SERUM INSULIN-LIKE GROWTH FACTOR I, BONE MINERAL DENSITY AND BIOCHEMICAL MARKERS OF BONE METABOLISM IN CHILDREN WITH IDIOPATHIC OSTEOPOROSIS

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Objective. To determine whether the serum concentration of insulin-like growth factor I (IGF-I) correlates with the occurrence of idiopathic osteoporosis in children, and whether serum levels of IGF-I correlate with selected bone metabolism markers in patients with osteoporosis.

Methods. The study comprised 24 patients aged 7-18 years, including 12 with idiopathic osteoporosis and 12 control children. Bone mineralisation disorders were diagnosed on the basis of complex clinical, densitometric and biochemical evaluation. In all children serum concentration of IGF-I was estimated by radioimmunoassay and the third fraction of IGF binding proteins (IGFBP3) by immunoradiometry.. In children with osteoporosis the indices of bone metabolism were also assessed, e.g. serum levels of osteocalcin and activity of bone isoenzyme of alkaline phosphatase (bone formation markers) and urine concentration of pyridinoline and deoxypyridinoline and collagen type I crosslinked C-telopeptyde (resorption markers).

Results. It was found that in children with osteoporosis IGF-I concentration was significantly lower than in the control group (mean values were 583 and 850 ng/ml, respectively; $P<0.05$). These differences were independent on biological age of the studied children and were present in all adolescence stages. Concentrations of IGFBP3 did not differ significantly between groups (3593 vs. 3955 ng/ml), while that of IFG-I correlated positively with total and spinal bone mineral density ($R=0.85$ and $R=0.80$, respectively; $P<0.00001$). In children with osteoporosis there was also a significant relationship between IGF-I concentration and elimination of pyridinoline and deoxypyridinoline with urine ($R=0.64$ and $R=0.65$, respectively; $P<0.05$). There was no significant correlation between the concentration of IGF-I and IGFBP3 and other studied bone metabolism markers.

Conclusions. The conducted study revealed that lower IFG-I concentrations correlate with higher bone resorption markers values and decreased mineralisation. These results suggest the importance of insulin-like growth factor in the ethiopathogenesis of idiopathic osteoporosis, which needs to be confirmed in further studies.

Key words. Idiopathic osteoporosis – Children – Insulin-like growth factor I – Bone metabolism markers

Recently conducted studies showed a significant influence of cytokines, including growth factors, on the process of bone remodelling. These factors operating locally in the microenvironment of the bone effect the activity of bone cells and take part both in bone formation and bone resorption (MAROWSKA 1991; WLODARSKI et al. 1993a; WLODARSKI et al. 1993b). Both in vitro and in vivo studies indicate that bone formation process is in particular influenced by such growth factors as insulin-like growth factor I (IGF-I), transforming growth factor β (TGFβ), platelet-derived growth factor (PDGF) and bone morphogenetic pro-

teins (BMPs). However, the key role seems to be played by IGF-I (Canalis 1994; Dziedzic-Gocławska 1996; Pindel and Pluskiewicz 1996).

IGF-I is a polypeptide similar in structure to proinsulin (Robak 1995). It is produced mainly by hepatocytes, but also by cells of other organs, including bone and cartilage cells (Gesing and Lewinski 1999). Release and concentration of IGF-I in serum depends on the growth hormone, but also on the age, sex, nutritional status and other hormones, including estrogens, androgens, thyroxin, prolactin, insulin and parathormone (Gesing and Lewinski 1999; Niedzwiedzka 2000]. Peripheral activity of IGF-I is modulated by IGF-I binding proteins (insulin-like growth factor binding proteins-IGFBPs) [Hill and Pell 1998; Mohan and Baylink 1996). There are at least six types of these proteins, and the existence of further types is currently discussed. The prevailing type in the serum is IGFBP3 and it is thought to be the most important one. IGFBP3 increases the half time of IGF-I in serum, but it also inhibits the activity of IGF-I by decreasing its availability in peripheral tissues (Gesing and Lewiński 1999; Mohan and Baylink 1996; Niedźwiedzka 2000). Numerous studies on IGF-I and its binding proteins reveal that these factors, apart from their well known role in the physiology and pathology of growth, play an important role in the development of cardiovascular system diseases, nervous system diseases, neoplasms, wound healing and aging (Canalis 1994; Gesing and Lewinski 1999; Niedzwiedzka 2000; Pfeilschifter and Ziegler 1998).

