Acta Orthop. Belg., 2014, **80**, 216-221

ORIGINAL STUDY

The importance and the differences of bone morphogenetic proteins for osteoporotic hip fractures

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Bone morphogenetic proteins (BMPs), major contributors to tissue repair, have become one of the most exciting fields in rheumatic and orthopaedic research. In our study we aimed to evaluate the relationship between osteoporotic hip fractures and the serum levels of BMPs to reveal their potential roles in the diagnosis of patients. The study group included 62 patients with osteoporotic hip fracture (Group 1 ; intertrochanteric fracture, Group 2 ; collum femoris fracture) and the control group. All fractures were due to low energy trauma, simple falls. For all subjects BMD measurements were in agreement for osteoporosis and no significant differences were observed between the two fracture groups. Biochemical markers ; BMP-4 and BMP-7 (pg/mL) were determined by commercial Elisa kits from the serum samples. The mean and standard error values of serum samples for BMP-4 and BMP-7 in Group 1 (100.70 ± 10.03, 74.41 ± 6.31 respectively) and in Group 2 (112.34 ± 11.52, 81.91 ± 10.14 respectively) were not statistically different however for both groups only BMP-7 values increased statistically when compared to the control group. BMP-7 measurements may not only serve as potential biochemical markers for determining disease severity but also the increased levels, an osteogenic factor and bone stimulating agent in vivo, after trauma elevated levels are adaptive or protective and therefore may reduce the severity of the fracture.

Keywords : osteoporotic hip fracture, bone morphogenetic proteins, risk assessment

INTRODUCTION

Bone constantly undergoes remodelling to repair and replace its bone tissue. In aging the amount of bone tissue gradually declines and structural elements are lost. Elderly subjects are at high risk for bone fragility fractures because of senile alterations of bone mineral density (BMD), falls, nutrition and life-style, rate of bone remodelling, and changes in microstructure. In aging men and women, low BMD can be the result of increased rate of skeletal remodelling with bone loss. Besides the vast majority of osteoporotic hip fractures result from fall-related minor trauma to a proximal femur (*5,15*).

Bone morphogenetic proteins (BMPs) belong to the TGF-β family members. Up to date there are more than 30 BMPs' described and their primary task is to shape and guide body morphology and to maintain its integrity. BMPs can improve fracture healing as a local stimulator both through increasing osteogenesis and creating a favourable healing

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No benefits or funds were received in support of this study.

environment by altering cytokine release by endogenous cells. BMP2 and 7 have recombinant forms which are available for limited clinical use. BMPs can also activate the osteoblastic lineage from mesenchymal precursors (*2,16,20,22*).

Future concepts of bone pathologies will focus on a more complex management with the reduction or antagonism of destructive processes by including signals like TGF-β and members of its family, such as BMPs. Therefore analysing the expression, mechanism of action and gene regulation of BMP's, which are the major players of tissue repair, has become one of the most exciting fields in rheumatologic and orthopaedic research.

In our study we aimed to evaluate the relationship between the levels of BMPs and osteoporotic hip fractures in order to speculate on the diagnosis of patients.

MATERIAL AND METHODS

The study was approved by the Hospital Ethical Committee (0186/26.04.2006) and informed consent was obtained from all subjects before surgical procedures. It was a prospective cohort study. Thirty-eight of the total 62 hip fractures (Group 1 ; 23 female/15 male) were intertrochanteric fractures and 24 of the total (Group 2 ; 18 female/6 male) had collum femoris fractures. All fractures were due to low energy trauma, simple falls. None of the patients had neoplastic pathology of bone, long-term corticosteroid usage, bone metabolism disease or arthritis and any other metabolic disease. Control group had twenty-one subjects (Group 3 ; 18 female/3 male) whom were all healthy elderlies.

BMD (bone mineral density) measurements were done with Lunar DXA. The measurements were performed on the intact side of the hip at fracture groups. BMD measurements were obtained as femoral neck, wards, trochanteric and total BMD values.

Peripheral venous blood samples were taken in to tubes in the fasting state (12 h) from all subjects within the first twenty-four hours after the fracture and early in the morning. They were centrifuged at 3,000×g for 10 min for serum separation. All samples were stored at -80°C until analyses. Human BMP-7 (pg/mL) levels were measured by R&D Systems Quantikine Human BMP-7 (Lot No : 277375) and RayBio ELISA kit. Human serum BMP-4 (pg/mL) values were determined by Cell sciences ELISA kit.

