Interleukin-6 and tumor necrosis factor alpha levels in the serum and synovial fluid in relation to bone mineral density and turnover in children with juvenile idiopathic arthritis

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Abstract

Aim of the study: *To evaluate the relationships between levels of proinflammatory cytokines (TNF-α and IL-6) and bone metabolism in juvenile idiopathic arthritis (JIA) children.*

Methods: *Twenty children with active JIA were included, 12 with polyarticular type of JIA and 8 with oligoarticular onset. The control group consisted of 15 healthy children. TNF-α and IL-6 were measured in the serum and synovial fluid using ELISA Quantikine kits (R & D Systems, USA). The levels of osteocalcin (N-MID Osteocalcin) and C-terminal type I alpha-collagen chain telopeptide (CTx) were determined in the Elecsys® 2010 system. Bone mineral density (BMD) was assessed by DXA method in the total bone (Total BMD, g/cm^2) and in the L2-L4 vertebrae (SpineBMD, g/cm^2) as Z-score.*

Results: *In children with JIA, the mean serum IL-6 level was elevated as compared with the control group (34.5 pg/ml ± 31.9 and 1.4 ± 1.6 pg/ml, respectively, p<0.01). The level of TNF-α in JIA children was higher (9.3 pg/ml ± 3.9) in comparison with the control group (4.7 pg/ml ± 0.5), but the difference was not statistically significant. In both JIA forms, the level of IL-6 in synovial fluid was high (442.8 pg/ml ± 162.2). The mean levels of N-MID Osteocalcin and CTx were increased as compared with the control group (96.3 ng/ml ± 54.0 and 70.2 ng/ml ± 48.3; 1.7 μg/dl ± 0.5 and 1.2 μg/dl ± 0.4, respectively). A significant positive correlation was observed between ESR and serum IL-6 level (p<0.04) and the degree of articular damage (p<0.001). There was a trend toward negative correlation between serum IL-6 and TNF-α levels and bone mineral density (p=0.05 for both).*

Conclusions*: Elevated levels of IL-6, TNF-α, N-MID osteocalcin, CTx and significant correlations between inflammatory parametrs, articular destruction and bone mass loss were observed in patients with JIA.*

Key words: *juvenile idiopathic arthritis, pro-inflammatory cytokines, bone mineral density.*

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Introduction

Juvenile idiopathic arthritis (JIA) is a disease closely related to osteoporosis, both local and generalized type [1, 2]. It is believed that the chronic immunologicaly-driven inflammatory process that underlies JIA has an undoubtful effect on osteoporosis incidence/contributes to the development of osteoporosis [3, 4]. Activation of the immune and inflammatory cells as well as enhanced production of pro-inflammatory cytokines, of which tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) are considered to play a major role, are responsible for bone destruction and periarticular osteoporosis in the early stage of the disease [5, 6]. In adults with rheumatoid arthitis (RA) the involvement of cytokines in bone resorption and osteoporosis is well documented, while in children with JIA the

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reports are rare. The objective of the current study was to determine whether there is a relationship between the levels of TNF-α and IL-6 in the serum or synovial fluid and biochemical markers of bone turnover and bone mass in children with JIA [7, 8].

Material and Methods

The study involved 20 children and adolescents with active JIA diagnosed according to the ILAR criteria (International League Against Rheumatism of 1997), 12 boys and 8 girls aged 6-17 years (mean – 12.1±4.4), including 12 with polyarticular JIA and 8 with oligoarticular onset. The mean duration of the disease was 8.9±4.3 years. Articular damage was graded based on X-ray examination as: 1° – lack or minimum changes (7 children), 2°° – moderate changes (7 children) and 3°° – advanced damage (6 children).

