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Evaluation of interleukin-1 and -6 in the etiopathogenesis of idiopathic osteoporosis and osteopenia in children

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Introduction: The aim of the study is to determine whether serum concentrations of interleukin (IL)-1 and IL-6 correlate with indices of bone mineral metabolism in children with idiopathic osteoporosis and osteopenia.

Materials and Methods: The study comprised 62 patients aged 6–18 years (20 with idiopathic osteoporosis, 22 with idiopathic osteopenia, and 20 controls). In 10 children, investigations were repeated after one year of treatment. Serum concentrations of IL-1α, IL-1β, IL-1 receptor antagonist (IL-1ra), as well as IL-6 and its soluble receptor (IL-6sR) were determined by the ELISA method. In patients with decreased bone mass, selected calcium-phosphorus metabolism indices and bone turnover markers were assessed.

Results: Higher values of IL-6 were recorded in those with idiopathic osteoporosis than in controls (2.79 vs. 1.43; $p<0.05$). In these patients there was also a tendency towards higher values of IL-6sR ($p=0.05$). IL-1α and IL-1β were not markedly elevated in any of the patients. No significant differences between groups regarding IL-1ra were observed. Negative correlation between IL-6, IL-1α, cytokine/receptor indices, and spinal bone mineral density was determined. Positive correlation was found between IL-α, IL-1/IL-1ra, and parathormon as well as between IL-1α, IL-6sR, and bone formation markers. Increase in bone mass after treatment was accompanied by a decrease in IL-6sR.

Conclusions: The higher serum levels of IL-6 in children with idiopathic osteoporosis/osteopenia and the decrease in IL-6sR after treatment reveal an involvement of IL-6 in the etiopathogenesis of these disturbances. The results suggest that IL-1 may also participate in the primary decrease of bone mass in children.

Key words: cytokine • bone mineralization • children

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INTRODUCTION

Disorders of bone mineralization constitute a major problem not only in adults, but also in children. Literature data and clinical observations draw attention to more frequent diagnoses of both secondary and idiopathic osteoporosis and osteopenia in the developmental stage of life. Lack of sufficient knowledge about the etiopathogenesis of idiopathic disorders unfavorably affects the process of their treatment, which is in consequence associated with persistence of ailments, occurrence of subsequent fractures and complications, as well as with a higher risk for developing osteoporosis later in life[2].

In spite of enormous technical advances, concepts relating to the probable causes of idiopathic disorders of bone mineralization are still controversial. Of particular interest among the latest studies on the etiopathogenesis of such disorders in adulthood are reports showing the effect of various cytokines on the process of bone tissue remodeling[16, 22]. These low-molecular proteins have been found to affect bone cells and to be involved both in bone formation and bone resorption. Recent studies concerning a role of these proteins in mineralization disorders have been limited to adult populations; most of these reports focus on postmenopausal osteoporosis, the most frequently recognized, and only a few deal with idiopathic osteoporosis in young adults[10, 11, 18]. In most of the studies, alterations in cytokine concentrations were observed not only locally in the bone environment, but also in the serum[6, 9, 10, 11, 18], although there were some reports in which similar relationships were not confirmed[17]. Evidence indicates that the stimulation of bone resorption is particularly mediated by such proinflammatory cytokines as interleukin (IL)--1, IL-6, tumor necrosis factor α, and transforming growth factor α. However, the key role seems to be played by IL-6 and IL-1[16].

IL-1, affecting mainly osteoblast receptors, also stimulates resorption through enhancement of synthesis and collagenase release by osteoblasts, inhibits osteocalcin synthesis, and stimulates the production of an osteoclast precursor recruitment-inducing factor by osteoblasts and their fusion into polynuclear osteoclastic cells[4]. IL-1 also directly stimulates osteoclasts through receptor binding[25]. Khosla et al.[10] detected a higher index of IL-1α bioactivity in the serum of patients with postmenopausal osteoporosis. Estrogen deficiency has been suggested to induce a higher IL-1 secretion[4]. Sunyer et al.[25] reported in their recent studies that the production of cytokine receptors on osteoclasts is modulated by estrogens with lower cell response to IL-1. Cohen-Solal et al.[3] reported a positive correlation between IL-1β concentration and the resorptive activity of peripheral monocytes. Ghazali et al.[5] observed a relationship between IL-β and the decreased bone mineralization in patients with idiopathic hypercalciuria, similarly to findings by Salamone et al.[20] in postmenopausal women and by Zheng et al.[27] in premenopausal subjects. However, some other studies did not confirm such correlations[6, 9, 21].

