



Analysis of selected pro-inflammatory cytokines in patients with fracture of the proximal end of the femur

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ABSTRACT

Fracture of the proximal end of the femur is one of the most common fractures in elderly patients. Worldwide, this type of fracture occurs in 2.5 m of people annually including approximately 30,000 Poles. It usually occurs as a complication of osteoporosis. In the bones, changes in the level of cytokines following osteoporosis are observed. These changes refer mainly to pro-inflammatory cytokines: tumor necrosis factor α (TNF- α), interleukin 1 (IL-1) and interleukin 6 (IL-6). The aim of this preliminary study was to assess if there are any changes in the blood level of these cytokines in postmenopausal patients with low-energy fracture of the proximal end of the femur. Additionally, the changes in cytokines concentration were also analyzed in conjunction with the body mass index, sex hormones (FSH and estradiol) as well as parameters connected with bone turnover (calcium, phosphorus). The study showed a significantly higher concentration of interleukin 1, interleukin 6 and TNF- α in patients with a low-energy fracture of the proximal end of the femur and confirmed the relationship between blood estradiol level and body mass index versus the tested fracture.

Keywords: fracture of the proximal end of the femur; IL-1; IL-6; osteoporosis; TNF- α

INTRODUCTION

Fractures of the proximal end of the femur are one of the most common types of fractures affecting elderly people. Worldwide, this type of fracture annually affects approximately 2.5m people, including 30,000 Poles, thus this phenomenon is a serious health, sociological and economic problem. Moreover, along with the extending human lifespan and an aging population it may become increasingly important. Many patients affected by such a fracture are immobile for several months, hospitalized or require constant care at home. It is predicted that thousands of patients will never regain their normal physical activity and as many as 10-20% will die in the aftermath of it [3, 5, 9, 13].

These fractures are usually a consequence of osteoporosis. They may occur even due to a minor low-energy

injury and therefore such cases are classified as low-energy fractures. Currently, the used methods of assessing a risk of fracture affecting the proximal end of the femur are not flawless. The main criteria in the treatment of osteoporosis are based on bone mineral density assessed using a densitometric examination. However, a recent research has shown that a significant number of fractures of the proximal end of the femur (55-75%) occurs in patients with normal densitometric parameters [1]. Thus, it seems that this measurement alone is not sufficient to qualify patients for osteoporosis treatment [6]. For this reason, more effective markers of the risk factors of these fractures, are looked for. This preliminary study aimed to establish if there are any changes in the blood level of pro-inflammatory cytokines in patients with a fracture of the proximal end of the femur.

METHODS

Sixty women were qualified for the examination at the age of 64-96. The investigated group (average age: 78.48 \pm 7.37 years) consisted of forty patients treated for a low-

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energy fracture of the proximal end of the femur. A low-energy fracture was recognized as a fracture which occurred due to a fall from one's own height or an injury of lower energy. The diagnosis was based on clinical examination and radiographic images of the fracture of the proximal end of the femur. The main criteria for the patients to participate in the examination included having had menopause, suffering from osteopenia or osteoporosis. Osteoporosis or osteopenia were defined based on dual-energy X-ray absorptiometry (DXA) measurement – according to the definition proposed by WHO [18]. It means that the value of mineral density lower than 2.5-fold standard deviation below the average value for a population of Caucasian young adults is considered as osteoporosis. Twenty healthy women (average age 75.8±4.93) having no fracture and without osteoporosis or osteopenia were qualified for control group. The criterion excluding from the examination was the use of pharmacotherapy with established influence on bone metabolism. All patients signed a consent to the examination.

For the purpose of the examination blood samples were taken from the basilic vein within 24 hours after the patients' injury prior to operative treatment. In all individuals serum concentrations of IL-1 and IL-6 (BioVendor, Czech Republic), TNF- α (Biomedica, Austria) and level of FSH and 17 β -estradiol (Dia Metra, Italy) were determined using ELISA tests according to the manufacturer's recommendations. The determinations were made with the use of a microplate reader Power Wave XS (Bio-tek, USA). The serum level of total calcium and inorganic phosphorus (BioSystem S.A., Spain) was determined spectrophotometrically using a biochemical analyzer Cobas Mira Plus (Roche, Switzerland). In addition, the body mass index (BMI) was analysed in patients.

