Autoantibodies against some selected appetite-regulating peptide hormones and neuropeptides are present in serum of short children with *Candida albicans* colonisation and *Helicobacter pylori* infection.

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Background:

Short stature is one of the most common reasons why children should be referred to endocrinologists. However, only in a few percent of them, the endocrine disorders, i.e. growth hormone (GH) deficiency (GHD), are recognized. In most of them, idiopathic short stature (ISS) is diagnosed with normal GH secretion and (sometimes) lower insulin-like growth factor I (IGF-I) secretion. The causes of the secondary IGF-I deficiency may be (besides GHD) malnutrition and gastrointestinal diseases.

Ghrelin, a 28-amino-acid octanoylated peptide, predominantly produced by X/A cells in the gastric oxyntic mucosa, was discovered to be a natural ligand of the type 1a growth hormone secretagogue (GH) receptor. Thus, the peptide is considered as a natural GH secretagogue, exerting GH-releasing hormone (GHRH)-like effect, which regulates growth in children.

Moreover, ghrelin and other gastrointestinal (GI) or adipose tissue-derived peptide hormones such as leptin and insulin signal to the brain the state of hunger or satiety or energy storage. In the brain, these peptides interact with neuronal circuitries expressing orexigenic neuropeptides such as i.e. neuropeptide Y (NPY) and orexin or anorexigenic neuropeptides such as alfa-melanocyte-stimulating hormone (alfa-MSH). In addition to appetite regulation, orexigenic and anorexigenic neuropeptides are involved in mechanisms related to stress, sleep/wakefulness, and reproductive, defensive/aggressive, and social behaviors, thereby integrating appetite, emotions, and other homeostatic functions.

The GI microflora (i.e. *Candida albicans* – CA and *Helicobacter pylori* – HP) is an antigenic source. Based on the molecular mimicry hypothesis, intestinal microbe-derived antigens may trigger the production of autoantibodies cross-reacting with regulatory peptides.

The aim of the study was to assess whether in short children (both with GHD or ISS) with CA colonisation and HP infection the autoantibodies anti selected neuropeptides (Ab anti-NP) are more prevalent than in the control group.

Method:

The study group comprised 77 short (height below -2.0 SD), children (28 girls and 49 boys), mean age: 10.2 3.6 SD years. The control group comprised 14 children with normal height (9 girls and 5 boys), mean age: 11.9 3.8 SD years.

In order to assess the growth hormone (GH) secretion, in each child two stimulation tests: after oral administration of clonidine (with the dose of 0.15 mg/m^2 and GH measurements at time 0 and at 30th, 60th, 90th and 120th minute of the test) and after intramuscular administration of glucagon (in the dose of 30 µg/kg, with GH measurements at time 0 and at 90th, 120th, 150th and 180th minute of the test) were performed. Peak GH (EIA) concentration (GH_{max}) was determined in both tests. The children with GH_{max} values < 10 ng/ml were qualified as GHD, while in the patients with GH_{max} value ≥ 10 ng/ml, ISS was diagnosed. In each child fasting ghrelin (RIA), leptin (EIA) and IGF-I (EIA) concentration was assessed.

To detect HP infection, the serology test was performed. It is currently based on the quantitation of IgG antibodies against HP by means of an enzyme-linked immunosorbent assay. In order to diagnose *Candida albicans* colonization, the stool samples from patients were cultured for *Candida sp*. but only the significant levels of *Candida albicans* were taken into consideration of candidiasis mucosae of GI. In every child, the prevalence of anti-ghrelin, anti-leptin, anti-alfaMSH and anti-orexinA antibodies were assessed (Elisa, Immuniq).

Results:

In the analysed group of 77 short children, GHD was diagnosed in 20, while ISS - in 57 of them. The CA colonisation was found in 35 children with short stature and 7 children from the Control Group, while HP infection in 15 children with short stature and 2 from the Control Group, however in some children both HP and CA were observed. Thus, we observed HP and/or CA in 12 cases (60.0%) with GHD and 30 cases (52.6%) with ISS, together in 42 children (54.5%) with short stature and in 7 cases (50%) from the Control Group (Table 1 and Table 2).

