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Original article

Clinical Characteristics and Growth Hormone Treatment in Patients with Prader-Willi Syndrome

Dağdeviren Çakır A et al. Nationwide Study of Prader Willi Syndrome

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What is already known?
Prader-Willi syndrome (PWS) is a genetic disorder characterized by short stature, low lean body mass, muscular hypotonia, mental retardation, behavioral abnormalities, dysmorphic features, and excessive appetite with progressive obesity. Growth hormone (GH) treatment is beneficial for children with PWS. It improves linear growth, increases lean body mass, basal energy expenditure, muscle strength and reduces fat mass.

What this study adds?
Although clinical and genetic characteristics of PWS are well defined, national Turkish data regarding patients with PWS is lacking. This study reports clinical and genetic characteristics, the rate and timing of GH treatment initiation, and response to GH treatment in Turkish PWS patients. Additionally, by increasing pediatricians' awareness of PWS, it is hoped that earlier diagnosis and therefore earlier treatment may occur.

Abstract
Objective: To investigate clinical characteristics and response to growth hormone (GH) treatment in patients with Prader-Willi syndrome (PWS) in Turkey.
Methods: The data of 52 PWS patients from ten centers was retrospectively analyzed. A nation-wide, web-based data system was used for data collection. Demographic, clinical, genetic, and laboratory data and follow-up information of the patients were evaluated.
Results: The median age of patients at presentation was 1.5 years, and 50% were females. Genetic analysis showed microdeletion in 69.2%, uniparental disomy in 11.5%, imprinting defect in 1.9% and methylation abnormality in 17.3%. Hypotonia (55.7%), feeding difficulties (36.5%) and obesity (30.7%) were the most common complaints. Cryptorchidism and micropenis were present in 69.2% and 15.3% of males, respectively. At presentation, 25% had short stature, 44.2% were obese, 9.6% were overweight and 17.3% were underweight. Median age of obese patients was significantly higher than underweight patients. Central hypothyroidism and adrenal insufficiency were present in 30.7% and 4.7%, respectively. Hypogonadism was present in 75% at normal age of puberty. Growth hormone treatment was started in 40% at a mean age of 4.7±2.7 years. After two years of GH treatment, a significant increase in height SDS was observed. However, BMI SDS remained unchanged.
Conclusion: The most frequent complaints were hypotonia and feeding difficulty at first presentation. Obesity was the initial finding in 44.2%**.** Growth hormone treatment was started in less than half of the patients. While GH treatment significantly increased height SDS, BMI SDS remained unchanged, possibly due to the relatively older age at GH start.
Keywords: Prader-Willi syndrome, endocrine dysfunction, growth hormone treatment, body composition

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Introduction
Prader-Willi syndrome (PWS) is a genetic disorder resulting from lack of paternally inherited

imprinted genes on chromosome in the 15q11-q13 region, either due to deletions from the paternal chromosome, maternal uniparental disomy or, rarely, defects in the imprinting center (1). The estimated incidence of PWS is around 1 in every 15,000–30,000 births. Both sexes are affected equally (2).

PWS is a complex disorder with different phenotypic features developing at different ages. It is characterized by severe hypotonia with poor sucking and feeding difficulties in early infancy, followed by excessive eating and gradual development of obesity in later infancy or early childhood, if access to food is unrestricted (3-4). Hypothalamic dysfunction is characteristic of PWS and this is hypothesized to underlie many of the syndrome's cardinal features, such as hyperphagia, temperature instability, sleep-disordered breathing, and multiple endocrine abnormalities that include growth hormone deficiency, central adrenal insufficiency, hypogonadism and hypothyroidism (5). Global developmental delay, cognitive dysfunction and neurobehavioral problems are other features of the syndrome (1).

Growth hormone (GH) deficiency is very common in PWS (6). Recombinant human GH (rGH) is indicated in the treatment of growth failure in PWS and provocation testing to demonstrate GH deficiency is unnecessary for patients with genetically confirmed PWS (7). In addition, treatment with GH can improve body composition and physical strength, as well as motor and mental development (8).

