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Association of bone fracture type and degree of callus formation with leptin concentration in children with long bone fractures

Povezanost tipa preloma kosti i stepena formiranja kalusa sa koncentracijom leptina kod dece sa prelomima dugih kostiju

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Abstract

Background/Aim. Recent studies indicate that adipokines have an important role in bone physiology and pathology. Recent data indicate that adipokine leptin functions as a regulator of bone growth at multiple levels, systemically and locally. So far, it has been shown that leptin influences bone volume and bone mineral density in a population with metabolic and/or hormonal abnormality. Data concerning leptin values in non-obese children with fractures are scarce. **Methods.** This study included 93 non-obese children with long bone fractures (LBF), 14 children with short bone fractures (SBF), and 19 healthy children. Leptin concentration was determined in two blood samples (day 0 and day 21) and analyzed according to gender, fracture type, anatomical localization of the fracture, fracture topography, callus formation, and the healing outcome. **Results.** Children with LBF demonstrated significantly increased leptin levels compared to the control group (both day 0/day 21). In the control group, girls had significantly more leptin than boys. Leptin value was significantly influenced by anatomical localization since boys and girls with humerus fracture and girls with femur fracture had the highest average leptin concentration in the initial sample. Boys with incomplete callus formation had the highest leptin concentration (both day 0 /day 21), significantly elevated compared to boys' samples in the control group, boys' samples with an intermediary and well-formed callus, and also increased compared to the initial samples of girls with incomplete callus. Better callus formation in girls was associated with an increment of leptin concentrations in the second over the initial sample. Girls with partially and satisfactorily formed callus had significantly increased leptin concentration in the second sample (day 21) compared to the boys' group. **Conclusion.** Leptin concentration was significantly increased (both samples) in children with LBF compared to children with SBF and corresponding controls. Leptin concentration was highly influenced by gender. High blood leptin concentrations in boys or low leptin concentrations in girls immediately upon fracture could be used to identify groups of children with incomplete callus formation.

Key words:
fractures, bone; humeral fractures; radius fractures; tibial fractures; ulna fractures; child; leptin; bony callus; gender; prognosis.

Apstrakt

Uvod/Cilj. Novije studije pokazuju da adipokini imaju važnu ulogu u fiziologiji i patologiji kostiju. Takođe, najnoviji podaci pokazuju da adipokin leptin funkcioniše kao regulator rasta kostiju sistemski i lokalno. Pokazano je da leptin utiče na volumen kostiju i mineralnu gustinu kostiju u populaciji sa metaboličkom i/ili hormonskom

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abnormalnošću. Podaci o vrednostima leptina kod negojazne dece sa frakturama su oskudni. **Metode**. U ovu studiju bila su uključena 93 negojazna deteta sa prelomima dugih kostiju (LBF), 14 dece sa prelomima malih kostiju (SBF) i 19 zdrave dece. Koncentracija leptina određena je u 2 uzorka krvi (0. dana i 21. dana) i analizirana prema polu, tipu frakture, lokalizaciji anatomske frakture, topografiji frakture, formiranju kalusa i ishodu zarastanja. **Rezultati.** Deca sa LBF imala su značajno povećane nivoe leptina u poređenju sa kontrolnom grupom u oba uzorka krvi (0. dana/21. dana). U kontrolnoj grupi devojčice su imale značajno više nivoe leptina od dečaka. Na vrednost leptina značajno je uticala anatomska lokalizacija, jer su dečaci i devojčice sa prelomom humerusa i devojčice sa prelomom femura imali najveću prosečnu koncentraciju leptina u početnom uzorku. Dečaci sa nepotpuno formiranim kalusom imali su najveću koncentraciju leptina (u oba uzorka, 0. dana/21. dana), značajno višu u odnosu na kontrolne uzorke dečaka, uzorke dečaka s intermedijarnim i dobro formiranim kalusom, a takođe višu u odnosu na koncentracije leptina u početnim uzorcima djevojčica s nepotpunim kalusom. Bolje formiranje kalusa kod devojčica je bilo povezano sa povećanjem koncentracije leptina u drugom (21. dan) u odnosu na početni uzorak (0. dan). Devojčice sa delimično i zadovoljavajuće formiranim kalusom imale su značajno višu koncentraciju leptina u drugom uzorku (21. dan) u odnosu na grupu dečaka. **Zaključak**. Koncentracija leptina je značajno povećana (u oba uzorka krvi) kod dece sa LBF u poređenju sa decom sa SBF i odgovarajućim kontrolama. Koncentracija leptina je zavisna od pola. Visok nivo leptina u krvi kod dečaka ili niska koncentracija leptina kod devojčica odmah nakon preloma može se koristiti za identifikaciju grupa dece sa nepotpunim formiranjem kalusa.

