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Serum Ghrelin and Glucagon-like Peptide 1 Levels in Children with Prader-Willi and Bardet-Biedl Syndromes

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What is already known on this topic?

Ghrelin stimulates appetite and secretion of growth hormone, and induces a positive energy balance and leads to weight gain. Glucagon-like peptide-1 (GLP-1) exerts its central effects through the GLP-1 receptor in the central nervous system, reducing the rate of absorption of food into the blood by suppressing appetite, reducing the rate of gastric emptying and and inhibiting glucagon secretion.

What this study adds?

There was no evidence for a definite role for ghrelin and GLP-1 in the pathogenesis of Prader-Willi syndrome in pediatric patients. There is only one study in which the plasma ghrelin levels do not differ between the patients with Bardet-Biedl syndrome (BBS) and control groups, and there is no study evaluating serum GLP-1 levels in BBS patients. However, similar studies with larger series are needed.

Abstract

Objective: Prader-Willi syndrome (PWS) and Bardet-Biedl syndrome (BBS) are causes of pediatric syndromic obesity. We aimed to investigate a possible role for ghrelin and glucagon-like peptide-1 (GLP-1) in the pathophysiology of PWS and BBS.

Methods: The study included 12 children with PWS, 12 children with BBS, 13 pediatric obese controls (OC) and 12 pediatric lean controls (LC). Fasting serum ghrelin and GLP-1 levels were measured by ELISA.

Results: In the PWS group, no significant difference was detected for median ghrelin levels when compared with OC and LC, which were 0.96 (0.69-1.15), 0.92 (0.72-1.20) and 1.13 (0.84-1.29) ng/mL, respectively. Similarly, there was no difference in PWS median GLP-1 levels when compared with OC and LC; 1.86 (1.5-2.94), 2.24 (1.62-2.78) and 2.06 (1.8-3.41) ng/mL, respectively. In the BBS group, there was no difference in median ghrelin levels when compared with OC and LC; 1.05 (0.87-1.51), 0.92 (0.72-1.20) and 1.13 (0.84-1.29) ng/mL, respectively. Neither was there a significant difference in median GLP-1 levels; 2.46 (1.91-4.17), 2.24 (1.62-2.78) and 2.06 (1.8-3.41) ng/mL for BBS, OC and LC, respectively.

Conclusion: There were no differences in median fasting ghrelin or GLP-1 levels when comparing patients with PWS and BBS with obese or lean peers. However, similar studies with larger series are needed.

Keywords: BBS, PWS, ghrelin, GLP-1

Introduction

Prader-Willi syndrome (PWS) is the most common cause of pediatric syndromic obesity. The clinical characteristics of PWS consist of hyperphagic obesity beginning in early childhood, decreased fetal movement, neonatal hypotonia, developmental delay, cognitive deficits, short stature, and hypogonadotropic hypogonadism (1). Subjects with PWS develope uncontrolled appetite resulting in weight gain, usually starting after two years of age, although there is almost universal poor feeding and appetite in infancy in babies with PWS. PWS is seen in all races, occurring equally in the sexes, with an incidence of approximately 1 in 10,000 to 1 in 15,000 live births, and results mostly from the absence of functionally active paternal inheritance in the 15q11.2-13 chromosome region (2,3).

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Bardet-Biedl syndrome (BBS) may be described as a genetically heterogeneous ciliopathy with an autosomal recessive inheritance. The characteristics of BBS is described by six primary attributes: progressive rod-cone dystrophy, early-onset obesity, postaxial polydactyly, hypogonadism, cognitive impairment, and genitourinary tract malformations with progressive renal dysfunction. Its prevalence is about 1 to 9 in 1,000,000. Mutations in 24 different genes have been described that are associated with BBS (4).

