

Serum BMP-2 and BMP-4 levels and their relationship with disease activity in patients with rheumatoid arthritis and ankylosing spondylitis

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ABSTRACT

Objectives: This study aims to investigate the levels of bone morphogenic proteins (BMPs), one of the pathways affecting bone turnover in these diseases, and to investigate their relationship with disease activity.

Patients and methods: Between September 2013 and July 2015, a total of 100 ankylosing spondylitis (AS) patients (53 males, 48 females; median age: 40 years; range, 18 to 62 years), 58 rheumatoid arthritis (RA) patients (25 males, 33 females; median age: 40.5 years; range, 26 to 59 years), and 102 age- and sex-matched healthy controls (55 males, 47 females; median age: 38 years; range, 18 to 55 years) were included in the study. In all groups, serum BMP-2 and BMP-4 levels were measured using enzyme-linked immunosorbent assay (ELISA). Demographic data (age, sex, duration of disease) and acute phase reactants of the patients at the final visit were recorded. Disease activity was assessed through the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Ankylosing Spondylitis Disease Activity Score C-Reactive Protein (ASDAS-CRP) for AS patients and through the Disease Activity Score-28-CRP (DAS-28-CRP) for RA patients.

Results: The median BMP-2 values were found to be significantly higher in the RA group compared to the other groups and in the control group compared to the AS group ($p < 0.001$ for both). There was no significant difference between the groups in terms of median BMP-4 values ($p > 0.05$). No significant relationship was found between serum BMP-2 and BMP-4 levels and disease activity in both AS and RA patients, while there was a weak positive correlation between erythrocyte sedimentation rate and CRP levels with BMP-2 level in RA patients ($p = 0.014$, $r = 0.320$ and $p = 0.029$, $r = 0.287$, respectively).

Conclusion: Our study results suggest that the BMP pathway may have different dual effects in AS and RA patients depending on the underlying pathogenesis, and that local effects are more prominent than serum levels.

Keywords: Ankylosing spondylitis, bone morphogenetic protein, bone turnover, disease activity, rheumatoid arthritis.

Ankylosing spondylitis (AS) is a chronic inflammatory disease of mainly sacroiliac joints, spine, and entheses. As a result of chronic

inflammation, bone erosions and new bone formations (syndesmophytes) and ankylosis occur.¹ It is thought that syndesmophytes occur

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secondary to the reparative process that develops in response to inflammation and includes cartilage metaplasia. Syndesmophytes contribute to AS-related symptoms and signs and accelerate deterioration in functional status.² Rheumatoid arthritis (RA), which primarily affects articular and periarticular bone, is a prototype of inflammatory arthritis. Joint margins are usually site at which the inflamed synovium is in direct contact with bone, resulting in the bone erosions characteristic of RA, likely due to effects of cytokines and factors being expressed in neighboring joints.³

There are many pathways that affect bone turnover in inflammatory arthritis. The Wnt/ β -catenin pathway and its natural inhibitors Dickkopf-1 (DKK-1) and sclerostin (SOST), bone morphogenetic proteins (BMPs) and inhibitors of this pathway such as noggin and cytokines such as tumor necrosis factor- α (TNF- α), interleukin (IL)-17 IL-6 and IL-23 are the most emphasized factors.³⁻⁶ However, the underlying pathogenic processes that affect the bone metabolism in different ways (new bone formation versus erosion) in AS and RA patients have not been clearly elucidated.

