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Research Article

Role of Bone Morphogenetic Protein-2 in Primary Knee Osteoarthritis

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Abstract

Introduction: Many different factors contribute to the onset and progression of osteoarthritis (OA). The bone morphogenetic proteins (BMPs), that are subsets of the transforming growth factor (TGF- ß) superfamily, have a known protective role on cartilage. They are lately gaining attention for their questionable role in osteophyte synthesis and osteoarthritis pathogenesis.

Aim of the work: The aim of this study was to investigate the role of plasma BMP-2 in primary knee osteoarthritis and its relation to disease severity.

Methodology: The study included 30 patients with primary knee O.A and ten apparently healthy matched individuals as a control group. Pain intensity as well as, assessment of functional status using the Western Ontario and McMaster University Osteoarthritis Index (WOMAC), plasma levels of BMP-2, and radiological severity of the disease, were done.

Results:

- Plasma levels of BMP-2 were significantly higher in patients than in control group.
- Patients with palpable osteophytes had the highest BMP-2 levels.
- A strong positive correlation was found between plasma levels of BMP-2 and each of: radiological severity, disease duration and WOMAC score.

Conclusion: BMP-2 levels correlate with radiographic severity of O.A. which make such biomarker measurement may not only act as a substitute marker for the disease, but also as an indicator of the disease severity and a potential future tool to assess the processes underlying the pathogenesis of O.A.

Keywords: *Bone Morphogenetic Protein; BMP-2; Knee Osteoarthritis*

Abbreviations

BMPs: Bone Morphogenetic Proteins; TGF- ß: Transforming Growth Factor; WOMAC: Western Ontario and McMaster University Osteoarthritis Index

Introduction

Osteoarthritis, is the most common degenerative chronic disorder of the joints, affecting nearly half the elderly population worldwide. The disease results from an imbalance in the dynamic equilibrium between the breakdown and repair of the joint tissues [1].

In contrast with degradation occurring in O.A, new bone formation is often observed elsewhere in the joint. This might be a remodeling process initiated as a response to injury, resulting in osteophytes at the joint margins. The molecular pathways principally involved in cartilage and bone changes in knee OA are gaining more interest [2].

The bone morphogenetic proteins (BMPs), which are subset of the transforming growth factor B (TGF- ß) superfamily, are produced by mesenchymal cells, osteoblasts and chondrocytes [3]. They are involved in protection against cartilage destruction and in formation of new cartilage, as can be found during osteophyte formation. They stimulate production of extracellular matrix (ECM) components by chondrocytes and have the ability to counteract catabolic cytokines like interleukin 1 (IL-1) [4].

It has been proposed that BMP-2 particularly, may be one of the most potent inducers of mesenchymal cell differentiation to osteoblasts, while the remaining BMPs promote the maturation of committed osteoblasts [5]. Moreover, BMP-2 controls the expression of several other BMPs and when its activity is blocked, marrow stromal stem cells fail to differentiate into osteoblasts [6].

Although BMP-2 can induce cartilage formation, it was found that its expression in healthy cartilage was low but that its expression was elevated in areas surrounding cartilage lesions and in OA cartilage [7]. This could indicate that BMP-2 is not just upregulated as a reparative response as have been previously thought, but may be as a pathological side effect, thereby stimulating further injury. Whatever the explanation might be, it raises the possibility that BMP-2 plays a critical role in the pathogenesis of knee OA that should be furtherly investigated. It would be attractive if we find a biological marker playing a definite role in joint remodeling and OA progression.

Objective

The present study aimed to investigate the role of plasma BMP-2 in primary knee osteoarthritis and its relation to disease severity.

Materials and Methods

This study was conducted on thirty symptomatic patients with primary knee osteoarthritis according to the American College of Rheumatology (ACR) criteria [8]. In addition, 10 healthy asymptomatic individuals matched for age and sex were included serving as a control group. Patients were recruited from Physical Medicine, Rheumatology and Rehabilitation outpatient clinic of Ain-Shams University Hospitals.