Ghrelin is a 28-amino acid peptide that is the natural ligand for the growth hormone secretagogue-receptor. Ghrelin is produced mainly by the stomach and concentrations increase in fasting. Ghrelin stimulates appetite and secretion of growth hormone, and induces a positive energy balance, leading to weight gain. Ghrelin levels are low after meals or in hyperglycemia, and also in obesity (5). Hyperghrelinemia has been reported in older children and adults with PWS in many studies but the correlation with obesity is still unclear (6). However, there is only one study evaluating ghrelin levels in BBS (7).

Glucagon-like peptide-1 (GLP-1) is mainly synthesized by L-cells in the duodenum, small intestine and in small quantities by the pancreas and hypothalamus. Its secretion in the gastrointestinal tract is modulated by glucose and fatty acid levels after food intake, or as a result of stimulation of the vagus nerve. The main mechanisms of action of GLP-1 include stimulating insulin secretion by β-cells in the islets of Langerhans and inhibiting glucagon secretion by α-cells. GLP-1 also exerts its central effects through the GLP-1 receptor in the central nervous system, reducing the rate of absorption of food into the blood via appetite suppression, and reducing the rate of gastric emptying (8). The relationship between GLP-1 and obesity is not clear and controversial in terms of underlying pathophysiology (9). There are only two studies evaluating fasting plasma GLP-1 levels in PWS; in the first one, fasting GLP-1 concentrations in adults with PWS were similar in individuals with obesity and lean control (LC) groups. In the second one, fasting GLP-1 concentrations in adults with PWS were higher than in the obese and LC groups, but GLP-1 concentrations in the obese and lean groups were similar (10,11). However, there was no study evaluating fasting plasma GLP-1 levels in BBS patients.

In this study, we aimed to investigate the role of ghrelin and GLP-1 in the pathophysiology of pediatric syndromic obesity caused by either PWS or BBS.

Methods

Patients with a diagnosis of BBS or PWS and aged between three and 18 years were selected from patients attending pediatric endocrinology and clinical genetics clinics. The diagnosis in all patients with PWS was genetically confirmed. In the patients with BBS, the majority were confirmed genetically but a quarter of diagnoses had been made clinically. The clinical diagnosis of BBS was made according to criteria published by Beales et al. (12).

The control subjects were matched for age, sex and pubertal stage and were selected from our pediatric clinics. Exclusion criteria were any patient with endocrine (diabetes mellitus, hypothyroidism, and adrenal deficiency), systemic or infectious diseases, and those taking any kind of medication. The study protocol was approved by the University of Health Sciences Turkey, Antalya Training and Research Hospital Local Institutional Review Board (decision no: 10-12, date: 18.05.2022). Informed written consent from the subjects (>8 years old) and their parents were obtained. The study was conducted according to the principles of the Declaration of Helsinki.

Anthropometric Measurements

Body weight of subjects was measured using a pre-calibrated digital scale and height was measured with a 0.1 cm sensitivity in a Harpenden Stadiometer, both produced by Densi Industrial Scale Systems San. and Tic. Ltd. Şti. (Tuzla/İstanbul/Turkey). The obesity was defined as a body mass index (BMI)-standard deviation (SD) score (SDS) greater than or equal to 2 SDS using national BMI data defined according to age and gender (13). Pubertal developmental stage was evaluated using Marshall and Tanner's standards (14).

Assays

Venous blood samples were taken from the subjects in the morning after a 12 hour fast. After centrifugation for 20 min at 2000 RPM, serum samples were stored at -20 °C. Serum ghrelin and GLP-1 levels were measured by the ELISA method [Shanghai Korain Biotech (BT-LAB) Co., Shanghai, China]. The detection range of the ghrelin assay by the competitive inhibition method was 0.05-10 ng/mL with a sensitivity of 0.01 ng/mL. The detection range of the GLP-1 assay by competitive inhibition was 0.05-30 ng/mL with a sensitivity of 0.026 ng/mL. The inter-assay coefficient of variation (CV) and intra-assay CV for both assays was given as <10% and <8%, respectively.