In Turkey, the data regarding the purpose of GH treatment in patients with PWS are not clear. In addition to describing the prevailing current situation, an aim of this study was to determine the clinical, demographic and accompanying endocrine and non-endocrine co-morbid conditions of pediatric Turkish patients with PWS.

Methods and Subjects

In this study, we retrospectively analyzed the data of 52 patients with PWS who were being followed in 10 centers in Turkey. PWS patients aged between 0 to 18 years were enrolled in the study. A nation-wide, web-based, CEDD-NET Data System (http://cedd.saglik-network.org/) was used for data collection between March 2016 and February 2018. A case recording form, including demographic, clinical, genetic, and laboratory findings and follow-up information on the patients was uploaded to the website and completed by the patient's managing physician. The study was conducted according to the principles of the Declaration of Helsinki and approved by the institutional ethical review board (Approval Number:2016-16, 02.19.2016).

Short stature was defined as a height that was two standard deviation score (SDS) or more below the mean height for age and sex (9). Overweight and obesity were defined as body mass index (BMI) that was between >85th and <95th percentile and ≥95th percentile for age and sex, respectively. Underweight was defined as BMI that was <5th percentile for age and sex (10). SDS and percentiles of height, weight and BMI were calculated according to national data (11).

Preterm delivery was defined as a gestational age of <37 weeks. Low birth weight was defined as birth weight below 2500 gr. Small for gestational age (SGA) was defined as birth weight below the 10th percentile for gestational age (12).

Growth hormone deficiency was diagnosed after two stimulation tests (L-dopa, clonidine, glucagon or insulin, depending on each center's normal practice). Complete GH deficiency was diagnosed after a stimulation test with a GH peak <5 µg/L and partial GH deficiency with a GH peak between 5 and 10 µg/L. Diagnosis of central hypothyroidism (CH) was made with low free T4 (fT4) concentrations associated with low/normal serum thyroid stimulating hormone (TSH) levels. Primary hypothyroidism was diagnosed with low fT4 associated with elevated TSH levels (13). Presence of central adrenal insufficiency (CAI) was investigated by estimation of serum adrenocorticotrophic hormone (ACTH)-cortisol levels in blood samples obtained in the early morning and by low-dose ACTH stimulation test, when needed. The cut-off level for appropriate cortisol response was accepted as 18 mcg/dL (14). Hypogonadism was investigated when puberty was delayed. Low sex steroid levels along with high gonadotropin levels suggested primary hypogonadism. Diagnosis of secondary hypogonadism was made with low sex steroid levels and negative luteinizing hormone-releasing hormone (LHRH) stimulation test in patients above pubertal age (15). Micropenis was defined as penile length smaller than 2.5 SD below the mean; SDS of penile length was calculated according to national data (16). Osteoporosis was considered present when the child had sustained: (a) one or more low-traumatic vertebral fractures in the absence of local disease or high-impact injury; or (b) two or more low impact fractures of the long bones if less than **ten** years of age or three or more low impact fractures before 19 years of age, together with bone mineral density (BMD) as assessed by dual-energy X-ray absorptiometry (DXA) that was < -2 SDS below the mean for sex, chronological age, and height/height age (17).

Clinical and laboratory characteristics of the patients were evaluated by each center at presentation and during follow up. Clinical characteristics including birth weight, gestational age at delivery, history of developmental milestones, feeding difficulties in infancy, complaints, anthropometric measurements (height, weight, BMI) and pubertal status were recorded. A detailed PWS-specific questionnaire regarding cardiac, renal, endocrine, otorhinolaryngological and skeletal systems, as well as neuromotor, psychosocial and sleep problems relevant to PWS were completed by managing physicians. Cardiac findings were based on echographic examinations. If available, sella magnetic resonance imaging (MRI) and polysomnography (PSG) results were requested. Confirmatory genetic test results were recorded. In addition, any other clinical features present in individual patients but not specifically queried in the questionnaire were also requested and recorded. Patient's anthropometric measurements were recorded yearly over two years follow up. Annual laboratory assessments were collected which included serum insulin-like growth factor 1 (IGF1), glycated hemoglobin (HbA1c), fasting glucose and insulin, and lipid profile consisting of total triglyceride, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) concentrations. SDS values were calculated for IGF-1, according to age- and sex-matched reference values for the Turkish population (18). Dyslipidemia was defined according to the guidelines of the National Heart, Lung, and Blood Institute as total cholesterol ≥200 mg/dL, LDL ≥130 mg/dL, HDL<40 mg/dL, triglyceride ≥100 mg/dL for younger than 10 years old and ≥130 mg/dL for those older than 10 years (19). Homeostasis model of assessment of insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR = [fasting insulin (µIU/mL) × fasting glucose (mg/dL)]/405 (20).