IL-6 is a powerful stimulator of resorption, but it also regulates the development and functions of both osteoclasts and osteoblasts[15]. It can be produced, among others, by osteoblastic cells. Low levels of IL-6 have been implicated in the stimulation of osteoclast induction from their precursors, while higher levels activate mature osteoclasts[4]. Numerous studies of peripheral monocyte cultures supported the relationship between IL-6 and higher resorption activity and decreased bone mineral density[3, 5, 20, 27]. In some reports evidence has been provided for its higher concentration both in the serum of patients with postmenopausal osteoporosis[18] and in men with involutional osteoporosis, in whom IL-6 concentration negatively correlated with dehydroepiandrosteron concentration[23]. According to Scheidt-Nave et al.[21] and Straub et al.[23] hormonal replacement therapy results in lower IL-6 values in serum. However, other authors did not find changes in this cytokine concentration in the course of postmenopausal osteoporosis[8, 17]. Lakatos et al.[11] revealed secondary osteoporosis involvement in hyperthyroidism, and Grey et al.[6] in hyperparathyroidism, together with elevated levels of IL-6. Recently, Libanati et al.[13] showed a negative correlation between IL-6 concentration and bone mineral density in healthy girls at puberty.

Considering the available literature, there have been no such studies conducted so far in children with disorders in bone mineralization. The aim of the study was thus to determine a possible relationship between IL-1 and IL-6 concentrations in serum and the occurrence of idiopathic osteoporosis and osteopenia in developmental age as well as to establish whether cytokine concentrations correlated with selected markers of bone mineral metabolism in children.

MATERIALS AND METHODS

The study comprised 62 children aged 6–18 years, including 42 patients with idiopathic decrease in mineralization (20 with osteoporosis and 22 with osteopenia) and 20 children in the control group. In 10 patients (7 with osteoporosis and 3 with osteope-

nia) examinations were repeated after one year of treatment (the treatment in this group of patients was similar: calcium preparations, vitamin D, physiotherapy).

The diagnosis of idiopathic osteoporosis or osteopenia was based on complex clinical, biochemical, and densitometric examinations. In the clinical evaluation, attention was paid to fractures, complaints of pain involving the skeletal and muscular systems, and also walking disorders. Fractures of long bones were confirmed in 12/20 with osteoporosis and 13/22 with osteopenia, and vertebral fractures in 5/20 with osteoporosis. Pain complaints were stated in 18/20 with osteoporosis and 17/22 with osteopenia, and walking disorders in 6/20 with osteoporosis. Patients with factors which could cause secondary mineralization disorders, such as long-term corticosteroid treatment, prolonged immobilization, and chronic diseases enhancing osteoporosis, such as diseases of the adrenal glands, thyroid, and parathyroid glands as well as hypogonadism, diabetes, anorexia nervosa, malabsorption syndrome, and liver and kidney diseases, were excluded from the study. Due to determination of cytokine concentrations, children with acute and chronic inflammations and with "fresh" healing bone fractures were also excluded; in order to eliminate any latent inflammatory processes the number of peripheral leukocytes, serum C reactive protein concentration, and erythrocyte sedimentation were determined in all patients.

The control group included healthy children without any symptoms of diseases, with normal results of laboratory (together with normal inflammatory indices) and densitometric examinations.

The study has been approved by the Ethics Committee of the Medical University of Łódź (No. RNN/154/02KE). Parents gave written consent for participation of their children in the study.

In all children a detailed questionnaire was completed and a physical examination conducted. The questionnaire included questions concerning diet (daily intake of calcium), physical activity, past diseases, and family history. Basic anthropometric measurements of body weight and height were done and the body mass index was calculated. The results were compared with the norms for children in Łódź[14]. Additionally, the stage of puberty was determined according to the Tanner criteria. Children with somatic development disorders were not included in the study.

All patients were subjected to bone densitometry performed by dual-energy X-ray absorptiometry on a DPX device (Lunar, USA) in the Regional Center of Menopause and Osteoporosis in Łódź. The bone mineral density (BMD) of the total body (total body projection) and the lumbar spine region (AP spine projection) were evaluated. BMD values in both projections were interpreted as a Z-score, i.e. a reference to standard values for the given age and sex. Z-scores were calculated based on reference data provided by the manufacturer of the DPX apparatus and the results were additionally verified by comparison with values gathered for the Polish population. According to the accepted criteria for children[2, 12, 26], osteoporosis was diagnosed at a Z-score <-2.00 (less than 2 units of standard deviation), and osteopenia was diagnosed at a Z-score between –1.00 and –2.00 (at least in one projection, with other concomitant signs suggesting these diseases, such as fractures, pain complaints, walking disorders). In the control group the Z-score was within the range of –1.00 to +1.00.