The role of IGF-I in the pathogenesis of bone mineralisation disorders in adults is gaining increasing interest. Its role in the development of idiopathic involution osteoporosis (OP) (Janssen et al. 1998; Nicolas et al. 1994; Ravn et al. 1995; Sugimoto et al. 1997; Sugimoto et al. 1998), idiopathic osteoporosis in young males (Kurland et al. 1998a,b) and secondary osteoporosis (Foeldes et al. 1997; Hotta et al. 2000] has been demonstrated, although some authors did not find similar relationships (Johansson et al. 1997; Kassem et al. 1994). To our knowledge there have been no such studies have been conducted in children with primary mineralisation disorders.

The aim of the study was thus determination of a possible relationship between IGF-I concentration in serum and occurrence of idiopathic osteoporosis in developmental age and establishing whether in children with osteoporosis IGF-I concentration correlates with selected bone metabolism markers.

Subjects and Methods

The study comprised 24 children aged 7-18 years, including 12 patients with idiopathic osteoporosis (4 girls and 8 boys) and 12 children from the control group (3 girls and 9 boys). The diagnosis of osteoporosis was based on complex clinical, biochemical and densitometric examination. We have excluded factors which may cause secondary mineralisation disorders such as: long-term corticosteroid treatment, prolonged immobilisation, chronic diseases enhancing OP – diseases of the adrenals, thyroid, parathyroids, hypogonadism, diabetes, anorexia, malabsorption syndrome, liver and kidney diseases. The control group comprised healthy children without any symptoms of diseases, with normal results of laboratory and densitometric examinations.

The study has been approved of by the Ethical Committee of the Medical University of Lodz.

In all children detailed questionnaire and physical examination were conducted. The questionnaire included questions on the diet (daily intake of calcium), physical activity, past diseases and family history. Basic anthropometric measurements of body weight and height were done, and body mass index (BMI) was calculated. The results were compared with the norm for children in Lodz. Additionally, the stage of pubescence was determined according to Tanner criteria. There were no cases of delayed pubescence or short stature.

OP was diagnosed on the basis of bone densitometry performed by dual energy X-ray absorptiometry (DEXA) on a DPX apparatus by Lunar (USA). Bone mineral density (BMD) at the total body (programme Total body) as well as the lumbar spine region (programme Spine) were evaluated. BMD values in both projections were interpreted as Z-score, a reference to standard values for a given age and sex. Osteoporosis was diagnosed at Z-score < -2.00 (below 2 units of standard deviation values) (Chlebna-Sokol 1998). In the control group Z-score was within the range –1.00 to +1.00.

Blood samples were taken from venous blood, in the morning, after overnight fasting. After centrifu-

Table 1
Clinical characteristics and bone mineral density in studied children – summary of mean values

Variable	Children with idiopathic osteoporosis	Children from control group
Age [years]	12.9 (7 – 18)	13.0 (7 – 17,5)
Body weight [kg]	41.3 ± 14.8	54.4 ± 18.5
Body height [cm]	155.6 ± 20.1	159.8 ± 20.2
BMI [kg/m^2]	16.5 ± 2.9 *	19.4 ± 3.3 *
Physical activity [hours/week]	18 ± 12.4	20.6 ± 10.2
Ca intake with diet [mg/day]	1038.5 ± 513.9	1414.4 ± 421.2
Total body bone mineral density [Z-score]	-1.87 ± 0.87 **	-0.03 ± 0.80 **
Lumbar spine mineral density [Z-score]	-3,11 ± 0.56 **	-0.22 ± 0.47 **

Results expressed as mean (range) or mean +/- standard deviation
* p<0,05
** p<0,0001

Table 2
Mean values of IGF-I, IGFBP3 and IGF-I/IGFBP3 index in studied groups of children

Variable	Children with idiopathic osteoporosis	Children from control group
IGF-I [ng/ml]	582.9 ± 261 *	849.8 ± 339 *
IGFBP3 [ng/ml]	3593.3 ±784	3955.4 ± 706
IGF-I/IGFBP3 index	0.16 ± 0.05 *	0.21 ± 0.06 *

Results expressed as mean +/- standard deviation
* p<0,05

gation the serum was frozen and kept at below – 20 ^{0}C for further analysis.