In all groups, mean ± standard deviation (SD) values were calculated for age, urea, creatinine and BMD. In order to establish a difference in these parameters between the two groups, Mann Whitney-U test was then used. Parameters with values of $P < 0.05$ were considered statistically significant. All statistical analysis was performed with the SPSS (Statistical Package for Social Sciences) for Windows v. 10 (SPSS Inc, Chicago, IL) program.

RESULTS

The total number of patients in the study group were 62 patients ; 41 females and 21 males, mean ages were 76.26 ± 6.65 and 77.28 ± 7.32 years respectively. Thirty-eight of them (Group 1 ; 23 female/15 male, mean age 77.52 ± 6.74 years) had intertrochanteric fractures and 24 of them (Group 2 ; 18 female/ 6 male, mean age 75.16 ± 6.89 years) had collum femoris fractures. The mean age of the control group (Group 3) was 73.88 ± 7.36 years. The neck, trochanter, wards and total BMD values of the patients were in agreement for osteoporosis and no statistically significant differences were observed between the patient groups for all BMD values. However, only the trochanter and total BMD values between the genders were significantly different in Group 2. From biochemical parameters ; urea (mg/dL) and creatinine (mg/dL) levels were not different between the two patient groups. However, within the groups creatinine levels were significantly different between the genders (Group 1 ; p = 0.001 and Group 2 ; p = 0.04) (Table I).

The mean and standard errors for BMP-4 (pg/mL) and BMP-7 (pg/mL) levels in Group 1 (adequate serum samples n = 34, 100.70 ± 10.03, 74.41 ± 6.31 respectively) and in Group 2 (adequate serum samples n = 21, 112.34 ± 11.52, 81.91 ± 10.14 respectively) were not significantly different. However for both groups only the BMP-7 values were statistically increased compared to the control group. The BMP-4 (pg/mL) and BMP-7 (pg/mL) levels for the control group (n = 21) were 123.22 ± 18.76 and 12.31 ± 2.21 years, respectively. According to the manufacturer, the minimum detectable level (LLD) for the BMP assays are ; 1.0 pg/ml for

Table 1. — The mean ± standard deviation (SD) of age, urea (mg/dL), creatinine (mg/dL) and bone mineral density (BMD) values of patient groups depending on gender classification. Parameters with values of $P < 0.05$ were considered statistically significant.

	Group 1 (n = 38) mean ± SD			**Group 2 (n = 24) mean ± SD**		
Numbers of Subjects (n)	*female (n = 23)*	*male (n = 15)*	*P*	*female (n = 18)*	*male (n = 6)*	*P*
Age (years)	77.30 ± 6.29	77.86 ± 7.59		74.94 ± 7.03	75.83 ± 7.05	
Urea (mg/dL)	51.78 ± 25.12	43.90 ± 22.90		51.05 ± 26.18	58.46 ± 19.09	
Creatinine (mg/dL)	0.75 ± 0.23	1.26 ± 0.58	*P = 0.001*	1.16 ± 1.34	1.31 ± 0.43	*P = 0.04*
BMD Parameters						
Neck BMD (gr/cm^2)	0.65 ± 0.19	0.72 ± 0.14		0.66 ± 0.11	0.81 ± 0.19	
Wards BMD (gr/cm^2)	0.58 ± 0.13	0.61 ± 0.16		0.56 ± 0.15	0.70 ± 0.21	
Trochanter BMD (gr/cm^2)	0.59 ± 0.11	0.64 ± 0.10		0.56 ± 0.09	0.76 ± 0.15	*P = 0.018*
Total BMD (gr/cm^2)	0.72 ± 0.11	0.72 ± 0.15		0.70 ± 0.13	0.97 ± 0.18	*P = 0.038*

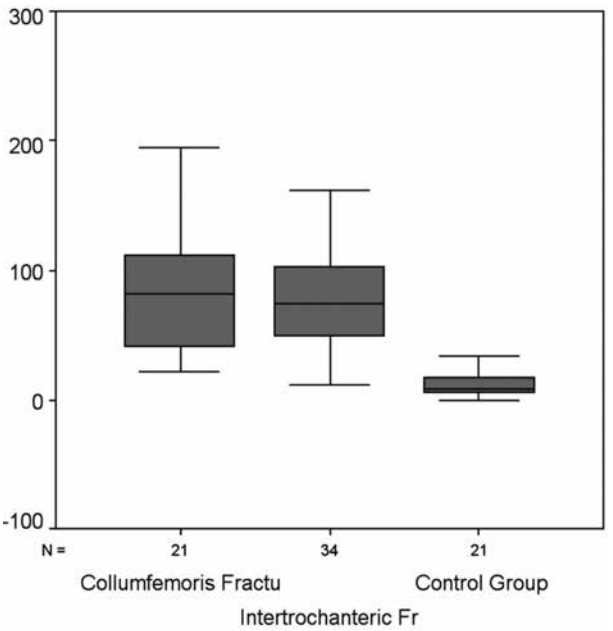

Fig. 1a. — Changes in serum BMP-7 (pg/mL) levels of both fracture groups within the first twenty-four hours of fracture and the control group. All values are expressed as median (IQR) values.