In all children, venous blood samples were collected in the morning after overnight fasting. Following centrifugation, the serum was frozen and kept at a temperature below –20°C for further analysis. Frozen serum specimens and investigation kits were moved to room temperature 30 min before the commencement of investigation procedures. All determinations were made according to the manufacturer's recommendations.

Concentrations of IL-1α, IL-β, IL-1 receptor antagonist (IL-1ra), and IL-6 as well as of soluble IL-6 receptor (IL-6sR) were determined by means of the enzyme-linked immunosorbent assay (ELISA) using commercially available kits. The concentration of IL-1α was determined with the Endogen Human IL-1 Alpha kit (Endogen Inc., USA). The sensitivity threshold of this method was <2 pg/ml and the intra-assay and inter-assay variability indices were $<10\%$. The concentration of IL-1β was determined with the Endogen Human IL-1 Beta kit (Endogen Inc., USA). The sensitivity threshold of this method was <1 pg/ml and the intra-assay and inter-assay variability indices were $<10\%$. The concentration of IL-1ra was determined with the Quantikine Human IL-1ra kit (Quantikine R&D Systems Inc., USA). The sensitivity threshold of this method was <22 pg/ml, the intra-assay variability index ranged 3.1–6.2%, and the inter-assay variability index ranged 4.4–6.7%. The concentration of IL-6 was determined with the Endogen Human IL-6 kit (Endogen Inc., USA). The sensitivity threshold of this method was <1 pg/ml and the intra-assay and inter-assay variability indices were $<10\%$. The concentration of IL-6sR was determined with the Quantikine Human IL-6sR kit (Quantikine R&D Systems Inc., USA). The sensitivity threshold

of this method was <7 pg/ml, the intra-assay variability index ranged 2.3–8.6%, and inter-assay variability index ranged 4.2–6.4%.

All the immunoenzymatic investigations were performed in the Unit of Hormone Chemistry at the Institute of Endocrinology of the Medical University of Łódź. In this unit the sensitivity of the Endogen kits for IL-1 and IL-6 had been previously studied and lower sensitivity thresholds (i.e. higher sensitivity) had been observed than in the data given by the manufacturer[24] (within the range 0.8–1.2 pg/ml for IL-1α and 0.3–0.6 pg/ml for IL-6). Therefore, the results below the sensitivity threshold of this method (presented by the kit manufacturer) were considered in the statistical analysis.

In the children with decreased bone mass, the calcium, phosphorus, and magnesium concentrations in serum as well as calcium excretion in 24-h urine samples (according to commonly accepted methods), and the concentrations of liver metabolite of vitamin D in serum (radiocompetitive method) and parathormone (radioimmunometric method) were determined. Moreover, in children with osteoporosis, bone formation markers in the serum were evaluated, such as bone isoenzyme of alkaline phosphatase and osteocalcin (ELISA method). Urinary excretion of bone resorption markers was determined in conversion to creatinine: the concentration of pyridinoline and deoxypyridinoline using high-performance liquid chromatography and collagen type I cross-linked C-telopeptide (CTx) using the ELISA method. Moreover, CTx was evaluated in serum, also by ELISA.

The statistical analysis was done using the Statistica 6.0 Program. The cytokine bioactivity (cytokine/receptor) index, i.e. the ratio of cytokine concentration to the main binding protein, was also calculated[10]. The arithmetic mean and standard deviation were calculated on the basis of the particular values of cytokine concentrations, cytokine receptors, and cytokine/receptor indices in the groups studied. Values of zero were analyzed as mathematical data. ANOVA analysis was performed to compare the values of the 3 groups studied. Dependencies between the studied features were evaluated using the Spearman R correlation coefficient. Changes in concentration values affected by treatment were studied by means of the Wilcoxon test. The results were accepted as statistically significant at $p<0.05$.

RESULTS

The clinical characteristics of the studied patients, mean bone mineral density, and inflammation indices in both groups are presented in Table 1. Children with osteoporosis, osteopenia, and healthy children did not differ significantly as to their age and somatic development, i.e. body weight and height. They also reported similar levels of physical activity and calcium intake with diet. The BMD was one of the conditions of the patient's inclusion into the particular group, thus Z-score values differed significantly ($p<0.01$).