Concentration of IGF-I in serum was evaluated by competitive radioimmunological method using MEDGENIX IGF-I-RIA-CT set from Biosource Europe S.A. (Belgium). Sensitivity threshold of this method is 0.4 ng/ml, intra-assay variability index ranges from 3.5 to 9.5 %, and inter-assay variability index ranged from 5 to 8 %. The percentage of cross-reactions with IGF-II comprised 0.15 %.

IGFBP3 concentration was evaluated by sandwich immunoradiometric method using IGFBP-3 IRMA set form Immunotech (France). Sensitivity threshold of this method is 50 ng/ml, intra-assay variability index ranges from 4.8 to 6 %, inter-assay variability index ranges from 4.3 to 9.5 %. There were no cross-reactions with other IGFBPs.

We have also calculated growth factor (cytokine) bioactivity index, i.e. the proportion of IGF-I compared to the main binding protein, IGFBP3 – the IGF-I/IGFBP3 index (Khosla et al. 1994).

In children with osteoporosis the bone formation markers in serum were also evaluated, such as bone alkaline phosphatase and osteocalcin. In urine the elimination of bone resorption markers was determined as creatinine: the concentration of pyridinoline (Pyr), deoxypyridinoline (DPyr) and collagen type I crosslinked C-telopeptide (CTx).

Statistical analysis was done using the programme Statistica 5.0. Mean values of the determined parameters were compared using t-test for individual variables. Relationships between studied features were assessed using Spearman R correlation index. The results were accepted as statistically significant at P<0.05.

Results

Clinical characteristics of the studied patients and mean bone mineral density in both groups are presented in Table 1. Children with osteoporosis and normal children did not differ significantly as to their age, somatic development – body weight and height and also reported similar levels of physical activity and calcium intake with diet. Only body mass index was higher in healthy children. Bone mineral density was one of the factors of inclusion into particular group, thus Z-score values differed significantly (P<0.0001).

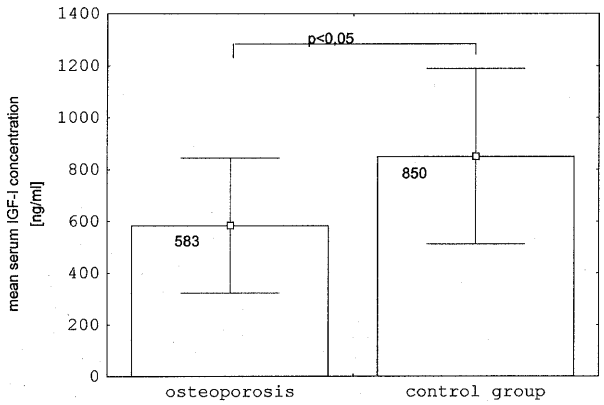

Fig. 1 Serum IGF/I concentration in children with osteoporosis and in control group (means and S.D.)

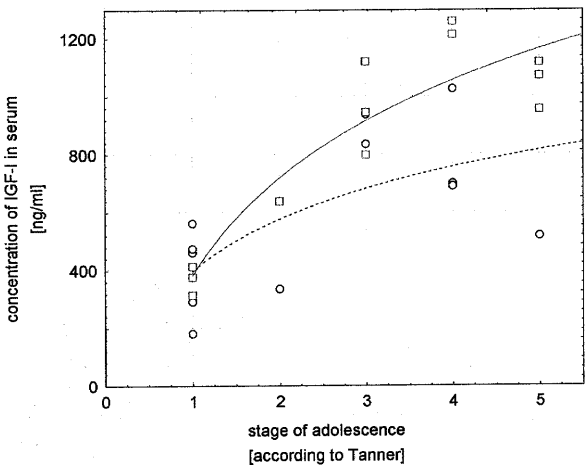

Fig. 2 Concentrationm of IGF-1 in serum in particular stages of adolescence (spheres – idiopathic osteporosis; squares – control group)