All patients and controls were subjected to full medical history taking, thorough clinical examination and laboratory testing. Pain intensity for the patients was determined using the Numerical Pain Rating Scale (NPRS). The patient was asked to make three pain ratings, corresponding to current, best and worst pain experienced over the past 24 hours and the average of the 3 ratings was used [9].

Assessment of functional status of the patients was done using the Western Ontario and McMaster University Osteoarthritis Index (WOMAC). (It is a self-administered, disease specific health related quality of life instrument that asks the patients questions regarding rheumatic symptoms, stiffness, pain and how it affects the ability to function. It produces one aggregate total score and scores for three subscales: pain, stiffness, and physical functioning.) The Likert version is simple to use and offers 5 response options ranging from 'none' to 'extreme'. A response of 'none' is scored as 0, 'mild' as 1, 'moderate' as 2, 'severe' as 3, and 'extreme' as 4 [10].

The laboratory investigations done included: CBC, ESR, CRP, serum uric acid, alkaline phosphatase, kidney and liver function tests, FBS, and RF. Plasma levels of BMP-2 was measured using direct ELISA technique.

For the patients, plain x-rays for both knees were done to assess the radiological severity of the disease using K-L grading system; Kellgren and Lawrence, 1957 [11].

The patients with the following disorders were basically excluded from our study: non-degenerative arthritis, seronegative spondylo-arthropathies, Diabetes Mellitus, previous knee injury or operation and all types of malignancies.

All patients and controls were informed about our research work in details and consents were taken before starting any procedure.

Statistics

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001). Data was presented and suitable analysis was done according to the type of data obtained for each parameter.

Results and Discussion

This study was conducted on thirty symptomatic primary O. A patients: 18 females and 12 males .Their ages ranged from 44 - 70 years with mean of 55.7 ± 7.46 and their disease duration ranged from 1 - 17 years with a mean of 5.63 ± 4.53.Their BMI (kg/m^2) ranged from 24.34 - 34.71 with a mean of 27.89 ± 2.49. Ten apparently healthy, asymptomatic individuals matched for age, sex and BMI served as control group. The main clinical findings of our patients are shown in table 1.

Clinical manifestations	Patients (30)	
	Number	%
Pain	30	100%
Crepitus	30	100%
Tenderness	30	100%
Palpable bony enlargement	2	6.7%
Patellofemoral test	21	70.0%
Synovial hypertrophy	8	26.7%
Effusion	10	33.3%
Quadriceps disuse wasting	10	33.3%
Ligament laxity	2	6.7%
Deformity	13	43%

***Table 1**: Incidences of clinical manifestations among our patients.*

Numerical clinical and laboratory data of the patients are shown in table 2.

Parameters	Range	Mean ± S.D.
Age (years)	44 - 70	55.7 ± 7.46
BMI (kg/m^2)	24.34 - 34.71	27.89 ± 2.49
Disease duration (years)	1 - 17	5.63 ± 4.53
NPRS for pain	1 - 9	4.6 ± 2.19
Morning stiffness (minutes)	0 - 10	2.43 ± 3.29
Thigh circumference (cm)	0 - 3.2	0.91 ± 1.32
Degree of flexion deformity	0 - 30	3.83 ± 7.50
WOMAC score	16 - 65	28.2 ± 11.34
ESR (mm/hr)	5 - 30	16.3 ± 6.29
Hb (g/dl)	9 - 15.1	11.87 ± 1.90
WBC ($10^3/mm^3$)	5 - 11.1	8.48 ± 1.87
PLT ($10^3/mm^3$)	180 - 426	292.03 ± 72.61
AST (u/l)	8 - 29	19.77 ± 5.98
ALT (u/l)	10 - 33	20.97 ± 6.43
ALP (u/l)	100 - 390	232.1 ± 68.85
BUN (mg/dl)	5 - 19	11.97 ± 3.59
S.creat. (mg/dl)	0.2 – 1.4	0.74 ± 0.34
S.uric acid (mg/dl)	1.3 - 6	3.86 ± 1.00
FBS (mg/dl)	60 - 105	81.07 ± 13.39
BMP-2 (pg/ml)	5500 - 17500	7473.33 ± 2336.21