In all children, concentrations of IL-1α were lower than 2 pg/ml (the sensitivity threshold of this method provided by the manufacturer). The values within the

Table 1. Clinical characteristics, bone mineral density (BMD), and inflammation markers in the studied groups of children (n=62)

Evaluated variable		Idiopathic osteoporosis n=20	Idiopathic osteopenia n=22	Control group n=20	p value
Age (years)		11.6 (6–18)	13.7 (8–18)	11.8 (6–17)	n.s
Body weight (kg)		38.1±13.9	51.2±17.1	46.3±18.4	n.s.
Body height (cm)		146.8±20.7	159.5±16.2	152.4±20.9	n.s.
Body mass index (kg/m^2)		17.2±3.2	19.6±3.4	19.4±3.5	n.s.
Physical activity (h/week)		15.6±11.6	17.5±10.7	22.5±9.2	n.s.
Calcium intake with diet (mg/day)		966.1±419.3	1034.2±326.5	1205.2±368.4	n.s
Total body BMD					
(g/cm^2)		0.840±0.123	0.960±0.127	0.960±0.140	<0.01
(Z-score)		–1.68±1.10	–0.62±0.66	0.25±0.81	<0.01
Spine BMD					
(g/cm^2)		0.590±0.215	0.860±0.183	0.890±0.210	<0.01
(Z-score)		–2.97±0.91	–1.51±0.28	0.10±0.74	<0.00001
Inflammation markers					
leukocytes (×10^6/l)		5700 (3100–9600)	5600 (4100–8600)	5900 (4100–9300)	n.s
CRP (ng/ml)		<0.5	<0.5	<0.5	n.s
erythrocyte sedimentation	after 1 h (mm)	8 (2–20)	4 (1–16)	6 (2–18)	n.s
	after 2 h (mm)	19 (4–40)	7 (3–40)	12 (4–42)	n.s

Results expressed as mean (range) or mean ±SD; n.s. – not statistically significant.

0–2 pg/ml range determined in 42 children (67%) were subject to the statistical analysis due to the lower sensitivity threshold of the kit achieved in the unit own investigations. Concentrations of IL-1β ranging 0–1 pg/ml were observed only in 3 children with osteoporosis and 1 with osteopenia; because of the small number of children, these values were not analyzed. Concentrations of IL-6 were analyzed for all the children studied, of which values >0 pg/ml were determined in 48 children (77%) and separately in 17 children (27%) in whom IL-6 concentrations exceeded the sensitivity threshold of the method provided by the manufacturer (1 pg/ml). In all children studied, IL-1ra and IL-6sR were detected in high concentrations.

Mean concentrations of cytokines, their receptors, and cytokine/receptor indices in the groups of children studied are presented in Tables 2 and 3. Most of the mean values did not differ between the groups. However, among patients with IL-6 >1 pg/ml, a higher IL-6 concentration was noted in children with idiopathic osteoporosis and osteopenia than in the control group ($p<0.05$; Table 3 and Fig. 1). In these patients a tendency towards higher IL-6sR values in comparison with the reverence group was observed, although the differences were not statistically significant ($p=0.05$; Table 3 and Fig.1).

A negative correlation was found by evaluating the relationship between the cytokine concentration and the bone mass. Concentrations of IL-1α and IL-6 as well as the cytokine/receptor indices showed statistically significant negative correlation with the BMD of lumbar spine ($p<0.05$); however, such dependencies were not found in the Total Body projection (Table 4 and 5).

In patients with osteoporosis and osteopenia, the relationship between the cytokine concentration and the selected biochemical indices of calcium-phosphate (Ca-P) metabolism were analyzed (Table 6), while in children with osteoporosis the bone turnover was also taken into account (Table 7). The concentration of IL-1α and the IL-1/IL-1ra index positively correlated with the parathormone concentration in serum. A significant correlation was observed between cytokines and bone formation markers:

Table 2. Mean values of serum IL-1α, IL-1β, IL-1ra concentration, and IL-1/IL-1ra index in the studied groups of children (n=62)

Evaluated variable	Idiopathic osteoporosis n=20	Idiopathic osteopenia n=22	Control group n=20	p value
IL-1α (pg/ml)	0.33 (0.00–1.10) n_1=15 (75%)	0.35 (0.00–1.10) n_1=14 (64%)	0.20 (0.00–0.70) n_1=13 (59%)	n.s
IL-1β (pg/ml)	0.05 (0.00–0.50) n_2=3 (15%)	0.002 (0.00–0.01) n_2=1 (4.5%)	0.00 (0.00–0.00) n_2=0 (0%)	n.s.
IL-1ra (pg/ml)	407 (187–1182)	427 (187–1060)	403 (142–996)	n.s.
IL-1/IL-1ra index ($\times 10^{-3}$)	1.03 (0.00–2.71)	0.92 (0.00–4.72)	0.56 (0.00–2.63)	n.s.