Anti-NP Abs were found in 15 (35.7%) out of 42 short children with CA and/or HP and in 2 (28.6%) out of 7 control children with CA and/or HP (Table 2).

Among short children without CA and/or HP infections, anti-NP Abs were detected in 3 cases only (out of 35) – 8.5%, while in the Control Group they were not found.

Next, we analysed the frequency of Ab anti-NP in GHD and ISS group, separately. Among 20 children with GHD, Ab anti-NP was confirmed in 4 cases - in each of them HP and/or CA were observed at the same time. Among 57 children with ISS, Ab anti-NP was confirmed in 8 children with HP and/or CA (in 1 girl two types of them), and in 4 children without HP or CA.

We divided the analysed group of children with short stature into 3 groups: short children with Ab anti-NP, short children with HP and CA, but without Ab anti-NP, and short children without HP, CA and Ab anti-NP. We found that ghrelin concentration is significantly lower in children with Ab anti-NP. (Table 3, Figure 1).

Table 1

		age (years)	HSDS	BMI SDS	ghrelin (pg/ml)	leptin (ng/ml)
Short stature, n=77	with HP and/or Calb , n=42	10.49±3.24	-2.47±0.82	-0.28±1.02	1515.34±1052	6.03±7.79
	without HP and Calb , n=35	9.82±3.79	-2.40±0.61	-0.16±0.96	2237.45±1373.72	5.48±9.73
Controls, n=14	with HP and/or Calb n=7	11.61±3.88	0.21 ±0.75	-0.04±1.12	NA	NA
	without HP and Calb n=7	11.3±3.25	0.33±0.68	-0.22±1.24	NA	NA

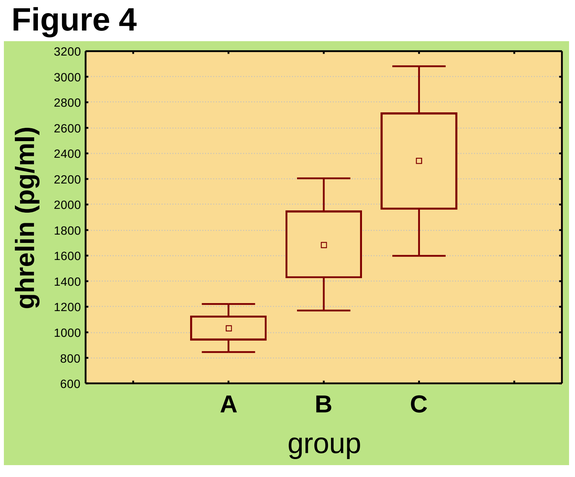
Table 2

		Ab anti-ghrelin	Ab anti-leptin	Ab anti-orexin A	Ab anti-alfaMSH	togehter
Short stature, n=77	with HP and/or Calb , n=42	1	5	2	8	15 (35.7%)
	without HP and Calb , n=35	1	2	0	0	3 (8.5%)
Controls, n=14	with HP and/or Calb n=7	0	0	0	2	2 (28.6%)
	without HP and Calb n=7	0	0	0	0	0 (0%)
together		2	7	2	10	21

Table 3

	Short children with Ab anti-NP	Short children with HP and CA, but without Ab anti-NP	Short children without HP, CA and Ab anti-NP
Group	A=18	B =28	C=31
GHD/ISS	4/14	8/20	8/23
age (years)	10.49±3.00	10.83±3.45	9.43±3.81
HSDS	-2.22±0.96	-2.63±0.66	-2.39±0.63
BMI SDS	-0.30±1.16	-0.20±0.98	-0.26±0.95
ghrelin (pg/ml)	1033.4±287.6*	1687.6±1180.2*	2340.0±1416.2*
leptin (ng/ml)	4.64±5.06	6.43±8.48	5.65±10.49
GH max (ng/ml)	11.64±8.17	14.52±8.51	14.42±9.27
IGF-I SDS	-0.68±0.80	-1.02±1.69	-1.03±0.59

Figure 4



Conclusion: In short children with *C. albicans* colonisation and/or *H. pylori* infection the incidence of antibodies against neuropeptides is elevated, which may be connected with the molecular mimicry phenomenon. It may be a reason of worse high velocity in these children due to disorders in neuropeptides activity. However, further studies are necessary to elucidate this issue.