***Table 2:** Descriptive parameters of the patients.*

BMI: Body mass index; NPRS: Numerical pain rating scale; WOMAC: The Western Ontario and McMaster University Osteoarthritis Index; ESR: Erythrocyte Sedimentation Rate; Hb: Haemoglobin; WBC: White Blood Cells; PLT: Platelets; AST: Aspartate Transaminase; ALT: Alanine Transaminase; BUN: Blood Urea Nitrogen; FBS: Fasting Blood Sugar BMP: Bone Morphogenetic Protein

Plasma levels of BMP-2 were significantly higher in patients than control group (Table 3 and Figure 1).

Parameter	Patients (N = 30)	Controls (N = 10)	t	P	Sig.
	Mean ± S.D.	Mean ± S.D.			
BMP-2 (pg/ml)	7473.33 ± 2336.21	3650 ± 1639.28	5.695	< 0.01	HS

Table 3: *Comparison between patients and controls as regards mean BMP2 levels.*

BMP: Bone Morphogenetic Protein; HS: Highly Significant

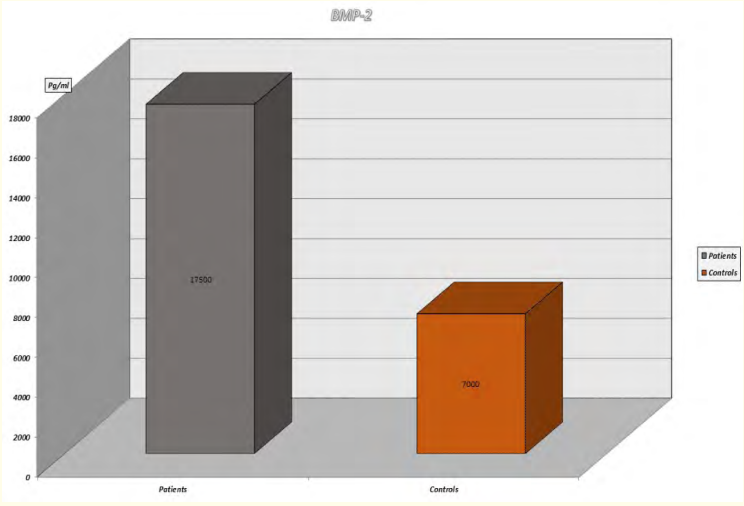

Figure 1: *Comparison between patients and controls regarding plasma BMP-2 levels.*

BMP2: Bone Morphogenetic Proteins 2

As for the radiological assessment, the highest percent of our patients were among Grade III K-L score (Table 4).

Parameter		No	%
K-L grading score	Grade 1	0	00.0%
	Grade 2	12	40.0%
	Grade 3	14	47%
	Grade 4	4	13%

Table 4: *Radiological classification of patients according to K-L score.*

KL: Kellgren and Lawrence

Comparison between the patients with K-L different grades and different parameters revealed a highly statistical significant increase in BMP-2 levels among higher K-L grades where patients with G4 scores had highest BMP-2 levels, age, disease duration and WOMAC score (P< 0.01) and no significant differences regarding other parameters (p > 0.05) (Table 5). Patients with palpable osteophytes had the highest BMP-2 levels.

Data	Grade 2 (N = 12)	Grade 3 (N = 14)	Grade 4 (N = 4)	P	Sig.
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.		
Age (years)	50.5 ± 5.63	57.64 ± 6.32	64.5 ± 4.43	< 0.01	HS
BMI (kg/m^2)	27.70 ± 2.10	28.01 ± 2.16	27.72 ± 4.81	> 0.05	NS
Disease duration (years)	2.58 ± 2.61	6 ± 3.1	13.5 ± 3.51	< 0.01	HS
Morning stiffness (minutes)	2.43 ± 2.59	0.33 ± 0.78	5.75 ± 1.5	> 0.05	NS
WOMAC score	20.67 ± 3.14	27.64 ± 4.48	52.75 ± 9.39	< 0.01	HS
BMP-2 (pg/ml)	6208.33 ± 492.6	7207.14 ± 599.3	12200 ± 3713.9	< 0.01	HS