Ključne reči:
prelomi; humerus, prelomi; radijus, prelomi; tibija, prelomi; ulna, prelomi; deca; leptin; kalus; pol; prognoza.

Introduction

The recovery of fractured bone represents unique biological phenomena in which the healing bone repairs itself through a complex interaction with immune cells, blood, bone marrow cells, and soft tissue cells. Finally, the healing bone again gains previous functional mechanical stability. According to classic understanding, there are three phases of this process, each represented with specific mediators. The initial, inflammatory, or the early remodeling phase, which starts immediately after bone injury, is characterized by increased secretion of IL-1, IL-6, and TNF-alpha [1, 2]. The reparative phase, which starts after a few hours upon fracture, is conducted by local and systemic production of numerous growth and differentiation factors, such as transforming growth factors (TGF), bone morphogenic proteins (BMP), platelet-derived growth factors (PDGF), fibroblast growth factor (FGF), and others [3, 4]. The third, remodeling phase, which starts with endochondral ossification, is mediated with metalloproteinases and angiogenic factors, like vascular endothelial growth factors (VEGF) and angiopoietins [5].

Data from a growing number of studies indicate that adipokines, mediators that are discovered primarily as fat and energy regulators, are also involved in the remodeling and development processes in bone physiology. Adiponectin, leptin, resistin, visfatin, and others are recognized as important regulators of bone metabolism [6]. Beyond its effects on fat tissue, leptin modulates immune response and inflammation [7]. Arguments demonstrating leptin's influence on bone tissue came both from experimental and clinical data. Mesenchymal stem cells and osteoblasts express functional leptin receptors [8, 9], and leptin ligation of specific receptor-induced cell proliferation [10] comparably intensive as seen in response to insulin live growth factor 1 (IFG-1), major anabolic bone stimulator [11]. Furthermore, it has been shown that leptin induces differentiation of mesenchymal cells to osteoblasts, increasing both messenger ribonucleic acid (mRNA) and protein levels of osteocalcin, type I collagen, alkaline phosphatase (ALP), and mineralization [12, 13]. Experimental leptin administration *in vivo* increased bone mineral density and bone length in the genetically manipulated ob/ob mice [14], significantly reduced bone fragility in male mice [11], and prevented bone loss in estrogen-deficient ovariectomized rats [15]. It seems that leptin acts directly on the bone because a study in which ovariectomized rats were treated with a virus vector expressing leptin directly in the hypothalamus showed no changes in bone parameters compared to peripheral administration of leptin [16].

Data connecting leptin with bone physiology in humans came mainly from studies of osteoporotic women. These results are still controversial; some of them indicate no leptin influence on bone metabolism [17], but others demonstrate association between leptin and bone mineral density [18, 19]. Newer results have indicated that leptin is valuable marker in osteoporosis [20], and that the increase in serum leptin level correlates with bone mineral density increase in postmenopausal women with primary knee osteoarthritis [21].

Studies in which leptin concentration was determined in children were mainly focused on the obese population, and they demonstrated a direct correlation between serum leptin values, body mass index (BMI), and obesity [22, 23]. Besides data from control children in some studies [24], we did not find any studies that investigated adipokines' influence on bone recovery after a fracture in the population of healthy children.

Methods

Participants

All children were admitted, diagnosed, and treated at the Department of Orthopedics and Joints/Bone Trauma, at

the Institute for Health Protection of Mother and Child, "Dr. Vukan Čupić", Belgrade, Serbia. This study was approved by the Ethics Committee of this Institute (No 8/26, 13/10/2015). Parents of all investigated children were informed by the attending medical doctor and gave written consent for their participation in the study.

Inclusion criteria

Nonobese children (BMI 15.0–24.0 kg/m^2), both boys and girls, aged 4 to 18 years, were included in the study. The investigated groups consisted of children with long bone fractures (LBF, n = 93), children with finger fractures – short bone fractures (SBF, n = 14), and a control group of children (control, n = 19) (Tables 1 and 2). The control group consisted of children admitted to the Clinic due to trauma of extremities but without any evidence of bone fracture.