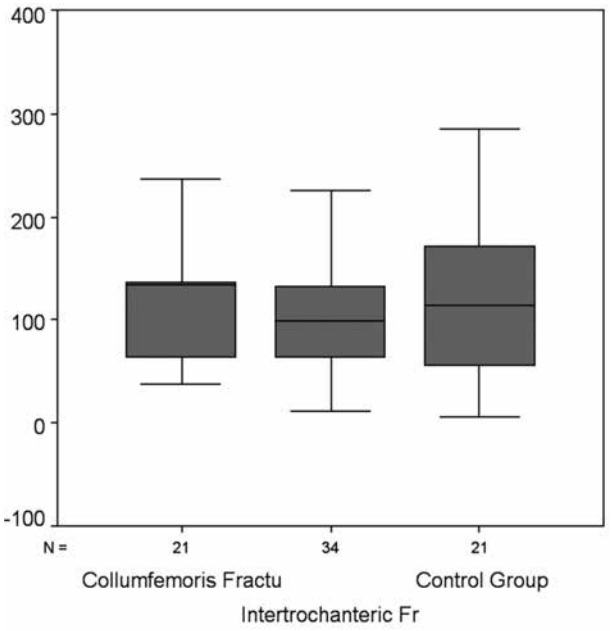

Fig. 1b. — Changes in serum BMP-4 (pg/mL) levels of both fracture groups within the first twenty-four hours of fracture and the control group. All values are expressed as median (IQR) values.

BMP-4, and 2.4 pg/ml for BMP-7 (In Figure 1a, 1b values are expressed as median, IQR).

DISCUSSION

The main outcome our study is the increased levels of BMP-7 (osteogenic protein-1, OP-1) values of patient groups compared to control group. BMP-7 is an osteogenic factor and an *in vivo* bone stimulating agent. BMP-7 was originally identified as having a bone induction activity and was a regulator of cartilage (*28*). Several studies have shown regeneration of articular cartilage defects by BMP-7 *in vitro* experiments and animal models (*13,21*).

In yet another of our studies discussing aspects of risk assessments of patients due to the fall-related moderate or minimal trauma and compared to non-fractured controls by BMD and proximal femur geometric measurements to assess whether geometric measurements of femoral dimensions were associated with femoral strength and hip fracture risk. We concluded as there were genetically determined differences among individuals concerning bone morphology and bone mineral distribution and these differences result in different bone strengths and fracture formation risk (*5*). In our different study in order to discuss the risk assessments for hip fractures due to fall-related, low energy traumas, we focused on possible allelic influences of the COL1A1, ESR, VDR, IL-6, and OPG genes. As a result we concluded as due to multifactorial inheritance and influential environmental factors, the molecular-genetic basis of osteoporosis had question marks. We hope that the differences in individual responsiveness to treatment, when clarified at the genomic level, would enable more effective, individualised guidance in clinical practice (*6*).

Proximal femoral fractures occur in both genders of the aging population as a result of osteoporosis as well as decreasing bone strength. One of the important approach to prevent this widespread, clinical problem involves the evaluation of bone strength and determination of fracture risks (*15,25*). There has been a FRAX tool developed by the World Health Organization (WHO) since 2008 to evaluate the fracture risk of patients. It is based on individual patient models that integrate the risks associated with clinical risk factors as well as BMD at the femoral neck (http://www.shef.ac.uk/FRAX/index. aspx). From the web of FRAX we can calculate the 10-year probability of fracture of our patients with the development of country-specific FRAX® tool. Tuzun *et al* (*27*) have completed a FRACTURK study that estimated current and future hip fracture risks and the prevalence of osteoporosis in Turkey. In an another related study by Tuzun *et al* (*28*) FRAX-based intervention thresholds in men and women from Turkey were determined and their impacts on the population discussed. The outputs obtrained by using this tool represent that approximately 8.6% of the female Turkish population aged 50 years or more had a prior fragility fracture and would be eligible for treatment. A further 13.6% without a prior fracture would be eligible for treatment. In contrast, the number of men aged 50 years or more eligible for treatment was 3.1%. However this tool does not include any biochemical markers and incorporate further risk factors suggested that FRAX had been known to have some limitations also. The use of biochemical biomarkers to diagnose and monitor treatment effectiveness for early/silent states shall have economical and healthcare benefits. To correlated the clinical state and biomarkers will help us to improve our expectations for the patients.