The researchers were asked to record the growth hormone dose, if treated, and whether the growth hormone treatment had been interuprted or discontinued completely, and if so, why.

Entering additional information not included in the questionnaire form was optional.

Genetic tests

DNA methylation analysis was performed as first line test to confirm the diagnosis of PWS. Polymerase chain reaction (PCR) along with Southern blotting of the small nuclear ribonucleoprotein polypeptide N (SNRPN) probe for the 15q11-q13 region or Methylation-Specific Multiplex Ligation- Dependent Probe Amplification (MS-MLPA) were used to determine methylation status, depending on each centers' preference. If the DNA methylation patterns were consistent with PWS, further tests were performed to identify the exact genetic etiology of PWS [deletion, uniparental disomy (UPD) or imprinting defect]. Fluorescence in situ hybridization (FISH) with high resolution karyotype, chromosomal microarray analysis with single nucleotide polymorphism (SNP) and copy number variant (CNV) probes or MS-MLPA methods were used to determine the deletion status of the 15q11-q13 region, again depending on each centers' preference. If no deletion was detected, DNA polymorphism analysis was performed to distinguish between maternal UPD and imprinting defects. The diagnosis of imprinting defect was made after exclusion of uniparental disomy. In some centers, the diagnosis of PWS was made only with DNA methylation analysis without further genetic tests.

Statistics

Statistical analyses were performed using SPSS, version 21.0 (IBM Inc., Chicago, Ill., USA). Descriptive statistics for categorical variables are presented as frequencies and percentages. Normality was tested using the Shapiro-Wilk test. Depending on the distribution of the data set, data are presented as mean±SD or median (25th to 75th percentile). Wilcoxon signed-ranks and Friedman tests were used to compare baseline values between first year and second year values in the group receiving GH treatment. A p-value <0.05 was assumed to indicate statistical significance.

Results

Baseline characteristics

Data of a total of 52 patients with PWS (26 males, 26 females) were collected. At first presentation, the median age of patients was 1.5 (0.08-15.4) years and 96.1% (n=50) of the patients were prepubertal. The most frequent complaints were hypotonia in 55.7% (n=29) patients, feeding difficulties in 36.5% (n=19) and obesity in 30.7% (n=16). Cryptorchidism and micropenis had been detected in 69.2% (n=18) and 15.3% (n=4) of male patients, respectively (**Table 1)**. Demographic and anthropometric data of the patients at presentation is shown in **Table 2**. Mean height and BMI SDSs were -1.25±1.23 and 0.96±2.56, respectively. Short stature was detected in 25% (n=13) of the patients. Height SDS was between -2 SDS and -3 SDS in 17.3% (n=9) and less than -3 SDS in 7.7% (n=4) of the patients. Among the patients 44.2% (n=23) were obese, 9.6% (n=5) were overweight and 17.3 % (n=9) were underweight. The median age of the obese patients was 4.1 (range 0.9-15.4) years, whereas the median age of the underweight patients was 0.12 (range 0.08-1.4) years. Median age of the obese patients was significantly higher than the underweight patients (p<0.001).

Birth characteristics

The mean gestational age and birth weight of the patients were 38±1.8 weeks and 2550±450 gr, respectively. Preterm delivery was present in 17.3% (n=9) of the patients. Of the patients 40.3% (n=21) were small for gestational age (SGA) and 42.3% (n=22) had low birth weight (LBW).