Results expressed as mean (range), there was not statistically significant difference between groups ($p>0.05$).
Explanations: n_1 – number of children (%) with concentration IL-1α = 0–2 pg/ml, n_2 – number of children (%) with concentration IL-1β = 0–1 pg/ml, n.s. – not statistically significant.

Table 3. Mean values of serum IL-6, IL-6sR concentration, and IL-6/IL-6sR index in the studied groups of children

Evaluated variable	Idiopathic osteoporosis	Idiopathic osteopenia	Control group	p value
All studied children (n=62)	n=20	n=22	n=20	
IL-6 (pg/ml)	1.14 (0.00–5.50) n_1=18 (90%)	1.14 (0.00–6.10) n_1=18 (82%)	0.44 (0.00–1.70) n_1=12 (60%)	n.s.
IL-6sR (pg/ml)	38 742 (25 456–59 508)	37 572 (24 528–65 212)	36 643 (22 588–64 632)	n.s.
IL-6/IL-6sR index ($\times 10^{-3}$)	0.03 (0.00–0.18)	0.03 (0.00–0.22)	0.02 (0.00–0.07)	n.s.
Children with concentration IL-6 >1 pg/ml (n=17)	n=7	n=6	n=4	
IL-6 (pg/ml)	2.79 (1.20–5.50)	3.33 (1.10–6.10)	1.43 (1.10–1.70)	<0.05
IL-6sR (pg/ml)	40 201 (30 352–59 508)	38 120 (27 588–52 916)	29 524 (25 744–33 386)	=0.05
IL-6/IL-6sR index ($\times 10^{-3}$)	0.07 (0.03–0.18)	0.10 (0.02–0.22)	0.05 (0.04–0.07)	n.s.

Results expressed as mean (range).
Explanations: n.s. – not statistically significant, n_1 – number of children (%) with concentration IL-6 >0 pg/ml.

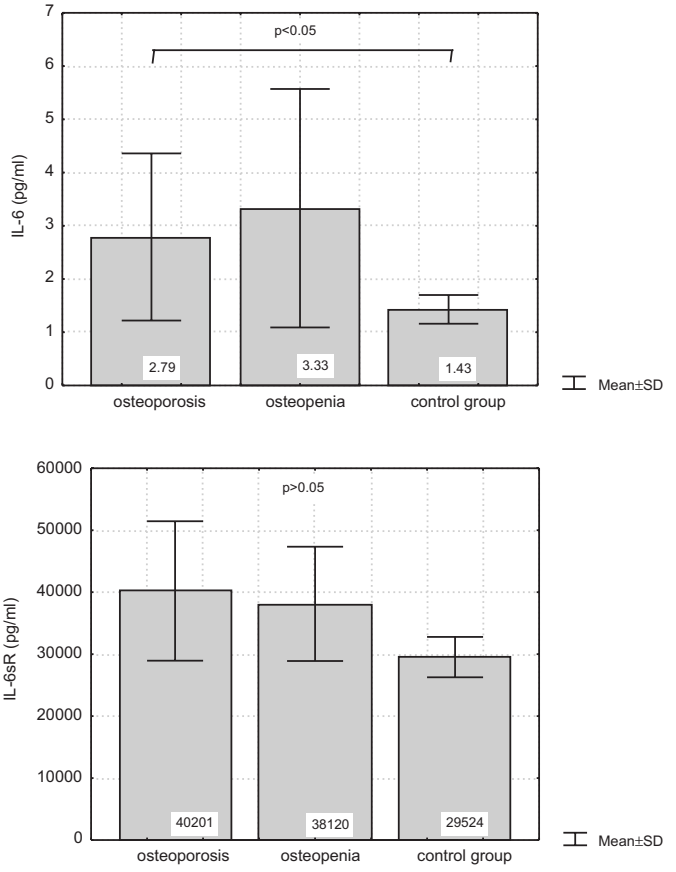

Figure 1. Mean concentration of IL-6 and IL-6sR in children with idiopathic osteoporosis, osteopenia and in control group (children with IL-6 >1 pg/ml, n=17)

IL-1α positively correlated with osteocalcin concentration, and IL6sR with bone alkaline phosphatase (Table 7). No significant correlations of cytokine concentrations with the remaining Ca-P metabolism indices and, in particular, with the bone resorption markers were recorded (Table 6 and 7).