Bone morphogenetic proteins are members of the transforming growth factor- β (TGF- β) family and play a critical role in osteoblast differentiation by binding to the surface receptors of mesenchymal cells.³ These proteins are subclassified based on phylogenetic analysis of nucleotide similarity creates particular subgroups of these ligands such as BMP2/4, BMP5/6/7/8, BMP9/10, BMP12/13/14 and BMP2/4 is the most emphasized in inflammatory arthritis.⁷ In a study, autoantibodies developed against noggin, a BMP inhibitor, were higher in AS patients, which, in turn, increased the BMP function and induced new bone formation.⁸ In another study by Chen et al.,⁵ AS patients with high BMP-2, BMP-4 and BMP-7 levels were more prone to radiographic progression. In a study by Bleil et al.,⁹ on the contrary, BMP-2 and BMP-7 in facet biopsies were low in patients with AS. In a study of patients with RA, serum concentrations of BMP-2 and BMP-7 were found to be higher in patients with RA compared to healthy controls.¹⁰ In another study, BMP-4 and BMP-6 were expressed less in peripheral blood cells of patients with RA compared to controls.¹¹ In a study examining synovial tissue in patients with RA, the expression

of BMP-4 and BMP-5 messenger ribonucleic acid (mRNA) was significantly reduced in the synovial tissue of patients with RA compared to controls.¹² These results, which seem to contradict each other, can be attributed to the lack of studies on this subject, particularly which should include large number of patients and relatively homogeneous groups. In addition, these contradictory results make us think to what extent local factors may be effective in the pathogenesis of inflammatory arthritis.

In the present study, we aimed to examine BMP-2 and BMP-4 levels, which have an effect on structural damage in bones in inflammatory arthritis, and to investigate the relation of these markers to disease activity in patients with AS and RA.

PATIENTS AND METHODS

This single-center, cross sectional study was conducted at Ankara University Medical School, Department of Physical Medicine and Rehabilitation, Division of Rheumatology between September 2013 and July 2015. A total of 100 AS patients (53 males, 48 females; median age: 40 years; range, 18 to 62 years), 58 RA patients (25 males, 33 females; median age: 40.5 years; range, 26 to 59 years), and 102 age- and sex-matched healthy controls (55 males, 47 females; median age: 38 years; range, 18 to 55 years) were included in the study. Patients who fulfilled the modified New York criteria and/or the Assessment of SpondyloArthritis international Society (ASAS) classification criteria for AS and those fulfilling the 1987 American College of Rheumatology (ACR) and/or 2010 ACR/European Alliance of Associations for Rheumatology (EULAR) classification criteria for RA were enrolled in the study.¹³⁻¹⁶ 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 were followed in the outpatient setting were included in this study during their routine control. Patients who had a new diagnosis during the study period were excluded. Additionally, patients who had a history of bone fracture within the last two 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, and concomitantly with any other rheumatic disease, were excluded from the study.

Disease activity was assessed through the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Ankylosing Spondylitis Disease Activity Score C-Reactive Protein (ASDAS-CRP) 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 An ASDAS-CRP score of < 1.3 , indicated inactive disease, between 1.3 and < 2.1 indicated low disease activity, between 2.1 and 3.5 indicated moderate disease activity, and > 3.5 indicated high disease activity.¹⁸ In RA patients, disease activity was assessed through Disease Activity Score in 28 joints (DAS-28) and patients with a DAS-28 score of ≤ 2.6 were considered to have inactive disease, between 2.6 and 3.2 were considered to have low disease activity, between 3.2 and 5.1 were considered to have moderate disease activity, and ≥ 5.1 were considered to have high disease activity.¹⁹

All assays were performed in the same biochemical laboratory. Among acute phase reactants, the erythrocyte sedimentation rate (ESR) and CRP were measured in all patients at their final visit. Human leukocyte antigen-B27 (HLA-B27) for AS patients and rheumatoid factor (RF) and cyclic citrullinated peptide (CCP) values for RA patients were recorded from patient files. Venous blood samples were also obtained after a minimum of 8 h of fasting to quantify plasma BMP-2, and BMP-4 levels. Samples for these biomarkers were collected in sterile containers and centrifuged within a maximum of 120 min at 4,000 rpm for 10 min and, then, stored at -80° until analysis. The serum concentrations of BMP-2 and BMP-4 were assessed using the commercial kit enzyme-linked immunosorbent assay (ELISA; Boster Biological Technology, Fremont, CA, USA). The levels of the markers mentioned above were measured according to the manufacturer's instructions. The sensitivity of the kits was < 2 pg/mL for the BMP-2 and BMP-4.