Exclusion criteria

Obese children (BMI over 24.0 kg/m^2), children with other injuries beyond long bone fracture, children with malignant diseases, systemic connective tissue diseases, metabolic diseases/disorders, and children with congenital anomalies of the joint/bone were not included in the study.

For each patient, radiological records were made as standard two X-ray projections [anteroposterior (AP) and profile], a total of 5 records at different time intervals (immediately before the orthopedic procedure, immediately after the intervention, follow-up after 7 days, follow-up after 21 days, and after removing the cast immobilization). All fractures were classified according to type (open, closed fracture), anatomical localization (humerus, radius, radius + ulna, femur, tibia, tibia + fibula), the topography of the affected bone (fracture of the proximal or distal segment,

Table 1

Demographic characteristics of investigated children

Groups of patients	All	Boys	Girls
LBF (n = 93)			
age (years)	9.3 ± 3.4	9.3 ± 3.7	9.3 ± 3.0
weight (kg)	32.5 ± 11.8	32.7 ± 12.6	31.3 ± 11.4
height (cm)	136.7 ± 18.4	139.2 ± 20.2	135.2 ± 17.2
BMI (kg/m^2)	16.6 ± 1.9	16.3 ± 1.6	16.9 ± 2.1
SBF (n = 14)			
age (years)	9.7 ± 5.9	9.8 ± 6.6	9.6 ± 5.1
weight (kg)	32.5 ± 10.8	31.7 ± 18.6	31.3 ± 12.2
height (cm)	134.1 ± 20.0	137.1 ± 26.5	131.1 ± 14.2
BMI (kg/m^2)	16.4 ± 1.6	16.1 ± 1.7	16.8 ± 1.5
Controls (n = 19)			
age (years)	9.1 ± 5.0	9.1 ± 5.4	9.0 ± 4.6
weight (kg)	31.4 ± 10.7	31.5 ± 10.5	31.1 ± 10.9
height (cm)	134.1 ± 13.8	135.2 ± 14.5	133.0 ± 13.1
BMI (kg/m^2)	16.4 ± 1.8	16.1 ± 2.0	16.7 ± 1.5

***Note*: Results are given as mean ± standard deviation.**
LBF – long bone fractures; BMI – body mass index;
SBF – small bone fractures.

Table 2

Number (%) of LBF patients according to fracture properties

Fracture properties	All	Boys	Girls
Anatomical localization			
humerus	25 (27)	16 (30)	9 (23)
radius	7 (7)	4 (8)	3 (7)
radius + ulna	33 (35)	18 (34)	15 (37)
femur	10 (11)	5 (9)	5 (13)
tibia	11 (12)	6 (11)	5 (13)
tibia + fibula	7 (7)	4 (8)	3 (7)
Total	93 (100)	53 (100)	40 (100)
Type			
oblique	21 (23)	14 (27)	7 (18)
transverse	58 (62)	33 (62)	25 (62)
spiral	14 (15)	6 (11)	8 (20)
Total	93 (100)	53 (100)	40 (100)
Callus formation (%)			
< 25	17 (18)	9 (17)	8 (20)
< 50	43 (46)	24 (45)	19 (48)
> 75	33 (36)	20 (38)	13 (32)
Total	93 (100)	53 (100)	40 (100)

LBF – long bone fracture.

diaphysis fractures), the severity of bone injury (easy, heavy, complicated), callus formation (based on radiological analysis as < 25% – incomplete, < 50% – partial, > 75% – satisfactory) and the healing outcome (unsatisfactory, incomplete, complete). According to the data obtained from parents, children were analyzed for previous bone fractures, propensity, and history of upper respiratory tract infections, allergies, previous nursery or school residence, and feeding habits.

Study design

The study was designed as a cross-sectional investigation. From all investigated children, the first initial blood sample (on the day 0 – 0d), 2 mL of venous blood from the cubital vein, was taken within the 1st hour upon admission. Where possible, a second blood sample (on the day 21 – 21d), 2 mL of venous blood from the cubital vein, was taken after 21 days of bone fracture. After separation of the serum, samples were frozen at –70ºC until testing.