In this study we specifically evaluated BMPs 4 and 7 levels in order to discuss the risk factors for hip fractures and to evaluate the fracture healing mechanisms. There are limited studies dealing with these markers as risk factors and how they can be used for treatment and prevention. In our study the increased levels of BMP-7 in Group 1 compared to control group might indicate the adaptive or protective stage which may also decline the severity of clinical outcome after the fracture. Besides, we did not observed any differences between the BMP levels of both fracture groups. Bouxsein (*3*) emphasized strong interest in enhancing healing of fractures and preventing complications associated with delayed union that both biophysical, local biologic and systemic pharmacologic interventions have been tested for their ability to improve fracture healing. Regarding the ability of interventions to accelerate fracture healing, they mainly focused on bone morphogenetic proteins and PTH. So all preclinical data's supporting the potential for new therapeutic pathways will challenge of conducting efficacy of interventions designed to enhance fracture healing.

Bone morphogenetic proteins are low molecular weight glycoproteins and have important functions, in normal physiology. However, misleading signals can also cause severe, destroyed pathophysiological diseases such as various type of cancers (*23,24*). Besides they have potent osteogenic activity *in vitro* (*4*) and constitutive activation, or exogenous application of them can induce ectopic bone formation *in vivo* (*10,14*). Their signalling is mediated by activa-

tion of type I and type II serine-threonine kinase receptors. BMPR1A (or ALK3) is a type I receptor that is known to have high affinity for BMP-2 (*17*) and BMP-4 (*12*). BMPs induce osteogenesis, and BMP-2 and BMP-7 are approved therapies for treatment of non-union fractures and spinal fusions (*10,14,30*). Although bone is one of the main sources of BMP-7, other extra skeletal tissues such as kidney may play a part in plasma BMP-7 elevation, constantly releasing BMP-7 in to the circulation of healthy individuals. In our patient groups and control group, we also did not record any statistically significant differences between the levels of serum urea and creatinine levels but within the groups creatinine levels were significantly different between genders. Our results were also in accordance with the results of Grgurevic *et al* (*11*).

Generally, researchers worked on patients with osteoarthritis and cartilage tissue to observe the differences about BMP-7. Merrihew *et al* had evaluated the levels of BMP-7 in cartilage tissue and observed a negative correlation between the increased degenerative disorders and BMP-7 levels (*19*). BMP-7 can stimulate collagen synthesis and the formation of cartilage matrix proteins and proteoglycans while inhibiting the catabolic effects of IL-1 and finally have an anabolic effect on cartilage tissue (*1*).

It was shown that BMPs 2, 4, 7 and 9 stimulate the intramembranous and endochondral osteogenesis, however BMPs 2, 6 and 9 mostly stimulate the differentiation of mesenchymal cells to osteoblasts. BMP-7 is widely used to stimulate the bone formation and as a treatment after the surgery of spinal fractures (*7,9,18,26*).

Generally experimental studies had been done to evaluate the BMPs that depend on animal studies and include invasive protocols. The detection of the local increase in BMPs means the increase in biological fluids. So that to monitor the patients' progression and prognoses BMPs might have a good choice (*4*).

Hofbauer (*12*), described the major discoveries over the last decade have provided key insights into bone cell biology and bone remodelling with regards to osteoblast biology, characterization of BMPs provided novel translational prospects to improve local or systemic bone regeneration. A detailed knowledge of bone biology with molecular insights into the communication between bone-forming osteoblasts and bone-resorbing osteoclasts and the orchestrating signalling network has led to the identification of novel therapeutic targets. So, BMP-7 measurements may not only serve as a potential biochemical marker for determining disease severity but might be predictive of prognosis with respect to the progression of the fracture. They also contribute to the fundamental processes underlying the pathogenesis of the fracture. The interest in signal pathway proteins which control the bone remodelling status are seriously increasing for the last years. The therapeutic strategies have been developed aimed at inhibiting excessive bone resorption and by increasing bone formation.

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