Statistical analysis

The study power and sample size calculation were performed using the G*Power software

3.1.9.5 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). With alpha error 0.05, beta error 0.20, and effect size 0.30, the minimum total number of patients required to achieve 80% power was calculated as 190.

Statistical analysis was performed using the SPSS version 21.0 software (IBM Corp. Armonk, NY, USA). Normal distribution was analyzed using the Shapiro-Wilk test and data were determined not to fit the normal distribution. Continuous data were expressed in mean \pm standard deviation (SD) or median (min-max), while categorical data were expressed in number and frequency. The Mann-Whitney U test was used to compare two independent groups, while the Kruskal-Wallis test was used to compare three or more independent groups. The Bonferroni corrected Mann-Whitney U test was used to evaluate the parameters with significant difference. The chi-square test was used to compare categorical data. Correlation of markers with disease activity and the correlation of acute phase reactants with BMP-2/BMP4 was evaluated using the Spearman correlation test. A *p* value of < 0.05 was considered statistically significant.

RESULTS

A total of 260 participants, including 100 AS (38.5%), 58 RA (22.3%) and 102 (39.2%) controls, were included in the study. There was no significant difference among the groups in terms of age and sex ($p=0.077$ and $p=0.401$, respectively). The mean disease duration in AS patients was 8.5 ± 5.6 years, while it was 6.2 ± 4.3 years in RA patients. According to the mean disease activity scores, the mean BASDAI score was 3.7 ± 2.41 , the mean ASDAS-CRP score was 1.82 ± 1.02 in AS patients, and the mean DAS-28 score was 2.92 ± 1.28 in RA patients. A total of 46% of AS patients and 36.2% of RA patients were receiving biological therapy. Demographic, clinical, and laboratory features of the patients and controls are given in Table 1.

The median value of BMP-2 was found to be 29.5 pg/mL in AS patients, 619.4 pg/mL in RA patients, and 201.6 pg/mL in the control group. The BMP-2 value was found to be significantly higher in the RA group than in the other groups and in the control group compared to the AS

Table 1. Demographics, clinical, and laboratory features of participants

	AS patients (n=100)			RA patients (n=58)			Healthy controls (n=102)			p	
	n	%	Mean±SD	Median	Min-Max	n	%	Mean±SD	Median		Min-Max
Age (year)				40	18-62				38	18-55	0.077
Sex											
Male	52	52			25	43.1				55	53.9
Female	48	48			33	56.9				47	46.1
Disease duration (year)			8.52±5.63					6.18±4.27			-
ESR (mm/h)				11.5	1-51				25	1-83	<0.001
CRP (mg/L)				2.9	1-61.5				6.1	0-96.8	0.002
Biologic treatment	46	46			21	36.2					-
HLA-B27	68	68			-	-					-
RF+	-	-			43	74.1					-
CCP+	-	-			40	69					-
BASDAI (range 0-10)			3.7±2.41								-
ASDAS-CRP (units)			1.82±1.02								-
DAS-28			-					2.92±1.28			-

AS: Ankylosing spondylitis; RA: Rheumatoid arthritis; SD Standard deviation; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; HLA: Human leukocyte antigen; RF: Rheumatoid factor; CCP: Cyclic citrullinated peptide; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; ASDAS: Ankylosing Spondylitis Disease Activity Score; DAS-28: Disease Activity Score-28; p<0.05 was considered statistically significant.