Leptin determination

The concentration of leptin was determined with a commercial flow cytometric test (LEGENDplex 13-plex Human Adipokine Panel) on a flow cytometer Beckman Coulter FC500.

Statistical analysis

Parameters of descriptive statistics were used to estimate the central tendency of data (mean, median), and to analyze group variability (standard deviation, standard error, range, 95% interval of confidentiality). The analysis between more than two groups, groups according to fracture type (oblique, transverse, spiral), anatomical localization of fracture (humerus, radius, radius + ulna, femur, tibia, tibia + fibula), and between groups according to the degree of callus formation (< 25%, < 50%, > 75% of formed callus) was performed using the one-way analysis of variance (ANOVA), with Bonferroni post-testing. Mann-Whitney test was used for all other comparisons between two independent groups. The sensitivity and specificity of leptin determination were analyzed with receiver-operating characteristic (ROC) curves constructed on the basis of values detected in the control boys' and girls' groups. All statistical analyses were done using the statistical package GraphPad Prism 5.01 (GraphPad Prism Software Inc. California, USA).

Results

Leptin concentration in children with long bone fractures vs. children with small bone fractures and control healthy children

Average leptin concentration in samples of all investigated children with long bone fractures was insignificantly increased compared to the control group in both time intervals (Tables 3 and 4). Same data were demonstrated in groups of boys and girls; both groups had an increased leptin value compared to their adequate peers. Both groups showed a slight leptin increase in the second sample. Boys with long bone fractures had insignificantly more leptin compared to boys with fractures of short bones (SBF). Leptin was significantly increased in the samples of girls compared to boys, both from the control group (Table 5).

Table 3

Average leptin concentration (ng/mL) in initial blood samples (on the day 0) of children with LBF, SBF and control children

Parameters	All	Boys	Girls
LBF	2.46 ± 1.29	2.27 ± 1.22	2.59 ± 1.16
long bone type			
oblique	2.85 ± 0.17	2.73 ± 0.19	3.01 ± 0.58
transverse	2.31 ± 0.11	2.24 ± 0.13	2.46 ± 0.84
spiral	2.14 ± 0.71	2.12 ± 0.76	2.17 ± 0.80
long bone localization			
humerus	2.72 ± 1.61	2.72 ± 1.74	2.73 ± 0.71 [b]
radius	1.61 ± 1.12	2.10 ± 0.97	1.12 ± 0.14 [b]
radius + ulna	2.27 ± 1.10	2.34 ± 1.46	2.19 ± 0.75
femur	2.76 ± 1.41	1.98 ± 0.72	3.54 ± 1.78 [b]
tibia + fibula	1.70 ± 0.34	1.88 ± 0.51	1.52 ± 0.15
Callus (%)			
< 25	3.23 ± 2.05	4.47 ± 2.05 [c, d, e, f]	1.73 ± 0.32 [f]
< 50	2.42 ± 1.53	2.27 ± 1.29 [c]	2.60 ± 1.06
> 75	2.31 ± 0.75	2.20 ± 0.77 [d]	2.56 ± 0.68
SBF	2.16 ± 0.41	1.91 ± 0.50	2.47 ± 0.29
Control	2.02 ± 0.22	1.67 ± 0.49 [a, e, h]	2.38 ± 0.41 [a]

Note: **Results are given as mean ± standard deviation.**
LBF – long bone fractures; SBF – small bone fractures.
Superscript letters mark pairs of groups that differ significantly (see Table 5).

Table 4

Average leptin concentration (ng/mL) in blood samples of children with LBF, SBF, and control children on the day 21

Groups of patients	All	Boys	Girls
LBF	2.59 ± 1.43	2.40 ± 1.26	2.85 ± 1.57
long bone type			
oblique	3.15 ± 1.81	3.63 ± 2.28	2.51 ± 0.98
transverse	2.39 ± 1.27	2.17 ± 0.95	2.99 ± 1.85
spiral	2.21 ± 0.89	1.82 ± 0.22	2.78 ± 1.41
long bone localization			
humerus	2.16 ± 0.71	2.20 ± 0.87	2.14 ± 0.53
radius	1.96 ± 0.52	2.10 ± 0.65	1.82 ± 0.38
radius + ulna	2.99 ± 1.30	2.65 ± 2.01	3.32 ± 0.59
femur	2.06 ± 0.32	2.14 ± 0.11	2.00 ± 0.54
tibia + fibula	2.23 ± 1.25	2.04 ± 0.61	2.42 ± 1.61
Callus (%)			
< 25	2.87 ± 2.01	3.92 ± 2.24 [g, h]	1.61 ± 0.33 [i]
< 50	2.27 ± 1.34	1.86 ± 0.78 [g, j]	3.13 ± 1.89 [j]
> 75	2.64 ± 0.83	2.36 ± 0.72 [k]	3.13 ± 0.83 [i, k]
SBF	2.16 ± 0.41	1.91 ± 0.50	2.47 ± 0.29
Control	2.02 ± 0.22	1.67 ± 0.49 [a, e, h]	2.38 ± 0.41 [a]