Table 5: *Comparison between patients with K-L different grades as regards parametric clinical data:*

WOMAC: The Western Ontario and McMaster University Osteoarthritis Index; BMP: Bone Morphogenetic Protein

NS: non-significant; HS: Highly significant

Correlation studies between BMP-2 levels and different studied clinical and radiological parameters revealed a highly significant positive correlation between BMP-2 and disease duration as well as WOMAC score ($p < 0.01$) and no correlation with other parameters ($p > 0.05$); (Table 6, Figures 2 and 3).

Parameters	BMP-2		Sig.
	R	P-value	
Age (years)	0.358	> 0.05	NS
Disease duration (years)	0.778	< 0.01	HS
BMI (kg/m^2)	0.042	> 0.05	NS
Pain (NPRS)	0.475	> 0.05	NS
Morning stiffness (minutes)	0.677	> 0.05	NS
Synovial hypertrophy	0.051	> 0.05	NS
Effusion	0.293	> 0.05	NS
Thigh circumference (cm)	0.67	>0.05	NS
Degree of flexion deformity	0.699	> 0.05	NS
Varus deformity	0.721	> 0.05	NS
WOMAC score	0.507	< 0.01	HS
ESR (mm/hr)	0.077	> 0.05	NS
FBS (mg/dl)	0.168	> 0.05	NS

Table 6*: Correlation between BMP-2 level and the studied parameters.*

BMI: Body Mass Index; NPRS: Numerical Pain Rating Scale; WOMAC: The Western Ontario and Mcmaster University Osteoarthritis Index; ESR: Erythrocyte Sedimentation Rate; FBS: Fasting Blood Sugar; BMP: Bone Morphogenetic Protein