Blood for analysis was collected on an empty stomach, in the morning hours. The sera obtained after centrifugation were stored at a temperature of -70°C. Synovial fluid samples for Il-6 and TNF-α analysis were obtained from knee joints displaying clinical signs of active synovitis. The synovial fluid from knee effusions was drained using a sterile technique, and samples were separated and stored at a temperature of -70°C. Quantitative enzyme-linked immunosorbent assay kits (Quantikine; R & D Systems, USA) were used to assess IL-6 and TNF-α levels. The lowest detectable concentration of IL-6 and TNFa were: 0.50 pg/ml and 3.4 pg/ml, respectively. The levels of osteocalcin (N-MID Osteocalcin), C-terminal type I alpha-collagen chain telopeptide and fragment beta (βCTx) were determined in the Elecsys® 2010 system.

Dual Energy X-ray Absorptiometry (DXA) was used for densitometric examination. Bone mineral density (BMD) was assessed in the total bone (TB BMD, g/cm^2) and in the L2-L4 vertebrae (SpineBMD, g/cm^2) and expressed as Z-score for Spine BMD in relation to age and sex.

Findings concerning the cytokines and bone turnover markers were compared with the control group consisting of 15 healthy children matched for age and sex.

Statistical analysis was performed with U-Mann-Whitney's test and statistical packet Statistica 5.0 (StatSoft). The p value of less than 0.05 were considered statistically significant.

The study was approved by the Bioethics Committee, Medical University of Białystok.

Results

Children with polyarticular JIA had significantly higher joint damage score, ESR and CRP values than children with oligoarticular JIA.

In children with JIA, the mean serum IL-6 level was elevated as compared to the control group (34.5 pg/ml ± 31.9 and 1.4 ± 1.6 pg/ml, respectively, $p<0.01$). Children with polyarticular JIA had significantly higher serum IL-6 level than those with oligoarticular JIA (Table 1). The level of TNF-α in JIA children was higher (9.3 pg/ml ± 3.9) in comparison with the control group (4.7 pg/ml ± 0.5), the difference being statistically insignificant. No significant differences were observed between polyarticular JIA and oligoarticular JIA with respect to the serum TNF-α levels. Synovial fluid levels of IL-6 and TNF-α were higher than the serum levels in children with both oligoarticular and polyarticular forms of JIA with no difference between the oligoarticular and polyarticular groups (Table 1).

The mean levels of N-MID Osteocalcin and βCTx in JIA patients (96.3 μg/ml ± 54.0 and 1.7 μg/dl ± 0.5, respectively) were elevated as compared to healthy children (70.2 μg/ml ± 48.3 and 1.2 μg/dl ± 0.4, respectively), but these differences were not statistically significant.

Although there were no significant differences in the total BMD between chidren with oligoarticular JIA and polyarticular JIA, children with polyarticular JIA tended to have a lower Z-score for Spine BMD than those with oligoarticular JIA.

The mean biomarkers of bone turnover and Z-score for Spine BMD and total BMD according to the clinical form of JIA are presented in Table 1.

A significant positive correlation was observed between ESR and serum IL-6 level ($r=0.498$, $p<0.05$) and the degree of articular damage ($r=0.697$, $p<0.001$). ESR was negatively correlated with Z-score for Spine BMD ($r=-0.556$, $p<0.01$). There was also a borderline correlation between the serum IL-6 levels and Z-score for Spine BMD ($r=-0,443$, $p=0.05$), and the serum TNF-α level and the total BMD ($r=-0.487$, $p=0.05$). No significant correlations between IL-6 or TNF-α levels and the biomarkers of bone turnover were found (Table 2).

Discussion

The relationship between the immune system and the osseous system has attracted researchers for the last 10 years and even the term "osteoimmunology" has been proposed [9]. Since the discovery of the three-molecule system belonging to the TNF family: TRANCE (TNF-related activation cytokine) / RANKL (receptor activator of nuclear factor – kB ligand) / OPGL (osteoprotegerin ligand), the major role in bone biology has been ascribed to immune cells, such as macrophages and T lymphocytes, believed to be involved in osteoclastogenesis [11, 12]. It is assumed that TNF-α and IL-6 produced by macrophages-monocytes in a microenvironment of bone tissue, through induction of the RANKL/RANK pathway, indirectly increase formation of osteoclasts and are the most potent stimulators of bone resorption. This can be the major mechanism operating in local and generalized osteoporosis [12, 13].