LBF – long bone fractures; SBF – small bone fractures.
Superscript letters mark pairs of groups that differ significantly (see Table 5).

Table 5

Statistical analysis of differences in leptin concentrations according to level of callus formation inside groups of boys and girls, and between groups of boys and girls

Marker	Group	Sample	Callus (%)		Group	Sample	Callus (%)	Significance
a	Boys	Control	none	*vs*	Girls	Control	none	**
c	Boys	Day 0	< 25	*vs*	Boys	Day 0	< 50	**
d	Boys	Day 0	< 25	*vs*	Boys	Day 0	> 75	**
e	Boys	Day 0	< 25	*vs*	Boys	Control	none	***
f	Boys	Day 0	< 25	*vs*	Girls	Day 0	< 25	*
g	Boys	Day 21	< 25	*vs*	Boys	Day 21	< 50	**
h	Boys	Day 21	< 25	*vs*	Boys	Control	none	**
i	Girls	Day 21	< 25	*vs*	Girls	Day 21	> 75	*
j	Boys	Day 21	< 50	*vs*	Girls	Day 21	< 50	*
k	Boys	Day 21	> 75	*vs*	Girls	Day 21	> 75	*

***p* < 0.05; ** *p* < 0.01; ***p* < 0.001 (Mann-Whitney test).**

Leptin concentration in children with long bone fractures according to the type of fracture

The analysis of all investigated children showed no difference between groups with oblique, transverse, or spiral long bone fractures (Tables 3 and 4). There was a slight increase of leptin concentration in the samples after 3 weeks, irrespective of the fracture type. Girls with transverse and spiral types of fracture had an increased leptin concentration compared to boys, while boys with oblique fractures demonstrated an increased average concentration of leptin after 3 weeks.

Anatomical localization of bone fracture and leptin concentration in children with long bone fractures

Different localization of long bone fractures was associated with various leptin concentrations in investigated children (Tables 3 and 4). Initially, the highest average leptin concentration was detected in samples of girls with a femur fracture and boys and girls with a humerus fracture. After 21 days from fracture, the highest average leptin concentration was detected in children, both girls and boys, with fractures of radius and ulna. The analysis of boys' samples only demonstrated smaller variations in leptin concentration, both between different anatomical localization and between the samples and controls. On the contrary, girls' samples showed significant variations between those that suffered femur and humerus fracture as opposed to girls with radius fracture in the initial samples (Table 3).

Callus formation and leptin concentration in children with long bone fractures

Stratification of children according to the level of callus formation demonstrated an increased average leptin concentration in all investigated samples compared to the controls. Children with incompletely formed callus had the highest leptin value, both in the initial and second sample, insignificantly elevated compared to all others and controls (Tables 3 and 4).

A more detailed diversification of examinants in groups of boys and girls with long bone fractures according to the quality of formed callus showed significant differences, both inside and between the investigated groups (Tables 5). Boys with incomplete callus formation (< 25%) had the highest leptin concentration both at the time of trauma (initial sample) and after 3 weeks. The average concentration of leptin in their initial samples was significantly elevated compared to boys' control samples, samples of boys with an intermediary and well-formed callus and also increased compared to the initial samples of girls with incomplete callus. The average concentration of leptin in boys with incomplete callus formation was still significantly increased after 3 weeks compared to the second sample of boys with intermediary formed callus and the control values in boys' samples. According to callus quality, later incomplete callus formation was associated with the highest leptin values in boys' samples and with the lowest leptin concentration in girls' samples at the time of bone trauma. Leptin in the initial samples of boys and girls with an intermediary and well-formed callus did not differ significantly (Tables 3 and 4, Figure 1).