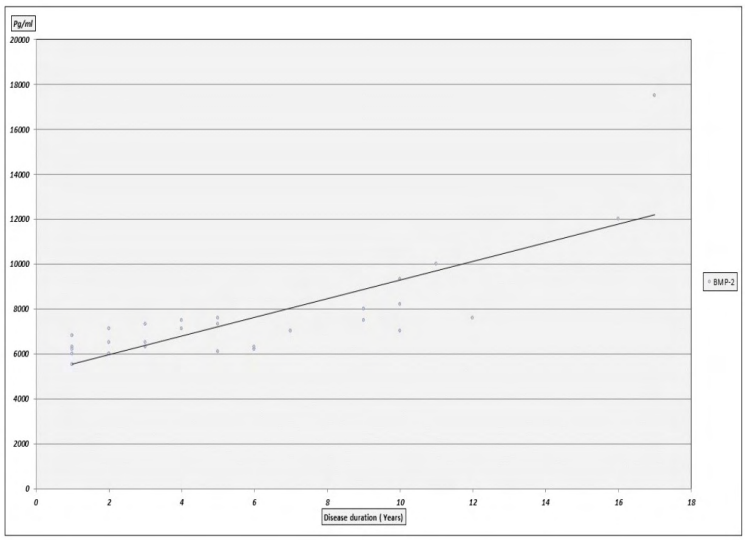

Figure 2: *Correlation between plasma BMP-2 levels and disease duration.*

BMP2: Bone Morphogenetic Proteins 2

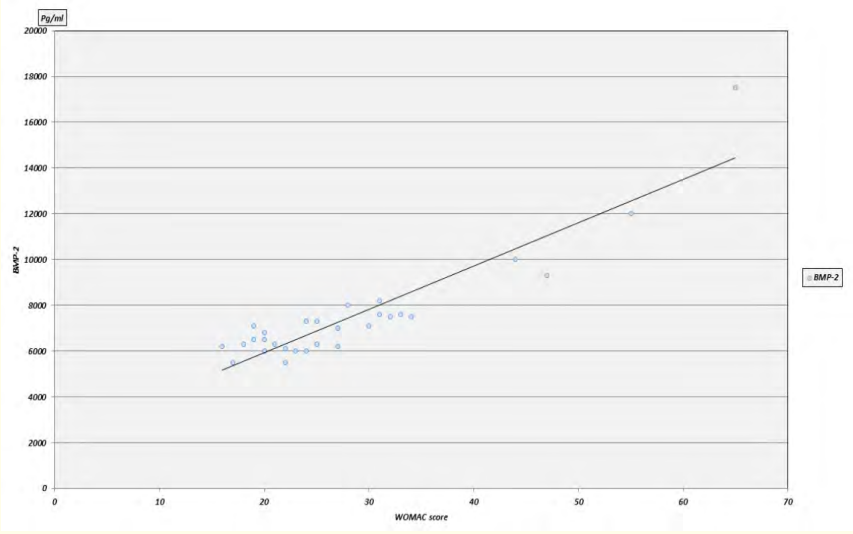

Figure 3: *Correlation between plasma BMP-2 levels and WOMAC score.*

BMP2: Bone Morphogenetic Proteins 2; WOMAC: Western Ontario and Mcmaster University Osteoarthritis Index

The validity of BMP2 was evaluated and the cut off value was calculated among the studies groups (Table 7, figure 4). Plasma BMP-2 levels were higher than cut-off value in all our patients.

	Efficacy	Cut-off value	Sensitivity	Specificity	PPV	NPV
BMP-2	95.0	4000	100.7	80.0	93.8	100.0

Table 7: *Validity of BMP-2 in diagnosis of O.A.*

PPV: Positive Predictive Value; NPV: Negative Predictive Value; BMP: Bone Morphogenetic Protein

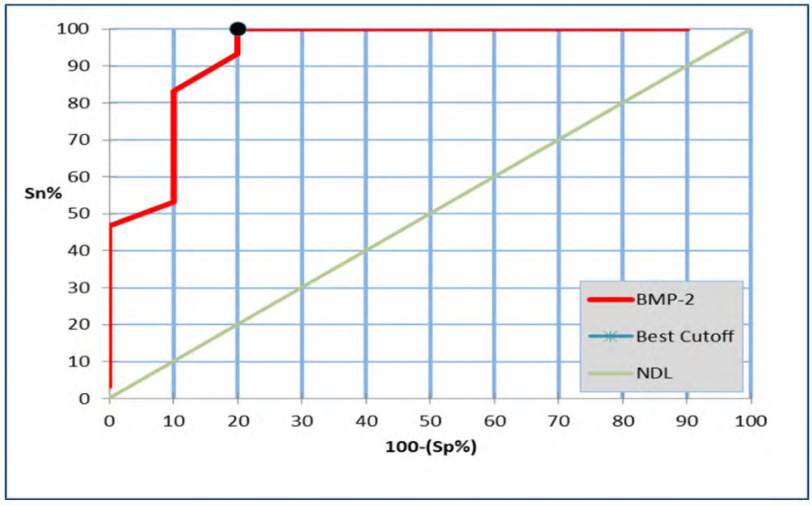

Figure 4: *Receiver operating characteristic (ROC) curve analysis showing the diagnostic performance of BMP-2.*

BMP2: Bone Morphogenetic Proteins 2; Sn: Sensitivity; Sp: Specificity; NDL: Non-Diagnostic Line.

Discussion

Using prognostic biomarkers in OA to identify patients at high risk for pro¬gression could reduce the mortality of the disease and may also help facilitate the development of disease-modifying os¬teoarthritis drugs [12] We have chosen to study BMP-2 for the lately rising debate about its role in knee OA (reparative or injurious), being among the most potent anabolic factors for articular cartilage [13]. Though it has been lately known of its capability to induce matrix synthesis and promote cartilage repair yet, it's puzzling why its role in knee OA is still unclear [13-17].