Statistical Analysis

The sample size calculation was performed using the DSS statistical software package (DSS, Locke Ave, Fort

Worth, TX, USA) for research sample size calculations. It was calculated that a minimum of 12 participants in each group would be required to demonstrate a difference of at least 10% for GLP-1 between the groups, with a power of 80% at the 5% significance level. The statistical analyses were performed using Statistical Package for the Social Sciences (SPSS), version 15.0 (SPSS, Chicago, IL, USA). The Shapiro Wilk-test was used to assess continuous variables for normal or abnormal distribution. Continuous variables with a parametric distribution were analyzed using analysis of variance and if the differences were significant, a *post-hoc* Tukey test was performed. Normally distributed data are presented as mean ± SD. The Kruskal-Wallis test was used for comparison of data sets containing at least one abnormally distributed continuous variable. Non-parametrically distributed data is presented as median and IQR [interquartile range (IQR), 25th-75th percentile]. When the Kruskal-Wallis test indicated statistically significant differences, the causes of those differences were determined using a *post-hoc* Dunn's test. The nominal variables were analyzed using the Pearson's chi-square or Fisher's exact test, where applicable. Categorical variables were presented as the number (%) of cases. Statistical significance was set at $p < 0.05$.

Results

The study included 12 patients with PWS (mean age; 10.6 ± 4.8 years), 12 patients with BBS (mean age: 10.3 ± 4.7 years), 13 obese controls (OC), (mean age: 11.1 ± 3.8 years) and 12 LC, (mean age: 10.9 ± 4.2 years). There was no significant difference between PWS, BBS, OC and LC in terms of age, gender or pubertal status. BMI-SDS was similar in the PWS, BBS and OC, while the BMI-SDS of LC was significantly lower than that of the PWS, BBS and OC groups ($p < 0.001$). In the PWS group, three (25%) were on both L-thyroxine and recombinant growth hormone, and one (8.3%) was on both L-thyroxine and hydrocortisone treatment at the time of the study. In BBS group, only one (8.3%) was on L-thyroxine because of central hypothyroidism.

The median (IQR) ghrelin level was 0.96 (0.69-1.15) in PWS group, 0.92 (0.72-1.20) in OC group, and 1.13 (0.84-1.29) ng/mL in LC group. There was no significant difference between the three groups. In addition, the median GLP-1 level was 1.86 (1.5-2.94) in PWS group, 2.24 (1.62-2.78) in OC group, and 2.06 (1.8-3.41) ng/mL in LC group. Again, these values did not differ significantly (Figure 1).

In the BBS group the median ghrelin level was 1.05 (0.87-1.51), while in the OC and LC groups it was 0.92 (0.72-1.20) and 1.13 (0.84-1.29) ng/mL, respectively ($p > 0.05$). In addition, the median GLP-1 level was 2.46 (1.91-4.17) in BBS group, 2.24 (1.62-2.78) in OC group, and 2.06 (1.8-3.41) ng/mL in LC group and did not differ between the groups ($p > 0.05$) (Figure 1). Furthermore, when the PWS and BBS groups were compared in terms of median ghrelin and GLP-1 levels, no significant difference was found ($p > 0.05$). The clinical characteristics and laboratory data of the subjects are shown in Table 1.

Discussion

Early onset obesity and hyperphagia are characteristic features of PWS. In many studies, hyperghrelinemia has been reported in these patients (15). In a large review by Tauber et al. (16), hyperghrelinemia was reported in PWS patients and the authors linked obesity and hyperphagia to