Table 2. Comparison of BMP-2 and BMP-4 levels between groups

	AS (n=100)		RA (n=58)		Control (n=102)		p*
	Median	Min-Max	Median	Min-Max	Median	Min-Max	
BMP-2 (pg/mL)	29.5	2-1776.2	619.4	106.3-7467.1	201.6	2-1145.9	<0.001
BMP-4 (pg/mL)	534.8	4-3464.9	522.7	210.5-50005	511.9	163.8-2624.4	0.292

BMP-2: Bone morphogenetic protein-2; BMP-4: Bone morphogenetic protein-4; AS: Ankylosing spondylitis; RA: Rheumatoid arthritis; p<0.05 was considered statistically significant; * Statistical significance among three groups.

group (p<0.001). The median value of BMP-4 was 534.8 pg/mL in AS patients, 522.7 pg/mL in RA patients, and 511.9 pg/mL in the control group, and no significant difference was found among the groups (p>0.05) (Table 2).

In the examination performed by classifying AS patients as active and inactive disease according to BASDAI values, no significant difference was found

between BMP-2 and BMP-4 levels and disease activity (p>0.05) (Table 3). Similarly, no significant difference was found between disease activity and BMP-2 and BMP-4 levels in AS patients according to ASDAS-CRP (p>0.05) (Table 4). There was no significant difference between both markers and disease activity evaluated according to DAS-28 score in RA patients (p>0.05) (Table 5).

Table 3. Levels of BMP-2 and BMP-4 according to BASDAI score in patients with AS

	Inactive disease (n=70)		Active disease (n=30)		p*
	Median	Min-Max	Median	Min-Max	
BMP-2 (pg/mL)	23.7	2-1776.2	59.8	2-1676.5	0.435
BMP-4 (pg/mL)	543.9	4-3464.9	465.3	64.8-1938.9	0.412

BMP-2: Bone morphogenetic protein-2; BMP-4: Bone morphogenetic protein-4; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; AS: Ankylosing spondylitis.

Table 4. Levels of BMP-2 and BMP-4 according to ASDAS-CRP score in patients with AS

	Inactive disease (n=22)		Low disease activity (n=34)		Moderate disease activity (n=40)		High disease activity (n=4)		p*
	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max	
BMP-2 (pg/mL)	21.2	2-1776.2	25.7	2-1264.8	38.9	2-1676.5	33.8	2-1236.2	0.505
BMP-4 (pg/mL)	513.3	4-2814.1	675.5	46.9-3464.9	429.9	64.9-2749.6	590.6	225.9-1938.9	0.431

BMP-2: Bone morphogenetic protein-2; BMP-4: Bone morphogenetic protein-4; ASDAS: Ankylosing Spondylitis Disease Activity Score; CRP: C-reactive protein; AS: Ankylosing spondylitis.

Table 5. Levels of BMP-2 and BMP-4 according to DAS-28 score in RA patients

	Inactive disease (n=22)		Low disease activity (n=19)		Moderate disease activity (n=20)		High disease activity (n=8)		p*
	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max	
BMP-2 (pg/mL)	606.4	106.3-7467.1	489.9	119-1596.1	638.9	195.2-1172.5	763.1	386.9-1105.9	0.323
BMP-4 (pg/mL)	504.8	421.8-50005	528.6	210.5-4869	546.5	268.9-13382.1	492.9	433.6-1274.1	0.869

BMP-2: Bone morphogenetic protein-2; BMP-4: Bone morphogenetic protein-4; DAS-28: Disease Activity Score 28; RA: Rheumatoid arthritis

Table 6. Correlations between acute phase reactants and BMP-2 and BMP-4 levels

			BMP-2	BMP-4
Rheumatoid arthritis	ESR	Rho	0.120	0.240
		<i>p</i>	0.429	0.069
	CRP	Rho	0.087	0.207
		<i>p</i>	0.884	0.120
Ankylosing spondylitis	ESR	Rho	0.010	0.106
		<i>p</i>	0.918	0.293
	CRP	Rho	-0.045	0.089
		<i>p</i>	0.658	0.379

BMP-2: Bone morphogenetic protein -2; BMP-4: Bone morphogenetic protein-4; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; Rho: Spearman correlation coefficient.

When the correlation between acute phase reactants and BMP levels was examined, no significant correlation was found between ESR and CRP levels and BMP-2 and BMP-4 levels in RA and AS patients (Table 6).