The follow-up investigations performed in 10 children after 1 year of osteoporosis/osteopenia treatment revealed that the increase in bone mineral density was accompanied by a decrease in serum IL-6sR concentration (Table 8 and Fig. 2).

DISCUSSION

An evaluation of dependencies between IL-1 and IL-6 concentrations and osteoporosis and osteopenia in children encounters problems related to their indeterminable values in serum. Lack of detectable concentrations in serum might mean that these cytokines do not play a crucial role in the pathogenesis of osteoporosis. On the other hand, it points to imperfection in the investigation method. Moreover, it cannot be precluded that the character of the cytokine activity is mainly local and confined to bone tissue.

In most of the patients studied, the elevated IL-1 and IL-6 concentrations in serum were very low, close to indeterminable values. Despite the lower sensitivity

Table 4. Spearman R correlation coefficients between serum concentration of IL-1α, IL-1ra, and IL-1/IL-1ra index, and BMD in all studied children (n=62)

BMD	IL-1α (pg/ml)		IL-1ra (pg/ml)		IL-1/IL-1ra index ($\times 10^{-3}$)	
	R	p	R	p	R	p
Total body (g/cm^2)	–0.23	0.07	0.01	0.99	–0.23	0.06
Spine (g/cm^2)	–0.26	0.04*	–0.15	0.24	–0.24	0.06
Total body (Z-score)	–0.10	0.45	0.17	0.18	–0.25	0.07
Spine (Z-score)	–0.15	0.23	0.04	0.73	–0.27	0.03*

*p<0.05

Table 5. Spearman R correlation coefficients between serum concentration of IL-6, IL-6sR, and IL-6/IL-6sR index, and BMD in studied children

BMD	IL-6 (pg/ml)		IL-6sR (pg/ml)		IL-6/IL-6sR index ($\times 10^{-3}$)	
	R	p	R	p	R	p
All studied children (n=62)						
Total body (g/cm^2)	0.16	0.21	0.01	0.96	–0.15	0.26
Spine (g/cm^2)	–0.26	0.04*	0.02	0.85	–0.24	0.03*
Total body (Z-score)	–0.06	0.61	–0.12	0.36	–0.02	0.84
Spine (Z-score)	–0.22	0.07	–0.16	0.20	–0.19	0.13
Children with concentration IL-6>1 pg/ml (n=17)						
Total body (g/cm^2)	–0.22	0.38	0.17	0.49	–0.18	0.48
Spine (g/cm^2)	–0.36	0.13	0.04	0.85	–0.31	0.20
Total body (Z-score)	–0.27	0.27	–0.24	0.34	–0.06	0.81
Spine (Z-score)	–0.41	0.09	–0.47	0.04*	0.14	0.56

* p<0.05

Table 6. Spearman R correlation coefficients between serum concentration of IL-1, IL-6, their receptors and cytokine/receptor index, and biochemical calcium-phosphate metabolism markers in children with idiopathic osteoporosis and osteopenia (n=42)

Evaluated variable	Serum calcium (mmol/l)	Serum magnesium (mmol/l)	Serum phosphorus (mmol/l)	Urine calcium (mg/kg/24 h)	25OHD (ng/ml)	PTH (pg/ml)
	R p	R p	R P	R p	R p	R p
IL-1α (pg/ml)	0.26 0.11	–0.07 0.67	–0.07 0.65	–0.04 0.79	–0.02 0.86	0.39 0.03*
IL-1ra (pg/ml)	0.25 0.11	–0.30 0.05	0.05 0.72	0.01 0.92	0.27 0.10	0.23 0.22
IL-1/ IL-1ra	0.02 0.86	0.04 0.77	–0.14 0.38	–0.05 0.75	–0.24 0.14	0.43 0.01*
IL-6 (pg/ml)	0.01 0.97	0.31 0.05	–0.15 0.35	–0.21 0.20	0.13 0.80	–0.01 0.98
IL-6sR (pg/ml)	–0.07 0.64	–0.15 0.33	–0.14 0.39	–0.08 0.59	0.19 0.25	–0.13 0.48
IL-6/IL6sR	0.02 0.89	0.31 0.05	–0.13 0.41	–0.20 0.22	0.10 0.54	0.01 0.92