After 3 weeks, leptin concentration in boys with incompletely formed callus decreased significantly but was still higher than the level measured in other groups. Intermediary and well-formed callus was associated with insignificant leptin change. In girls' samples after 3 weeks, incomplete callus formation was followed with a further decrement of leptin concentration. On the contrary, better callus formation in girls was associated with the increment of leptin concentrations in the second over initial samples.

Girls with partially (< 50%) and satisfactory (> 75%) formed callus had a significantly increased leptin concentration in the second sample (21d) compared to the boys' group (Table 4, Figure 1).

Finally, leptin concentration was significantly increased in girls' control samples compared to boys' control samples. Leptin was increased in all samples of boys with long bone fractures compared to their control values, both at the time of trauma and 3 weeks after. Girls with incomplete callus formation had lower leptin compared to their control values, while better callus formation was associated with an increased leptin concentration (Tables 3 and 4, Figure 1).

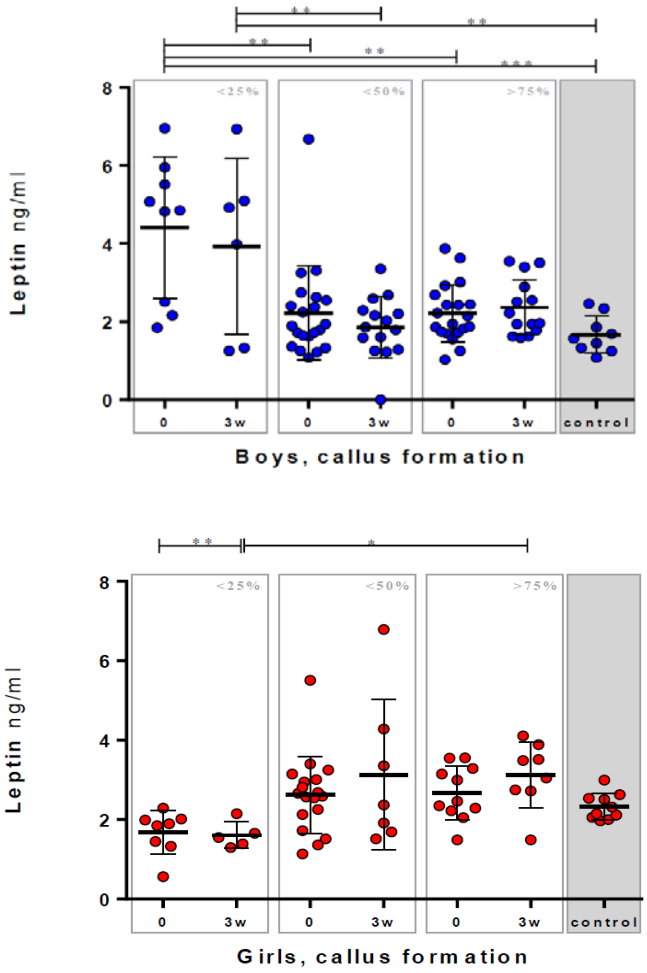

Fig. 1 – Serum concentration of leptin in children with long bone fractures according to the callus formation (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, Mann-Whitney test). 0 – initial values (before bone fracture); 3 w – values, three weeks after bone fracture.

Leptin ROC curve analysis in children with long bone fractures

ROC curve analysis comparing the diagnostic power of serum leptin concentration in children with long bone fractures demonstrated further differences between boys and girls (Figure 2). A more uniform distribution of blood leptin levels in boys resulted in a better area under the curve (AUC = 0.777) compared to the girls' group (AUC = 0.600). Cutoff leptin concentration for 100% specificity in boys was above 2.49 ng/mL, compared to 3.00 ng/mL in girls. Leptin concentration above the cutoff value in the boys' group was significantly associated with fractures localized exclusively in the arm and those with incomplete callus formation. Interestingly, the analysis showed that high leptin concentrations were detected in the majority of girls (67%) that were later subjected to a certain type of orthopedic therapy, namely open reposition and osteosynthesis.