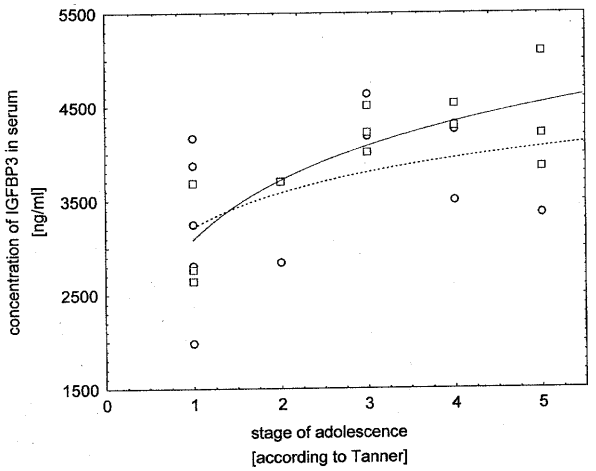

Fig. 3 Concentration of IGFBP3 in serum in particular stages of adolescents (spheres – idiopathic osteporosis; squares – control group)

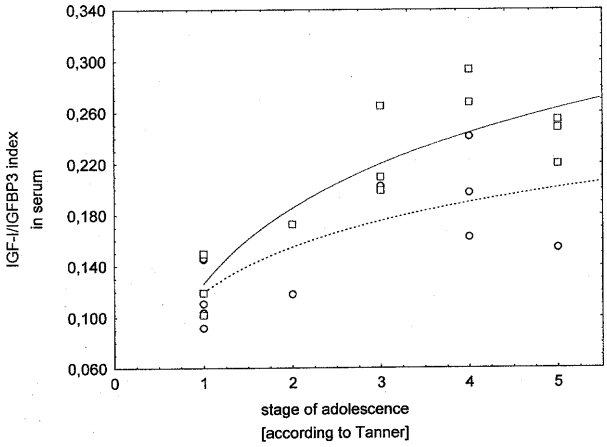

Fig. 4 IGF-I/IGFBP3 index in serum in particular stages of adolescence (spheres – idiopathic osteporosis; squares – control group)

Mean concentrations of IGF-I, IGFBP3 and IGF-I/IGFBP3 index in both groups of children are presented in Table 2. We have found statistically significantly lower concentration of IGF-I in patients with idiopathic osteoporosis as compared with the control group - 583 and 850 ng/ml, respectively; $P<0.05$ (Fig.1). The bioactivity index IGF-I was also significantly lower (0.16 vs. 0.21; $P<0.05$). There were no significant differences in IGFBP3 concentration.

In all studied cases concentrations of IGF-I and IGFBP3 increased, similarly to literature results, according to biological development of the child (Fig.2 and 3). The differences in IGF-I concentration and IGF-I/IGFBP3 index between groups did not depend on biological age of the studied children and could be seen in all stages of adolescence (Fig. 2 and 4).

The concentration of IGF-I, as well as IGF-I/IGFBP3 index, showed very strong positive correlation with total body and spine bone mineral density, as expressed in absolute units [g/cm^2], and in standard deviation units (Z-score) – Table 3. The correlation index values for IGF-I/IGFBP3 were slightly higher

Table 3
Spearman R correlation coefficients between the concentration of IGF-I, IGFBP3 and IGF-I/IGFBP3 index, and bone mineral density in all studied children (N=24)

Bone mineral density		IGF-I [ng/ml]		igfbp3 [ng/ml]		Igf-i/igfbp3 INDEX	
		R	p	R	p	R	P
g/cm²	*Total body*	*0.85*	0.000001 ***	*0.62*	0.0012 **	*0.87*	0.000001 ***
	Spine	0.80	0.000002 ***	0.61	0.0016 **	0.82	0.000001 ***
Z-score	Total body	0.54	0.006 *	0.33	0.12	0.59	0.023 *
	Spine	0.41	0.046 *	0.21	0.32	0.46	0.024 *

* P <0.05
** P<0.005
*** P<0.00001

Table 4
Spearman R correlation coefficients between the concentration of IGF-I, IGFBP3 and IGF-I/IGFBP3 index, and biochemical bone metabolism markers in children with idiopathic osteoporosis (N=12)