Explanations: PTH – parathormone, 25OHD – liver metabolite of vitamin D, *p <0.05

Table 7. Spearman R correlation coefficients between serum concentration of IL-1, IL-6, their receptors and cytokine/receptor index, and bone turnover markers in children with idiopathic osteoporosis (n=20)

Evaluated variable	Serum			Urine		
	BAP (U/l)	osteocalcin (ng/ml)	CTx (pmol/l)	Pyr/cr (pmol/μmol)	Dpyr/cr (pmol/μmol)	CTx/cr (μg/mmol)
	R p	R p	R P	R p	R p	R p
IL-1α (pg/ml)	0.08 0.74	0.58 0.008*	0.36 0.12	0.25 0.33	0.18 0.48	0.18 0.52
IL-1ra (pg/ml)	0.13 0.59	0.33 0.16	–0.06 0.80	0.30 0.22	0.29 0.25	–0.23 0.39
IL-1/ IL-1ra	0.02 0.93	0.43 0.06	0.44 0.05	–0.23 0.46	–0.31 0.21	–0.22 0.42
IL-6 (pg/ml)	0.10 0.66	0.01 0.97	0.14 0.55	0.16 0.52	0.08 0.74	0.07 0.79
IL-6sR (pg/ml)	0.47 0.04*	0.39 0.09	–0.38 0.10	0.33 0.19	0.37 0.14	–0.18 0.51
IL-6/IL-6sR	0.04 0.86	–0.05 0.82	0.19 0.41	0.10 0.68	0.01 0.97	0.11 0.67

Explanation: BAP – bone isoenzyme of alkaline phosphatase, CTx – C-telopeptide, Pyr – pyridinoline, Dpyr – deoxypyridinoline, cr – creatinine; *p<0.05.

Table 8. Concentration of cytokine and BMD before and after treatment of osteoporosis/osteopenia (n=10)

Evaluated variable	Mean values before treatment (±SD)	Mean values after treatment (±SD)	Evaluation of difference between groups	
			values of Wilcoxon test	p
IL-1α (pg/ml)	0.32 (±0.39)	0.47 (±0.38)	0.81	0.41
IL-1ra (pg/ml)	564 (±341)	556 (±151)	0.05	0.95
IL-6 (pg/ml)	1.87 (±1.8)	1.77 (±1.8)	0.06	0.95
IL-6sR (pg/ml)	40 851 (±10 989)	34 904 (±7 770)	2.19	0.02*
Total body (g/cm^2)	0.839 (±0.145)	0.867 (±0.143)	2.43	0.01*
Spine (g/cm^2)	0.613 (±0.292)	0.755 (±0.241)	2.66	0.007*
Total body (Z-score)	–1.38 (±1.06)	–1.29 (±1.12)	0.98	0.32
Spine (Z-score)	–2.90 (±1.36)	–1.83 (±0.83)	2.39	0.01*

*p<0.05

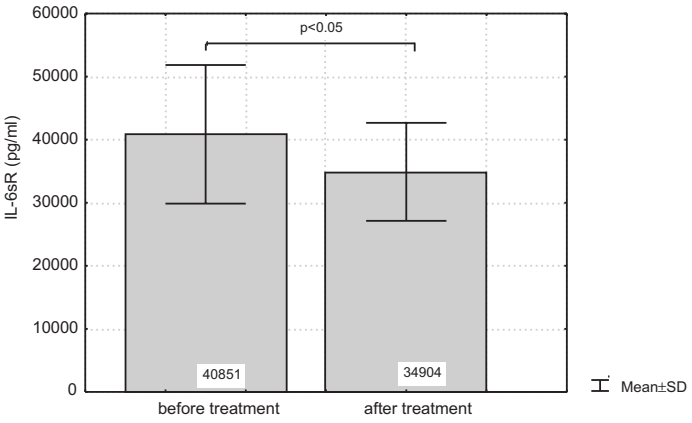

Figure 2. Mean values of IL-6sR serum concentration before and after treatment of osteoporosis/osteopenia (n=10)

threshold stated in the investigations conducted in the laboratory, it was not unlikely that they were equal to zero; thus, on the basis of these values, explicit and reliable conclusions cannot be drawn. Therefore the analysis of IL-6 concentrations was performed separately for 17 children in whom concentrations were higher than 1 pg/ml. In this subgroup, the IL-6 concentration was higher in children with osteoporosis than in the control group. A tendency towards a negative correlation between this cytokine's concentration and BMD index suggests an IL-6 effect on the decrease in bone mass. The results are in accordance with findings of other studies on adults with primary involutional osteoporosis[18, 21, 23], and also with reports of Libanati et al.[13], who demonstrated an inversely proportional dependence between IL-6 concentration and BMD in healthy girls.