Our findings confirm the data reported by other authors showing elevated levels of IL-6 and TNF-α in the

Table 1. Clinical parameters, cytokines levels, bone turnover markers, and bone mineral density in children with oligoarticular (Group I) and polyarticular JIA (Group II)

		JIA (n=20)		
Parameters		**Group I (n=8) Mean (SD)**	**Group II (n=12) Mean (SD)**	**p<**
Age [years]		11.0±3.6	12.1±4.4	NS
Disease duration [years]		4.1±1.54	4.8±2.5	NS
Joint involvement degree (X-rays)	1^o (n=7)	1.4±0.5	2.3±1.33	0.02
	2^o (n=7)			
	3^o (n=5)			
ESR/1h		30.1±6.8	59±23.8	0.001
CRP [mg/L]		7.8±2.6	15.6±4.8	0.01
N-MID Osteocalcin [µg/dl]		111.3±62.9	86.7±48.2	NS
CTx [ng/ml]		1.5±0.8	1.2±0.58	NS
IL-6 [pg/ml]	serum	16.2±27.8	49.2±36.2	0.01
TNF-α [pg/ml]		3.9±2.5	4.8±2.8	NS
IL-6 [pg/ml]	fluid synovium	442.8±162.2	487.5±107.5	NS
TNF-α [pg/ml]		10.3±4.3	8.3±4.4	NS
Z-score for SpineBMD		-0.80±0.74	-2.00±1.33	0.05
Total BMD [g/cm^2]		0.788±0.18	0.82±0.11	NS

Table 2. Correlation between cytokines level and both bone turnover markers and bone mineral density

Parameters	**IL-6 serum**		**TNF-α serum**		**IL-6 fluid synovium**		**TNF-α fluid synovium**	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**
N-MID Osteocalcin	-0.071	NS	-0.221	NS	- 0.343	NS	-0.223	NS
CTx	0.129	NS	0.146	NS	0.122	NS	0.109	NS
Z-score for SpineBMD	-0.443	0.05	-0.276	NS	-0.343	NS	-0.223	NS
Total BMD	-0.156	NS	-0.487	0.05	-0.371	NS	-0.290	NS
ESR	0.498	0.04	0.108	NS	0.138	NS	0.287	NS

serum and synovial fluid of patients with RA and JIA [14, 15]. Especially interesting is the finding of high IL-6 values in synovial fluid in active arthritis, irrespective of the clinical form of JIA. In half of the patients, the level exceeded the upper measurable limits (547.49 pg/ml). This is an argument for a considerable involvement of these cytokines, both in initiation and maintenance of the inflammatory process in joints, by intensifying their destruction and periarticular osteoporosis.

However, the effect of these cytokines on generalized bone mass loss is also likely [16, 17]. We found considerable generalized loss of bone mass in patients with higher activity of JIA, which was manifested as significant correlations between ESR, serum IL-6 and serum TNF-α level. Moreover, in 6/20 patients with Z-score below -2.5, the serum cytokine levels were markedly higher as compared to the mean values in the whole group.

According to many literature data, progression of inflammatory symptoms in both RA and JIA is not only accompanied by increased cytokine production but also by enhanced bone turnover [18-20].

Our attempt to assess bone metabolism showed elevated mean concentrations of the two biomarkers of bone turnover as compared to the control group. However, most researchers have observed reduced concentrations of N-MID Osteocalcin (bone formation marker) in patients with RA and JIA. In our study, reduced levels of osteocalcin were found in 5/20 patients with polyarticular JIA and disease duration >5 years. Ctx (bone resorption marker) that markedly exceeded the mean value for the whole group (above 2 µg/dl) was observed in 4/20 JIA children.

Concluding, our findings suggest a relationship between the immune and osseous system in chronic inflammatory process, which is manifested as significant correlations

between the levels of cytokines, ESR and loss of bone mass in JIA patients. This indicates that the future therapy of RA and JIA should include not only antiinflammatory actions but also those inhibiting osteoclastogenesis.