Bone metabolism markers		IGF-I [ng/ml]		igfbp3 [ng/ml]		Igf-i/igfbp3 index	
		R	P	R	p	R	p
Bone formation markers [serum]	Bone isoenzyme of alkaline phosphatase [U/l]	-0.34	0.311	-0.16	0.631	-0.33	0.325
	Osteocalcin [ng/ml]	-0.17	0.611	-0.08	0.811	-0.40	0.669
Bone resorption markers [urine]	Pyridinoline/creatinine [pmol/mmol]	-0.64	0.047 *	-0.51	0.127	-0.70	0.025 *
	Deoxypyridinoline/creatinine [pmol/mmol]	-0.65	0.042 *	-0.45	0.187	-0.74	0.013 *
	CTx/creatinine [mg/mmol]	-0.33	0.381	-0.25	0.516	-0.47	0.205

* P<0.05

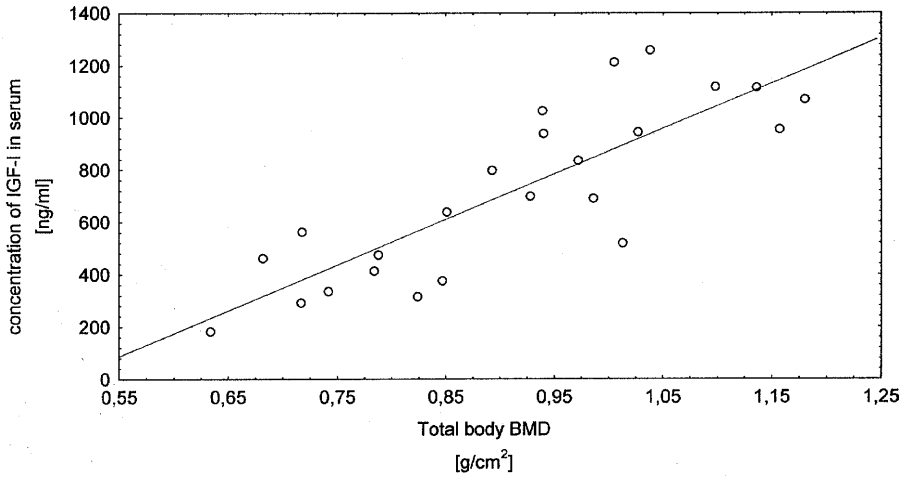

Fig. 5 Correlation between the concentration of IGF-I in serum and total body bone mineral density (R= 0.85; P<0.00001)

than those for IGF-I concentration. IGFBP3 concentration was not significantly dependent on Z score, but only on absolute value of BMD (g/cm$^{2)}$ increasing with age (Table 3). Figures 5 and 6 present graphical relationship between the concentration of IGF-I and bone mineral density, evaluated in programme Total body and Spine; the correlation index with total body BMD is slightly higher than that for the spine.

In patients with osteoporosis we have also analysed the correlation between IGF-I and IGFBP3 concentration and selected biochemical bone metabolism markers (Table 4). IGF-I concentration and IGF-I/IGFBP3 index correlated negatively with elimination of pyridinoline and deoxypyridinoline with urine. These correlations are presented in Figure 7. There was no significant correlation between the

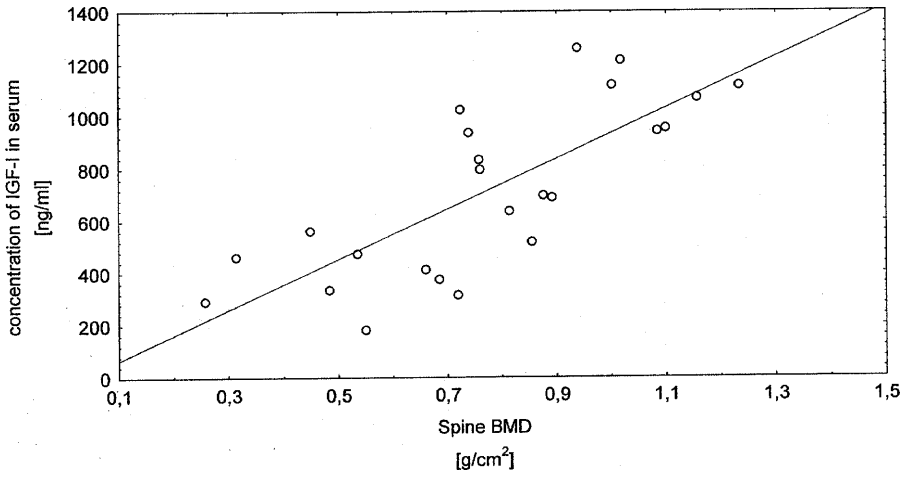

Fig. 6 Correlation between the concentration of IGF-I in serum and lumbar spine bone mineral density (R= 0.80; P<0.00001)

concentration of IGF-I and IGFBP3 and other studied bone metabolism markers, in particular with bone formation markers.