The higher values of plasma BMP-2 among our patients could be best explained by Blaney Davidon., *et al*. 2009 who noticed that TGF- β was found to be elevated in early O.A affected cartilage and tend to decrease until no longer expressed at later stages of osteophyte development and other factors such as BMP-2 are involved in further progression of osteophyte formation and maturation [18].

While searching in literature, we have found a single research by (Liu., *et al*. 2015) studying BMP2 levels in 38 primary knee OA patients and 20 controls where their patients manifested significantly higher BMP2 levels in serum and synovium. They suggested that the mechanism of this increase may be attributed to either secretion of BMP-2 residing in extracellular matrix, increased BMP-2 synthesis, or both [19].

Interestingly, our patients with G4 K-L scores had highest BMP-2 levels. Osteophytes result from bone deposition, which is linked to osteoblast activity. Their formation is directly controlled by BMPs, which potentially synergizes with other bone formation pathways such as those mediated by the TGF and Wingless signaling [20] Moreover, the chondrogenic phase of osteophyte formation is accompanied by high levels of proteoglycan synthesis and both TGF_ and BMP-2 have been shown to induce chondrogenesis in stem cells *in vitro* [13].

The positive correlation between plasma levels of BMP-2 and disease duration as well as WOMAC score can be enlightened by the progressive nature of O.A. So, as the disease progresses, osteophytes increase. While BMP-2 has been implicated in osteophyte formation and maturation and since in our study the patients with K-L different grades revealed a highly statistical significant increase in plasma BMP-2 levels, thus, patients with longer disease duration may show higher plasma BMP-2 levels.

Liu., *et al*. (2015) again agreed with our findings as they illustrated a pronounced positive correlation of se¬rum and synovial BMP-2 levels with the degree of radiographic and symptomatic severity in patients with knee OA. They assumed that the increased levels of BMP-2 in serum and SF may be a reparative response to degenerative changes in OA joints. The BMP-2 in SF may originate from synovial cells and chondrocytes in the local tissues [19].

On the other hand, Ay and Evcik, (2008) reported that radiographic diagnosis of osteoarthritis was not related to functional capacity and that function in knee OA is determined more by pain rather than structural changes seen on plain radiographs [21]. Actually, this is somehow compatible with our outcome as WOMAC score assess pain, stiffness and functional subscales and doesn't depend on radiological grading but as disease progress (evidenced by K-L grading), osteophytes and changes in the bone around the joint limit the function and increase pain decreasing functional ability increasing WOMAC scores.

Thus, a correlation of BMP-2 with all 3 categories of O.A entity pain, disability, and joint stiffness is implied. So, plasma BMP-2 levels, besides being higher in O.A, were increased in proportionally to severity.

Our findings revealed that, the plasma BMP-2 levels were higher than cut-off value in all our patients, suggesting the unquestionable contribution of BMP-2 to the pathogenesis of primary O.A. It might act as a future biochemical marker predicting disease severity. Per our results, we hypothesize that for diagnosis of primary knee O.A, plasma BMP-2 levels should be equal to or higher than 4000 pg/ml. As a test, it proved to be sensitive and specific (100% and 80% respectively).

We, too, assume it might be related to the reparative process occurring in knee OA though it is impossible to determine a definite cause-and-effect relationship and draw a strong final conclusion.

It should be pointed out, however, that a limitation of this study is that the sample size was not large enough to make wider correlations. Our preliminary data should be confirmed among a greater number of patients and controls. Additionally, the effect of joint sites other than the knee need to be considered.

Conclusion

Patients with primary knee O.A had elevated levels of plasma BMP-2 compared with healthy controls. Its concentration correlated significantly with the magnitude of O.A duration and radiographic progression. BMP-2 measurement may not only serve as a biochemical

marker for disease progression, but also as an indicator of the disease severity and a potential future tool to assess the processes underlying the pathogenesis of O.A.

Future research should be done to provide indications for either supplementation or inhibition of specific BMPs, or their various signaling pathways, to treat O.A.

Conflict of Interest

Authors declare no conflict of interest.

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Volume 6 Issue 2 April 2017