Results were then subjected to statistical analysis. The Shapiro-Wilk, *t*-Student, Mann–Whitney U test and rank Spearman (Rs) methods were used. Program STATISTICA 5.5 (StatSoft Inc., USA) was applied [15].

RESULTS

The higher, statistically significant concentrations of all analyzed cytokines were observed in patients from the investigated group (Table 1). A significant decrease in FSH and estradiol concentrations was observed in patients of the investigated group (Table 2). No statistically significant differences between both groups were observed (Table 2).

The average BMI value for the investigated group was 26.67 ± 4.39 kg/m². Median BMI in the control group was 26.77±2.94 kg/m². Individual parameters were assessed depending on BMI value, i.e. lower than median (lower than 26) vs. higher than median. In patients from the investigated group, there were no statistically significant differences in the serum concentration of IL-1, IL-6 and

TNF- α depending on median BMI (Table 4). Similarly, in patients from the investigated group there were no significant differences in the FSH concentration depending on median BMI (Table 5). However, a significant higher concentration of estradiol in the serum was found in patients with a higher than median BMI value (Table 5). There were no significant differences in the concentrations of serum calcium and phosphorus depending on median BMI in patients from the investigated group (Table 6).

Table 1. Serum concentration of cytokines (pg/ml)

	Control			Tested group			p
	N	M	SD	N	M	SD	
IL-1	20	8.75	0.93	40	10.27	1.83	0.0013
IL-6	20	9.51	1.24	40	10.57	1.81	0.0370
TNF- α	20	21.31	4.67	40	31.85	13.50	0.0029

Table 2. The FSH and estradiol serum level (pg/ml)

	Control			Tested group			p
	N	M	SD	N	M	SD	
FSH	20	95.36	37.74	40	70.95	41.53	0.0336
Estradiol	20	1.82	0.20	40	1.55	0.38	0.0002

Table 3. The calcium and phosphorus serum level (mg/dl)

	Control			Tested group			p
	N	M	SD	N	M	SD	
Calcium	20	8.22	0.85	40	8.03	1.11	0.4853
Phosphorus	20	3.76	0.55	40	4.04	1.18	0.4015

Table 4. Median BMI vs. serum cytokines levels in patients from the investigated group

	≤ Me			> Me			p
	N	M	SD	N	M	SD	
IL-1	22	10.47	2.02	18	10.02	1.59	0.407
IL-6	22	10.60	1.78	18	10.54	1.89	0.828
TNF- α	22	29.98	12.32	18	34.13	14.86	0.541

Table 5. BMI vs. serum FSH and estradiol level in the investigated group

	≤ Me			> Me			p
	N	M	SD	N	M	SD	
FSH	22	68.19	47.05	18	74.33	34.65	0.5960
Estradiol	22	1.38	0.35	18	1.75	0.32	0.0020

Table 6. Concentration of total calcium turnover and phosphorus in the serum of patients from the investigated group considering median BMI

	≤ Me			> Me			p
	N	M	SD	N	M	SD	
Calcium	22	8.05	1.03	18	7.99	1.24	0.8384
Phosphorus	22	4.01	1.51	18	4.07	0.65	0.2013

DISCUSSION

Epidemiological examinations, which have been conducted in recent years, show clearly that the problem of osteoporotic fractures is more and more common and the predicted number of adults treated for it within the next 50 years will almost triple [13]. Thus, now it is necessary to develop a special health policy encompassing new solutions to overcome this problem. New research explaining the molecular mechanisms of osteoporosis and enabling certain therapeutic actions may be helpful.

However, it is most important to predict and to prevent the main complication of osteoporosis, i.e. the fracture of the proximal end in the femur. The studies underline a great importance of determining the risk of the low-energy fracture in the population of each country as it has been practiced in Great Britain. Such a procedure will enable an effective prophylaxis for fractures, especially in postmenopausal women. A standard procedure now is the use of a fracture risk assessment system FRAX. The tool has been developed by WHO and is the basis for all decisions concerning the introduction of osteoporosis treatment in patients [5].