References

1. Cetin A, Celiker R, Dincer F, Ariyurek M (1998): Bone mineral density in children with juvenile chronic arthritis. Clin Rheumatol 17: 551-553.
2. Pepmueller PH, Cassidy JT, Allen SH, Hillman LS (1996): Bone mineralization and bone mineral metabolism in children with juvenile rheumatoid arthritis. Arthritis Rheum 39: 746-757.
3. Walsh NC, Crotti TN, Goldring SR, Gravallese EM (2005): Rheumatic diseases: the effects of inflammation on bone. Immunol Rev 208: 228-251.
4. Lien G, Flatř B, Haugen M et al. (2004): Frequency of osteopenia in adolescents with early-onset juvenile idiopathic arthritis: a long-term outcome study of one hundred five patients. Arthritis Rheum 50: 2036.
5. Yilmaz M, Kendirli SG, Altintas D et al. (2001): Cytokine levels in serum of patients with juvenile rheumatoid arthritis. Clin Rheumatol 20: 30-35.
6. Pietrewicz E, Urban M, Górska A (2004): Stężenie cytokin w surowicy u chorych z młodzieńczym idiopatycznym zapaleniem stawów w zależności od postaci choroby i stopnia jej aktywności. Pol Merk Lek 99: 232-234.
7. Bingham CO (2002): The patogenesis of rheumatoid arthritis: pivotal cytokines involved in bone degradation and inflammation. J Rheumatol Suppl 65: 3-9.
8. Verbruggen A, De Clerck LS, Bridts CH et al. (1999): Flow cytometrical determination of interleukin 1beta, interleukin 6 and tumour necrosis factor alpha in monocytes of rheumatoid arthritis patients; relation with parameters of osteoporosis. Cytokine 11: 869-874.
9. Rho J, Takami M, Choi Y (2004): Osteoimmunology: interactions of the immune and skeletal systems. Mol Cells 17: 1-9.
10. Walsh MC, Choi Y (2003): Biology of the TRANCE axis. Cytokine Growth Factor Rev 14: 251-263.
11. Targońska M, Kochanowska I, Ostrowski K, Górski A (2001): Osteoimmunology, new area of research on the associations between the immune and bone systems. Pol Arch Med Wewn 105: 435-440.
12. Takayanagi H (2007): Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. Nat Rev Immunol 7: 292-304.
13. Varsani H, Patel A, van Kooyk Y, Woo P, Wedderburn LR (2003): Synovial dendritic cells in juvenile idiopathic arthritis (JIA) express receptor activator of NF-kappaB (RANK). Rheumatology 42: 583-590.
14. Kotake S, Sato K, Kim KJ et al. (1996): Interleukin-6 and soluble interleukin-6 receptors in the synovial fluids from rheumatoid arthritis patients are responsible for osteoclast-like cell formation. J Bone Miner Res 11: 88-95.
15. Uson J, Balsa A, Pascual-Salcedo D et al. (1997): Soluble interleukin 6 (IL-6) receptor and IL-6 levels in serum and synovial fluid of patients with different arthropathies. J Rheumatol 24: 2069-2075.
16. Madhok R, Crilly A, Watson J, Capell HA (1993): Serum interleukin 6 levels in rheumatoid arthritis: correlations with clinical and laboratory indices of disease activity. Ann Rheum Dis 52: 232-234.
17. Walsh NC, Crotti TN, Goldring SR, Gravallese EM (2005): Rheumatic diseases: the effects of inflammation on bone. Immunol Rev 208: 228-251.
18. Pereira RM, Falco V, Corrente JE et al. (1999): Abnormalities in the biochemical markers of bone turnover in children with juvenile chronic arthritis. Clin Exp Rheumatol 17: 251-255.
19. Polito C, Strano CG, Rea L et al. (1995): Reduced bone mineral content and normal serum osteocalcin in non-steroid-treated patients with juvenile rheumatoid arthritis. Ann Rheum Dis 54: 193-196.
20. Lien G, Selvaag AM, Flatř B et al. (2005): A two-year prospective controlled study of bone mass and bone turnover in children with early juvenile idiopathic arthritis. Arthritis Rheum 52: 833-840.