Neuromotor development

The mean time for onset of developmental milestones were as follows: independent sitting at 17.9±8.9 months (n=30/52); walking at 33±13 months (n=27/52); and first spoken words at 31.3±16.1 months (n=20/52).

Nutritional characteristics

In infancy, need for assisted feeding with nasogastric tube, spoon and nursing bottle were recorded in 15.3% (n=8) of the patients.

Genetic evalution

Genetic analysis results of patients are shown in **Figure 1.** PWS was diagnosed based solely on methylation abnormality in 17.3% (n=9) of the patients. In these patients, microdeletion, UPD and imprinting center mutation could not be examined to define genetic etiology. In the remaining patients, further genetic tests revealed microdeletion, maternal UPD, and imprinting center defects in 69.2% (n=36), 11.5% (n=6), and 1.9% (n=1) of the patients, respectively

Endocrinologic evaluation

CH and acquired primary hypothyroidism were observed in 30.7% (n=16) and 1.9% (n=1) of the patients, respectively. Etiology of acquired primary hypothyroidism remained undetermined, thus autoimmune thyroid disease was excluded in the patient. Adrenal function was evaluated in 80.7% (n=42) and central adrenal insufficiency was detected in 4.7% (n=2) of them. These two patients had no clinical signs of adrenal insufficiency, CAI was detected with screening, and hydrocortisone replacement was provided in case of adrenal stress, for example, because of infection.

In 48% of the patients (n=25) GH stimulation tests were performed and 23/25 (92%) had a deficient GH response. Sella MRI was normal in all of the patients with GH deficiency.

Among patients at pubertal age (n=4), **two** had hypogonadotropic hypogonadism, and **one** had hypergonadotropic hypogonadism. The patient with hypergonadotropic hypogonadism was diagnosed due to arrest of pubertal development. One of the patients with hypogonadotropic hypogonadism presented with secondary osteoporosis. Overall, two patients had osteoporosis; one with normal pubertal development, thus the etiology of osteoporosis remained undetermined.

Cryptorchidism and micropenis were present in 69.2% (n=18) and 15.3% (n=4) of the male patients, respectively. Orchiopexy was performed in 57.6% (n=15) of male patients. In **one** patient, orchiectomy was performed due to atrophic testis.

Skeletal assessment

Skeletal problems were present in 30.7% (n=16). The most common problem was scoliosis, observed in 23% (n=12). Lower extremity abnormalities, including developmental dysplasia of hip, pes equino varus, pes cavus, x-bain and o-bain deformities, were present in 15.3% (n=8).

Otorhinolaryngological assessment

Seventy-one percent of patients (n=37) were formally evaluated by an otorhinolaryngologist. Pathologic findings, including adenoid vegetation and/or tonsillar hypertrophy, were reported in 43.2% (n=16) and surgical interventions (adenoidectomy and/or tonsillectomy) were performed in 50% (n=8) of these. One patient had conductive hearing loss.

Sleep apnea and polysomnography findings

Polysomnography was performed in 57.6% (n=30) and pathologic findings, including obstructive/central/mixed apnea and hypopnea, were detected in 70% (n=21). Narcolepsy was reported in one patient (1.9 %).

Other chronic diseases

Epilepsy was reported in 9.6% (n=5). Three patients were operated for strabismus. Echocardiographic evalution was performed in 53.8 % (n=28) and pathologic findings, including atrial septal defect, ventricular septal defect, subaortic ventricular septal hypertrophy, patent foramen ovale, pulmonary stenosis, pulmonary hypertension, minimal aortic insufficiency and tricuspid insufficiency, were present in 28.5% (n=8). One patient was operated due to atrial septal defect. One patient had a pacemaker due to arrhythmia. One patient died at the age of nine months due to lower respiratory tract infection. One patient had tracheostomy and she also had severe mental motor retardation.