The present study is closely linked with worldwide multicentre research aimed to discover reliable risk factors of such a fracture and to elaborate an effective schedule to treat it. Results of this study reveal that a low-energy fracture of the proximal end in the femur is associated with a higher blood level of all tested pro-inflammatory cytokines. The obtained in the study statistically significant differences between the study group and the control group have clinical relevance as well. Thanks to the study, there can be designated not yet determined reference values for IL-1, IL-6 and TNF- α . In that case there can be determined as mean \pm 2SD for healthy control group, thus for these cytokines, the "normal range" is as follows: 8.75 ± 1.86 , 9.51 ± 2.48 and 21.31 ± 9.34 , respectively. Comparing to the results obtained for the same parameters in the investigated group (mean \pm SD; 10.27 ± 1.83 , 10.57 ± 1.81 and 31.85 ± 13.50 , respectively) one can doubt in the clinical usefulness of such fracture risk "markers". However, assuming that statistical importance and clinical importance are comparable, the key question is what is the main cause of these risks? Is it related to or accompanied by osteoporosis process lasting before the fracture or is it result of the fracture?

In the studies of other authors, it was underlined that these cytokines play an important role in the process of developing osteoporosis [9]. Nowadays, it is known that in osteoporosis caused by inflammatory conditions of the bones or joints, e.g. in rheumatoid joints inflammation, the process occurs due to the activation of pro-inflammatory cytokines including IL-1 and IL-6 as well as TNF- α . TNF- α and IL-1 both stimulate gene expression for collagenase and stromelysin [16]. It was proved that activation of these cytokines is accompanied by a decrease in the mineral bone density and an increase in the concentration of serum osteocalcin [16]. Braun and Zwerina [2] also showed an influence of pro-inflammatory cytokines (TNF- α , IL-1, IL-17) on the loss of bone mass. Lacativa et al. [8] state that a high level of IL-1 and TNF- α can be treated as an independent risk factor for fractures. The role of these cytokines is quite significant due to the fact that anti-osteoporotic drugs have been developed.

They work as IL-1 receptor antagonists and antibodies against TNF- α [8]. O'Brien [12] and Sims [14] also suggested that IL-6 inhibits bone formation directly as well as by means of regulating OPG/RANK/RANKL system. The mechanism of indirect influence by means of the above mentioned system seems to be of greater importance than the direct one. The clinical effect of it is a significant loss of bone mass [12,14]. In the study conducted by Mysliwiec [10] it was underlined that IL-6 plays an important role as a factor facilitating osseous turnover in postmenopausal women and as a factor increasing the risk of developing osteoporosis. Regardless of this observation, in the present study there were no significant changes in blood calcium and phosphorous levels, but in other diseases a high level of RANKL and IL-6 was associated with hypercalcemia [4]. The authors suggested that OPG/RANKL system and inflammatory processes take part in increased bone resorption and lead to hypercalcemia [4].

It is also possible that the observed increase in the blood level of the tested cytokines is related to the inflammatory response playing a key role in postoperative organ dysfunction. According to the suggestion by Sun [17] it is worth checking whether the therapy with anti-cytokine antibodies will enable to decrease the mortality rate and the risk of complications after an operation on the hip [17]. Krämer et al. assessed the role of pro-inflammatory cytokines in hip fracture as well [7]. They showed an increase in the concentration of TNF- α in patients even one month after having fracture damage.

In the present study, it was revealed that the incidence of low-energy fractures is accompanied by a lower concentration of estradiol, which is a confirmation of the well-known scientific fact. Badurski [1] and Mysliwiec [11] also underline a huge role of pro-inflammatory cytokines, thyroid and sex hormones in the regulation of bone remodeling processes and osteoporosis. Hormonal deregulations in postmenopausal women also have an important influence on BMI elevation and thus the related incidence of fractures. This was observed in our study because a significantly higher concentration of estradiol in the serum was found in patients with a higher than median BMI value.

CONCLUSION

Concluding, significant statistically higher serum concentrations of IL-1, IL-6 and TNF- α were observed in patients with a low-energy fracture of the proximal end of femur. Thus future studies should be designed to answer the question if the observed changes are of clinical significance allowing use them as a risk marker; are related to osteoporosis process or are a direct response to bone injury.

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