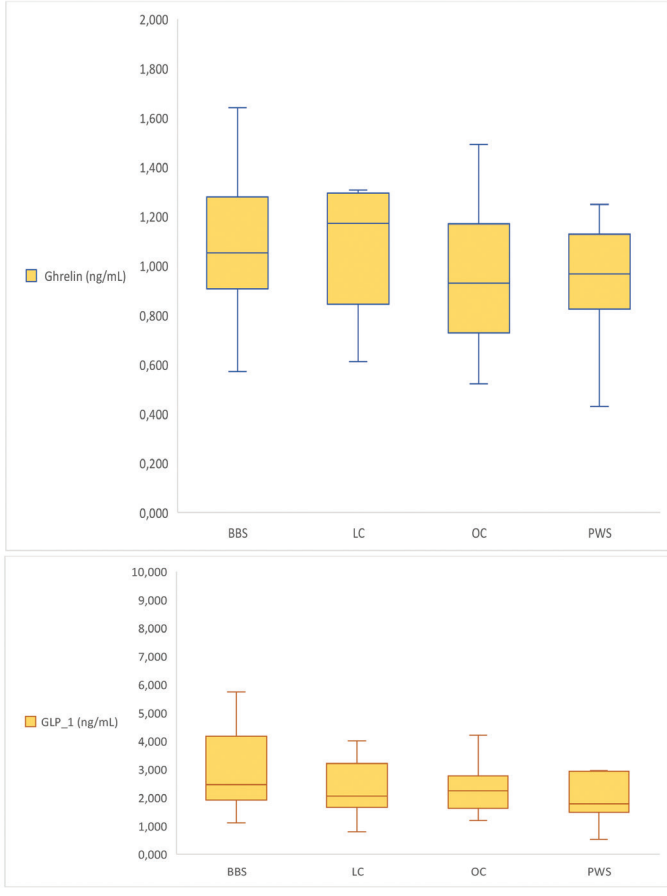

Figure 1. Box-plot presentation of serum ghrelin (ng/mL) and GLP-1 (ng/mL) levels in subjects with PWS and BBS, and in OC and LC. The lower and upper limits of the boxes represent 25th and 75th percentiles, and middle lines in each box represent 50th percentile, while the bottom and top end of the whiskers represent the minimum and maximum values, respectively. Dots represent the outlier data

PWS: Prader-Willi syndrome, BBS: Bardet-Biedl syndrome, GLP-1: glucagon-like peptide-1, OC: obese control, LC: lean control

Table 1. Clinical characteristics and laboratory findings of the patients and the control subjects

	PWS (n = 12)	BBS (n = 12)	OC (n = 13)	LC (n = 12)	p
Age (years) (min-max)	10.6 ± 4.8 (3-17.6)	10.3 ± 4.7 (5.3-19)	11.1 ± 3.8 (6.8-17.2)	10.9 ± 4.2 (2.8-16.4)	0.970
Female/male	6/6	4/8	6/7	8/4	0.412
Pubertal/prepubertal	4/8	6/6	4/8	6/6	0.136
BMI-SDS	2.7 ± 1.6[c]	3.13 ± 0.5[b]	2.81 ± 0.5[a]	1.18 ± 0.9[a,b,c]	**< 0.001**
Ghrelin (ng/mL)	0.96 (0.69-1.15)	1.05 (0.87-1.51)	0.92 (0.72-1.20)	1.13 (0.84-1.29)	0.485
GLP-1 (ng/mL)	1.86 (1.5-2.94)	2.46 (1.91-4.17)	2.24 (1.62-2.78)	2.06 (1.8-3.41)	0.460

Laboratory data are given as median for ghrelin and GLP-1 (interquartile range, 25th-75th percentile). One-way ANOVA (mean ± SD) was used for the age and BMI-SDS, Kruskal-Wallis was used for the rest.

[a]: OC vs LC $p < 0.05$.

[b]: BBS vs LC $p < 0.05$.

[c]: PWS vs LC $p < 0.05$.