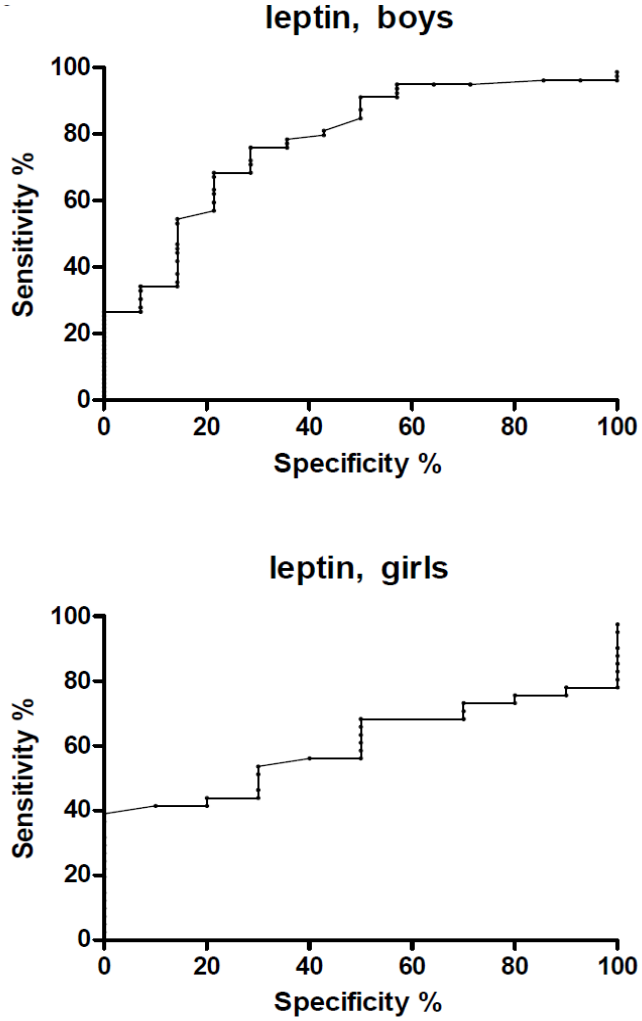

Fig. 2 – Receiver operating characteristic (ROC) analysis of serum leptin concentration in children with long bone fractures.

Discussion

Leptin is a representative adipokine produced by differentiated adipocytes, crucial in regulating energy balance and fat storage. Newer data demonstrated that leptin has almost the same relevance in numerous physiological processes, such as regulating both female and male reproduction, hematopoiesis and angiogenesis, glucoregulation, wound healing, inflammation, and osteogenesis [25]. Furthermore, leptin has a major role in chronic inflammatory mechanism characteristic for obesity and atherosclerosis [26], sepsis [27], chronic viral infection [28], and cancer [29]. Leptin exerts its action by binding specific membrane receptor (Ob-R), expressed on different cell types, including immune cells [30]. On the other hand, microbial products as lypopolysaccharide (LPS) together with inflammatory cytokines stimulate intense leptin production, indicating complex mutuality between this hormone and inflammatory processes. Mesenchymal stem cells and osteoblasts, same as immune cells, express both types of leptin receptors on their membrane [8, 9]. Leptin functions as a regulator of bone growth at multiple levels, both systemically and locally. After penetrating the blood-brain barrier and binding to specific receptors, leptin indirectly

regulates bone metabolism through the sympathetic nervous system [31–33]. There are still controversies about leptin and bone mineral density, at least regarding conditions without bone fractures. Several studies pointed out that there is no significant correlation between leptin levels and bone density [4, 34–40]. It must be emphasized that the most frequently studied population was among postmenopausal women, although from different ethnicities. Contrary to these data, Rhie et al. [41] reported a positive correlation of serum leptin level with bone density (measured as the spine and femoral bone mineral density) in a group of pre-pubertal obese girls compared to an age-adjusted group, indicating a positive role of leptin in bone metabolism.

In an experimental model where rats were subjected both to traumatic brain injury and femoral fracture, two studies demonstrated leptin significance in the process of healing bone [42, 43]. Callus formation was significantly associated both with increased serum leptin values and an abundant presence of leptin in various cells in fracture sites, osteoblasts, fibroblasts, and mesenchymal cells. The serum concentration of leptin and local expression of leptin documented by immunohistochemical analysis reached their peak in the 4th week after trauma.

There are also clinical data supporting leptin's importance in bone homeostasis. Women with hypothalamic amenorrhea demonstrate low serum leptin concentration together with decreased estrogen, growth hormones, and thyroid hormones. This hormonal disorder is associated with insufficient bone mass density, which is often the cause of bone fractures despite age [44, 45].