Growth hormone treatment

Twenty-one (40.3%) patients were treated with rGH (mean dose: 25±5 µg/kg/day) of whom 19 had GH deficiency. Growth hormone was started in **one** patient without GH stimulation testing and **one** patient without GH deficiency (Fig 2). The mean age at onset of GH treatment was 4.7±2.7 (range 1.6-9.4) years. At the beginning of treatment 28.5% of the patients had short stature, 52.3% were obese, 14.2% were overweight and 4.7% were underweight. Treatment was continued for one year in 21 patients and for **two** years in 11 patients. The mean growth velocity was 9.9±2.5 cm/year for the first year and 8.1±3.1 cm/year for the second year. Data showing the anthropometric and laboratory changes after the first and second year of GH treatment are shown in **Table 3** and **Table 4,** respectively. After one year of GH treatment, a significant increase in height SDS parallel to increase in serum IGF-1 SDS was observed. However, there was slight but significant increase in weight SDS (p=0.035) and BMI SDS remained unchanged. Serum glucose levels did not change in the first year of treatment, but insulin levels increased slightly (p=0.047). Fasting glucose levels were normal in all patients before GH treatment, while impaired fasting glucose was detected in only one patient after the first year of treatment. Insulin resistance was evaluated by HOMA-IR in fifteen patients before treatment and 2/15 (13.3%) had high pre-rGH treatment HOMA-IR values. At the end of the first year of rGH, high HOMA-IR values was detected in 5/15 patients (33.3%), three patients progressed from normal to abnormal HOMA-IR on GH treatment. Before GH treatment, elevated total cholesterol, LDL and triglyceride levels, and decreased HDL levels were detected in 22.2% (n=4/18), 27.7% (n=5/18), 31.2% (n=5/16) and 16.6% (n=3/18), respectively. After the first year of GH treatment, elevated total cholesterol, LDL and triglyceride levels were detected in 43.7.1% (n=7/16), 46.6 % (n=7/15) and 23% (n=3/13), respectively. When compared to baseline, there was no change in triglyceride levels, but total cholesterol, HDL and LDL levels increased after the first year of GH treatment **(Table 3).** None of the patients had low HDL levels after the first year of GH treatment.

In the eleven patients completing two years of GH treatment, a significant increase in height and IGF-1 SDS were observed, compared to baseline, but there were no difference in terms of weight and BMI SDS. After **two** years of treatment, despite no change in insulin and HOMA-IR levels, there was a slight increase in glucose levels compared to baseline. Since there was not enough data, the effect of growth hormone on lipid profile was not evaluated in the second year.

In all but two patients receiving GH treatment, polysomnography was performed before treatment (19/21). Sleep apnea was observed in **nine** patients before treatment and in **one** patient during GH treatment. In **three** of them, GH treatment was started after adenotonsilectomy. In **three** patients, GH treatment was started under continuous positive airway pressure (CPAP) or bilevel positive airway pressure (BiPAP) support. Due to exacerbation of apnea, **two** patients underwent adenotonsilectomy, which after GH treatment was continued. In **one** patient treatment was discontinued in the second year due to worsening of sleep apnea. In **one** patient who did not have sleep apnea before rGH treatment, sleep apnea was observed in the first year of treatment and treatment was continued under BiPAP support. In the remaining nine patients whose pre-treatment polysomnography was normal and in two patients who did not undergo polysomnography, no complication was reported during GH treatment. Growth hormone treatment was not started in 11 further patients with sleep apnea.

Adrenal insufficiency was not reported in patients receiving GH. In seven patients, CH was associated with GH deficiency. Hypothyroidism did not develop under GH treatment.

Discussion

A total of 52 patients (26 males, 26 females) with PWS who had been registered to the CEDD-NET Data System were involved in this study. In our cohort, 55.7% and 36.5% of the patients had presented with hypotonia and feeding difficulties, respectively. It is known that clinical signs of PWS vary by age. In infants, the most prominent findings are hypotonia and feeding difficulties. The characteristic findings like hyperphagia, obesity, and intellectual disability develop later in childhood (21-22). In a multicenter study investigating maternal and neonatal outcomes in patients with PWS, all neonates were hypotonic, and 99% had feeding difficulties (23). In our series, frequency of hypotonia and feeding difficulty were lower than in the literature. Clinical diagnosis of PWS is difficult during infancy because hypotonia is a non-specific feature and the typical clinical features of the later period are not yet present. Beside this, hypotonia is not an indication for admission to endocrine clinics. Thus, in our cohort, later age at presentation makes hypotonia a relatively less frequent symptom. It is recommended that PWS should be considered in any infant with significant hypotonia, particularly in the setting of poor feeding and genital hypoplasia (cryptorchidism, small penis, or small clitoris). Tuysuz et al (24) reported PWS in 11% of the patients referred for hypotonia. Infantile history should be actively sought during evaluation of older children (25).