Discussion

So far studies on the dependency between insulin-like growth hormone I and bone mineral density have been conducted only in normal children. The results of these studies revealed significant positive correlation (Libianti et al. 1999, Moreira-Andres et al. 1995). Objective interpretation of these correlations is difficult in the period of intense growth, when the bone mass often increases in "leaps", and the concentration of IGF-I changes. With the onset of pubescence gradual increase in IGF-I is observed, and the highest values occur in stage IV of adolescence according to Tanner scale (Argente et al. 1993; Juul et al. 1994; Libianti et al. 1999). In girls peak of IGF-I concentration in serum precedes that in boys by 2 years (Argente et al. 1993). These physiological processes may be disturbed in bone mineralisation disorders or abnormal secretion of IGF-I, however in available literature there are no reports on such studies in children with osteoporosis.

In our study we have evaluated the concentration of IGF-I and IGFBP3 in children with idiopathic osteoporosis. We have found lower IGF-I concentration in children with OP (independent on the stage of adolescence). The concentration of IGF-I was not significantly lower in comparison with reference values (as it may be in, for example, short stature syndrome), but were close to the lower normal range limit. These results are consistent with those of Kurland et al. (1998a; 1998b) concerning the ethiopathogenesis of idiopathic osteoporosis in young males. These authors found lower IGF-I concentration at normal axis growth hormone-IGF-I. It must be emphasized that in our study there were no children with decreased body height.

Relationship between low IGF-I concentration and osteoporosis may also be confirmed by high correlation index between IGF-I concentration and bone mineral density, expressed not only in absolute values, which change with age (g/cm^2), but also as Z-score (Table 3).

IGFBP3 concentration increased with the age of children, which was similar to literature data (Argente et al. 1993; Libianti et al. 1999), but there were no statistically significant differences between groups. This could support the hypothesis that in modulating activity of IGFBP3 the first place takes the inhibition of the peripheral activity of IGF-I (Gesing and Lewinski 1999; Mohan and Baylink 1996; Niedzwiedzka 2000). It is possible in patients with osteoporosis that relatively high concentrations of IGFBP3 in relation to IGF-I lead to a decrease of the bioavailability of IGF-I in tissues. Thus, it seems reasonable to determine the index of growth factor (cytokine) bioactivity in serum, as the biological activ-

ity of the growth factor depends more on the index than on the concentration itself (KHOSLA et al. 1994). This assumption was confirmed also in our study, as all observed dependencies were stronger for bioactivity index than for IGF-I concentration.

In our study we have showed significant negative correlation between the concentration of IGF-I and selected markers of bone resorption, but not bone formation. The results of our study are not consistent with those of other authors, who in their studies on adults found statistically significant positive correlation between IGF-I concentration and bone formation markers, such as the concentration of osteocalcin or activity of bone alkaline phosphatase (HOTTA et al. 2000). In the majority of studies, including experimental studies (in vitro and in vivo), stimulation of bone formation by IGF-I was also observed (DZIEDZIC-GOCLAWSKA 1996; MAROWSKA 1991; Pindel and Pluskiewicz 1996). Explanation of our results may lies in the fact that during intense growth bone formation markers are affected by a number of factors (external and internal), and thus their value as objective indices only of bone growth process is limited. Lower concentration of IGF-I together with decreased mineralisation and higher values of bone resorption markers suggests the role if insulin-like growth factor in the pathogenesis of osteoporosis in developmental age.

In conclusion, our study showed significantly lower values of IGF-I in children with osteoporosis as compared to the control group. We have demonstrated significant positive correlation between serum concentration of insulin-like growth factor-I and bone mineral density, and negative correlation between IGF-I concentration and biochemical markers of bone resorption. These results suggest that IGF-I plays a role in the ethiopathogenesis of idiopathic osteoporosis in developmental age. These results and observations need to be confirmed in larger groups of children in further studies.

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