DISCUSSION

In the present study, serum BMP-2 levels were significantly higher in the RA group compared to the other groups and in the control group compared to the AS group. No significant relationship was found between serum BMP-2 and BMP-4 levels and disease activities in both AS and RA patients, while there was a weak positive correlation between ESR and CRP levels with BMP-2 level in RA patients.

In inflammatory arthritis, inflammation induces bone resorption and causes insufficient bone formation, resulting in local and systemic bone loss. While bone loss is a predominant feature in RA, inflammation in AS also induces pathological bone formation in the enthesal regions.²⁰ In this process, a large number of inflammatory/non-inflammatory intertwined pathways are involved, and one of the most important pathways is BMPs. The BMP signaling pathways play a critical role in the regulation of osteoblast in rheumatic diseases. Proinflammatory molecules such as TNF- α , IL-6, and IL-17 in the inflamed joint are known to modulate BMP signaling, and altered BMP signaling is not only crucial for osteoblasts

and their progenitors, but also affects other cells such as fibroblasts and macrophages. Although it appears that inflammation may have a clear stimulatory effect on the BMP signaling pathway, their true effect in arthritis changes, whether the rate of BMP-dependent bone formation is sufficient to counteract the increased rate of bone resorption depending on the underlying disease and the result of these complex processes appears as erosion in RA and bone formation in AS.²⁰ In addition, a time-dependent activation of the BMP signaling pathway was demonstrated in synovium and cartilage of collagen-induced arthritis as a model of inflammatory arthritis, indicating that the level and effects of markers may vary depending on the duration of the disease.²¹ In their study, Park et al.²² demonstrated changes in serum BMP-2 and BMP-7 levels during follow-up of AS patients; however, there is no homogeneous group regarding the treatments received during the follow-up period. All these findings also complicate our understanding of these complex processes.

In AS patients, inflammation beginning in the vertebral entheses (particularly by TNF- α) leads to erosion in the cartilage and bone. Then, fibrous and adipose tissue infiltration occurs in these lesions and, eventually, ossification takes place, resulting in abnormal bone formation (syndesmophytes) which is associated with radiographic progression.²³ The BMPs are the important pathway contributing to radiographic progression in AS patients. In a mouse model, BMP-2/6/7 was shown to be immunohistochemically overexpressed in entheses.²⁴ In a study by Bleil et al.,⁹ BMP-2 and BMP-7 were contrarily shown to be at low levels in facet biopsies in AS patients. In a study conducted by Chen et al.,⁵ serum BMP-2, BMP-4, and BMP-7 levels were increased and associated with radiographic progression. In our study, serum BMP-4 levels were similar to those of the RA patients and control group; however, BMP-2 levels were lower than both of them. In another study on AS patients, fibroblasts cultured with BMP-2 exhibited more osteoblastic effects than osteoarthritis.²⁵ Thus, in pathogenetic understanding, a low serum BMP-2 level may not indicate less osteoinductive effect in terms of possible local effects in our study.

It was demonstrated that inflammatory cytokines had an inducing effect on BMP-2 and BMP-6 in the early period of the disease, which might possibly indicate the beginning of the bone formation process that started after inflammation and gained autonomy.²³ The TNF- α activates osteoclasts combined with IL-17 at the beginning of inflammation and leads to erosion. 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.^{3,26} All these results suggest that the levels of markers that contribute to bone turnover can change over time. On the other hand, we did not encounter any other studies in the literature investigating the levels of BMPs before and after anti-TNF- α or another treatment. Therefore, it is not certain to explain whether the change in BMPs is only associated with the duration of the disease, as possible effects of the treatment may contribute to this situation.

