serial measurement of biomarkers are needed to clarify this issue.

In our study, the mean MASES value was significantly lower in the group receiving anti-TNF-α, in fact, this is a normal situation considering the effectiveness of anti-TNF-α treatment on enthuses.[33] In fact, the point we want to emphasize here is that; although anti-TNF-α and NSAID treatments have similar effects on radiographic progression, the more pronounced effect of anti-TNF-α treatment in peripheral entheses makes us think that local efficacy of inflammatory or non-inflammatory pathways may differ in different localizations even in the same disease.

In addition to what we mentioned above, our study has some important limitations. First of all, when examining radiographic progression, prospective studies will give much clearer results due to the nature of the process. We did not include newly diagnosed patients in our study specifically to examine the difference between different treatment modalities, but examining the serum biomarkers of treatment-naïve patients could have made an additional contribution to our study. Another important limitation of our study is that the radiographic evaluation was performed by a single person (although it was evaluated twice). Taking the average of the evaluation of more than one person blinded to the patients could increase the objectivity in terms of mSASSS.

CONCLUSION

In conclusion, structural new bone formation is the most important cause of functional limitation in AS patients. There are many inflammatory and non-inflammatory pathways that contribute to this formation, and the relationship of these pathways with each other is extremely complicated. Both data in the literature and the results of our study indicate that these pathways are generally independent of each other, despite having a relative relation, particularly in the early disease period. Additionally, contradictory results in the literature indicate the importance of local factors, disease duration (early or late period), local functionality of the markers that may contribute to progression rather than their serum levels in radiographic progression. In our study, although it has been shown that BMPs may have effects on both radiographic progression and disease activation, it does not seem easy to fully clarify all these complex process, long-term prospective studies will illuminate this subject.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

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