In the present study a direct correlation between IL-6 concentration and Ca-P metabolism and also bone turnover markers, in particular bone resorption markers, was observed. This finding does not, however, contradict the commonly known activity of IL6, i.e. bone resorption stimulation[3, 5, 27].

The concentration of the soluble IL-6 receptor form seems to be associated with idiopathic osteoporosis, as after isolation of the subgroup with detectable values of IL-6 (n=17) there was a tendency towards higher IL-6sR values in children with decreased mineralization than in the control group. Moreover, this concentration significantly negatively correlated with trabecular bone mineralization determined as the Z-score for the lumbar spine region and at the same time positively correlated with the activity of bone alkaline phosphatase in serum. Consequently, the elevated concentration of the soluble receptor is likely to be a marker of the negative IL-6 effect on mineralization and bone metabolism (acceleration of bone turnover), and these dependencies are more easily detected due to higher receptor concentration in serum than interleukin concentration alone.

The results of this study correspond to literature data indicating that IL-6sR, as opposed to other soluble forms of the cytokine receptors, does not exhibit an inhibitory action but, on the contrary, seems to posses a stimulating effect on biological activity of the cytokine[7, 19]. This can be explained by the high affinity of IL-6 first to IL-6sR, and then of the whole complex to the membrane part of receptor, which results in transmission of the signal into the cell[7].

Such synergistic activity might be supported by the fact that in this study the value of the IL-6/IL-6sR index was not found to be more strongly associated with the bone mineralization than the concentration of the cytokine alone. However, in the case of soluble receptors with antagonistic action, different dependencies are usually observed: the cytokine/soluble receptor index correlates better with its bioactivity than the concentration of the cytokine alone[10].

In the present study, IL-1 concentrations in serum were not directly linked with the occurrence of osteoporosis and osteopenia, because in all children examined the values were lower than the sensitivity threshold of the method. No correlation between the concentration of the IL-1ra and bone mineralization was observed, either. However, in the range of low IL-1α values, below the threshold sensitivity, a significant negative correlation between IL-1α, IL-1/IL-1ra, and mineral density of the lumbar spine was recorded. Moreover, both IL-1α and the IL-1/IL-1ra index positively correlated with the concentration of parathormone and osteocalcin in serum. The results are not conclusive because of some limitations in the method of cytokine investigation (the discussed dependencies are related to concentrations approaching 0 values).

Interesting results were achieved by comparing "baseline" cytokine concentrations with the values obtained after one year of osteoporosis/osteopenia treatment. It appeared that significant improvement in mineralization was accompanied by a decrease in IL-6sR concentration. A similar decrease in this receptor concentration following treatment with biphosphonates in adults was also observed by Abildgaard et al.[1]. This could suggest that the applied management beneficially affects the change in biological activity of IL-6 in serum and, at the same time, confirms its role in pathogenesis of mineralization disorders. These suggestions require further investigations.

The obtained results show that certain proinflammatory cytokines, and predominantly IL-6, at higher concentrations are assumed to play a crucial role in inducing idiopathic osteoporosis and osteopenia in the developmental age. Such a hypothesis needs to be verified in further research on larger groups of children and with the use of more precise investigative techniques. Detailed identification of all pathogenic mechanisms is very difficult, also due to the fact that the functioning of a complicated "cytokine network" which causes a change in activity of one factor is associated with the activation or inhibition of others and also to the phenomenon of cytokine pleiotrophy and redundancy. What is more, changes in cytokine concentrations in serum do not always follow their changes in tissues.

In conclusion, higher serum IL-6 concentration together with lower bone mass in children with idiopathic osteoporosis/osteopenia reveals the involvement of IL-6 in the primary decrease in bone mineralization. The results of the study suggest that IL-1 may also participate in the etiopathogenesis of idiopathic osteoporosis and osteopenia in the developmental age.

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