Rheumatoid arthritis is characterized by synovial inflammation and hyperplasia, leading to cartilage and bone destruction.¹⁰ Several studies examining the relationship between RA and BMPs in the literature show contradictory results, as in AS. In a study by Biver et al.,¹⁰ serum concentrations of BMP-2 and BMP-7 were found to be higher in patients with RA compared to healthy controls. Similarly, in our study, we found serum BMP-2 levels were significantly higher in the RA group compared to the AS and control group; however, there was no significant difference in term of BMP-4 level. In another study, the mean BMP-2 levels of both AS patients and RA patients were significantly higher than that of controls, but the difference between AS and RA patients was not significant. Neither the mean BMP-4 level of the AS group nor that of the RA group was significantly different from that of controls, but the difference between AS and RA group was no significant. The mean BMP-7 level of AS patients was significantly higher than those of RA patients and controls in the same study.²² In a study examining local levels of BMPs in RA patients, the BMP-4 and BMP-5mRNA expression was found to be significantly reduced in the synovial tissue of patients with RA

compared to controls.¹⁰ This result makes us think that the effects of BMPs at the local level rather than the serum level are more prominent, similar to in AS patients, as locally decreased BMP levels in the context of RA result in impaired repair mechanism, which contributes to the development of erosion. Therefore, in our study, high BMP levels in RA patients may not be associated with local bone turnover.

In our study, we found no significant association between BMPs and disease activity scores in both AS and RA patients. The fact that there is no relationship between ESR and CRP values with BMPs supports this result. Studies examining this issue are very few in the literature, and conflicting results have been reported and most of them are not prospective and not included homogeneous treatment groups.^{5,10,11,22,27} An investigated RA model showed that TNF- α blockers had no effect on the BMP pathway in the synovium.¹⁰ Similarly, another study examining the human synovium showed that anti-rheumatic treatments had no effect on BMP activation in the synovium.²⁸ Although we reported the percentages of patients taking TNF- α blockers in our study, we could not find any publication in the literature examining BMP levels before and after treatment in a randomized fashion. Therefore, we have no clear information about the change in at least the serum level of BMPs after TNF- α blocker. Although there are conflicting publications in the literature, in the light of current information, our opinion is that these pathways would not be directly related to disease activity, except for probably in the early stages of RA and AS, that is, after the non-inflammatory pathways in the pathogenesis of the diseases gain autonomy.

It seems that the true effect of BMPs is strongly context-dependent and influenced by the local milieu of cells, cytokines, and growth factors and also the duration of disease (early or late period). Thus, local functionality of the BMPs that may contribute to progression of the disease may be more important than their serum levels.

On the other hand, one of the most important limitations to our study is its cross-sectional design and, therefore, that it does not contain homogeneous groups in terms of treatment. The fact that we did not examine BMPs other than BMP-2 and BMP-4 can be considered another limitation; however, previous studies have shown

that the osteoinductive effects of BMP-2 and BMP-4 are more compared to others.²⁹ The inclusion of HLA-B27 results in all AS patients who participated in our study, and those with RF and CCP results in RA patients may have caused bias and, thus, newly diagnosed patients were not included in our study. Again, the lack of radiological evaluations of the patients in our study can be regarded as a limitation, although we believe that radiological data would not provide sufficient information in a cross-sectional study. Additionally, examining the markers of other pathways such as Wnt in bone turnover would have made the study more valuable.

In conclusion, serum levels of BMPs may theoretically give inconsistent results while considering the pathogenesis of the underlying disease. Contrary to expectations, BMP-2 level was significantly higher in RA patients compared to AS patients in our study. As mentioned before, this finding highlights the importance of the activity at the local level, *albeit* indirectly. Moreover, the same bone marker may exert paradoxical effects on different arthritis prototypes; Differences in bone markers may be associated with marked differences in the pathways for bone remodeling in RA and AS. The BMPs can be viewed as part of the complex network of pathways that determine the outcome of the underlying arthritis remodeling, and to further elucidate this issue, we need the results of randomized longitudinal studies, particularly including early stage patients.

Ethics Committee Approval: The study protocol was approved by the Ankara University Faculty of Medicine Ethics Committee (no: 2013-04-162-13). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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