PWS: Prader-Willi syndrome, BBS: Bardet-Biedl syndrome, OC: obese controls, LC: lean controls, min-max: minimum-maximum, SD: standard deviation, GLP-1: glucagon-like peptide-1, BMI: body mass index, SDS: standard deviation score

hyperghrelinemia. However, several groups have reported that total ghrelin levels were not elevated in young children with PWS compared to control groups. Among these studies, Haqq et al. (17) found that plasma ghrelin values were similar to the control group in a study with 33 infants with PWS. Butler and Bittel (18) divided PWS patients into groups of patients under and over three years of age, but they did not detect hyperghrelinemia in these groups compared to controls. Lastly, Erdie-Lalena et al. (19) found that ghrelin values were similar in PWS patients under five years of age compared to controls. These studies showed that in obesity in PWS, which develops after the initial phase of poor feeding and usually starts in the second year of life, hyperghrelinemia did not precede or coincide with the development of hyperphagia. An increased number of ghrelin-producing cells in the stomach of PWS patients has been suggested as a cause of the rise in ghrelin levels (20). In the present study, hyperghrelinemia in PWS compared to OC was not found. Therefore, obesity in PWS patients may not be due to hyperghrelinemia. In contrast, in a study by Turkkahraman et al. (21), it was found that the mean α-melanocyte stimulating hormone level in the PWS group was significantly lower than in OC and therefore suggested that obesity in PWS might be due to MC4R upstream pathologies. Thus, there is no consensus regarding the role of ghrelin levels in children with PWS.

There are only two studies in literature investigating serum GLP-1 levels in PWS patients, both performed in adult patients. In the first study, fasting GLP-1 concentrations in PWS subjects were similar in individuals with obesity and LC groups (10). In the second adult study, fasting GLP-1 concentrations in PWS were higher than OC and LC groups, but GLP-1 concentrations in obese and lean group were found to be similar (11). Our results are consistent with the results of the first study. In the present study, we did not find a significant difference in GLP-1 concentrations in children and adolescents with PWS compared to control groups, and in OC compared to LC. Once again, there is no consensus regarding GLP-1 levels, especially in children with PWS, but there is very little published evidence.

The BBSome is a critical regulator of cilia function. Primary cilia are important signaling organelles, including for neuronal trafficking (22). Guo et al. (23) showed that selective disruption of the BBSome via *BBS1* gene deletion led to a significant increase in body weight and adiposity and to leptin resistance and hyperleptinemia. In the literature, there is only one study in which the plasma ghrelin levels are not different between BBS and control groups (7). However, there is no previous study evaluating serum GLP-1 levels in BBS patients. In the present study, both ghrelin and GLP-1 concentrations of BBS patients were compared with obese and LC groups, and no difference was found in median levels between the groups.

Study Limitations

The limitations of our study include the small numbers of PWS and BBS patients, the wide age ranges of the patients across both the child and adolescent ranges and that some patients were receiving additional treatments with recombinant growth hormone, levothyroxine and hydrocortisone.

Conclusion

In conclusion, there are conflicting results regarding ghrelin levels, and not enough data for GLP-1 levels in children with PWS. Similarly, there is scant data regarding ghrelin and GLP-1 levels in BBS patients. Even though no difference was found between median levels of ghrelin and GLP-1 levels in these two syndromes compared with obese and LC, we hope that our study will contribute to the understanding of the pathophysiology of PWS and BBS. However, multi-center studies with larger patient groups will be required.

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Ethics

Ethics Committee Approval: The study protocol was approved by the University of Health Sciences Turkey, Antalya Training and Research Hospital Local Institutional Review Board (decision no: 10-12, date: 18.05.2022).

Informed Consent: Informed written consent from the subjects (>8 years old) and their parents were obtained.

Authorship Contributions

Concept: Doğa Türkkahraman, Suat Tekin, Merve Güllü, Design: Doğa Türkkahraman, Suat Tekin, Merve Güllü, Data Collection or Processing: Suat Tekin, Analysis or Interpretation: Güzin Aykal, Suat Tekin, Merve Güllü, Literature Search: Doğa Türkkahraman, Suat Tekin, Merve Güllü, Writing: Doğa Türkkahraman, Suat Tekin, Merve Güllü, Güzin Aykal.

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