Interestingly, supportive therapy with synthetic leptin preparation not only improves hormonal abnormalities but also significantly mends bone density [44–47].

Increased serum leptin concentration immediately after bone trauma could be explained from several aspects. Bone fracture is accompanied by the hypermetabolic response, characterized by mobilization of free fatty acids, which cause leptin rise by the neuroendocrine mechanism [33, 48–50]. An early inflammatory response to acute bone trauma is mediated with inflammatory cytokine production, which also results in stimulating further leptin secretion [51, 52]. Another important source of posttraumatic leptin rise is the bone marrow, its adipose part (“medulla flava”), which abundantly releases leptin at the edges of a fractured bone [53]. Besides these, complex neuroendocrine response after bone trauma, represented in the release of multiple cytokines and hormones, influences the production and levels of leptin.

Several studies reported contradictory findings of improved healing in patients with long bone fractures and concomitant traumatic brain injuries, both at experimental and/or clinical levels [54–60]. Although far from clear understanding, data from these studies indicate that both serum and cerebrospinal fluid of patients with concomitant brain trauma and long bone fractures have stimulatory effects on bone healing. It is assumed that these osteoinductive factors are secreted and/or released from the injured brain. Based on these results, leptin is one of these factors.

Interesting data came from a study by Wang et al. [61]. They investigated the association between serum leptin concentration, bone density, and healing of long bone fractures in two groups of men, with fractures combined with spinal cord injury and one group with fractures only. Four and eight weeks after trauma, patients with spinal cord injury had significantly less formed callus, significantly reduced bone density, and significantly increased leptin concentration compared to the fracture-only group. Although our investigated population consisted of children with no other trauma (as one of the inclusion criteria), some children demonstrated an association of very high leptin concentration with unsatisfactory bone recovery. Namely, the group of boys with insufficiently formed callus had the highest leptin concentration, both initially after bone fracture and in the samples after 3 weeks. Gender conditionality of high leptin concentrations seems to be important because the leptin concentration was significantly decreased in girls with insufficient callus formation in both their samples.

Studies from the late 2000s demonstrated a strong association between leptin and gender, presented with increased serum leptin concentration in girls compared to boys of the same age [60–66]. This gender-based leptin domination was explained with a higher rate of leptin secretion from adipose tissue in girls, genetically determined higher subcutaneous/visceral fat ratio, and higher estrogen levels throughout puberty. Our data demonstrated a clear difference in leptin concentration between boys and girls, as well as in the control group of healthy children compared to the long bone fracture group. Girls generally have higher leptin serum concentration than boys, but this is not an absolute rule, at least not in our study. Average leptin concentration was higher in boys with an oblique type of fracture (21d), boys that had a fracture of the humerus (21d), radius (0d, 21d), combined radius and ulna (0d), and most strikingly, in boys with incomplete callus formation (0d, 21d).

Theoretically, fracture of larger bones should be associated with a more intensive systemic reaction, and a larger inner surface of the fractured bone should induce increased liberation of leptin from frontiers of lesion [53]. This is in compliance with our finding that boys and girls with humerus fracture and girls with femur fracture had the highest average leptin concentration in the initial sample. The association of high serum leptin with incomplete callus formation is even more complex to explain and leaves space for speculation. In the initial sample, the association of high serum leptin with unsatisfactory bone recovery in boys could indicate its compensatory increase as a result of the ongoing inflammatory process in incompletely formed callus. On the contrary, leptin could be considered as an adipokine necessary for bone recovery in a group of girls with insufficiently formed callus and extremely low leptin. Another possibility is that these boys and girls with incompletely formed callus had leptin abnormality before the resulting fracture. This issue should be investigated in the population of children with poor recovery after fractures of long bones. The determination of leptin concentration after 21 days from fracture demonstrated a greater variation. At that time, beyond the processes in a fractured bone, leptin concentration could be influenced by numerous factors, such as immobilization quality, the discipline of the patient, sleep, feeding, or infection.

Conclusion

Children with long bone fractures demonstrated a significant increase of leptin concentration in samples taken immediately upon bone trauma and three weeks after, compared to children with short bone fractures and corresponding controls. Leptin concentration is highly influenced by gender. High leptin concentrations in boys or low leptin concentrations in girls immediately upon fracture could be used to identify groups of children with incomplete callus formation.

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