Excessive weight gain follows the period of failure-to-thrive in early infancy in PWS (21). It is reported that obesity usually begins between the ages of one and six years, with an average age of onset of two years (3). In our series, 44.2% of the patients presented with obesity. Median age at presentation of obese patients was 4.1 years. In the 17.3% of patients presenting with underweight median age at presentation was 0.12 years. However, data regarding the age at onset of obesity was not present. As expected given the expected natural history of PWS, patients presenting with undernutrition were younger than those who were obese at presentation.

Toddlers with PWS have delayed motor and language development, with milestones achieved at about double the normal age (3). In our series, the average age at independent sitting, walking and speaking first words were at 18, 33 and 31 months, respectively. Developmental delay was a presenting feature in 26.9% of the patients.

The prevalance of preterm birth, SGA and LBW in our cohort was 17.3%, 40.3% and 42.3%, respectively, which was in concordance with the increased incidence of preterm birth, LBW and intrauterine growth retardation reported in PWS (26-28).

In our series, 17.3% were diagnosed by methylation analysis only. Unfortunately, further genetic tests were not performed in these patients. In the patients in whom further genetic analysis was performed the frequencies of microdeletion, UPD and genomic imprinting center defect were 69.2%, 11.5% and 1.9%, respectively. In the literature, paternal 15q11.2-q13 deletion is responsible for 65-75% of cases, maternal UPD is responsible for 20-30% of cases, and 1-3% of cases are sporadic or due to genomic imprinting center defect (1,29). Thus in this group of Turkish PWS patients the incidence of microdeletion and imprinting is in line with previous reports but the incidence of UPD is around half that expected. Nevertheless, if further genetic tests could have been performed in the group with only methylation analysis, these incidence rates may be different.

Hypothalamic dysfunction is thought to be responsible for some endocrinopathies in PWS including CH (30). In our series, the prevalence of CH was 30.7%, and one patient had acquired primary hypothyroidism due to unknown etiology. There are conflicting data in the literature regarding the prevalence of CH in PWS. In some studies, a prevalence of 2–4%, which is similar to that of the general population was reported, while others reported a prevalence of 20–30% (31-32) or even of 72% in a study conducted in patients with PWS during the first 2 years of life (33).

Children with PWS are at risk for CAI, also thought to be due to hypothalamic dysfunction (30). However, the frequency of CAI in patients with PWS is not clear and frequencies have varied widely between studies. In a cross-sectional study, CAI was detected in 60% of cases after metirapone test (34). Subsequent studies conducted with other methods did not confirm the reported high frequency. Corrias et al found CAI in 14.3% of the cases (35). Beauloye et al. reported CAI with a prevalance of 5% in children with PWS (36), similiar to the frequency of 4.7% found in our series. By contrast, some studies showed normal hypothalamic pituitary adrenal axis in PWS patients (37,38).

Hypogonadism is a common clinical feature of the syndrome and both hypothalamic and gonadal abnormalities can cause hypogonadism (39,40). In both genders, hypognadism manifests as genital hypoplasia, incomplete pubertal development, and infertility in the majority (1). Unilateral or bilateral cryptorchidism is a common finding, ranging from 66% to 100% of males (39,41). However, genital hypoplasia is often overlooked in females (1). Frequency of cryptorchidism and micropenis in our cases were 69.2% and 15.3%. Genital hypoplasia was not reported in females. In our series, 92.3% of the patients were in the prepubertal period and thus gonadal function was not evaluated. Among four patients with ages consistent with the normal pubertal period, **two** had hypogonadotropic hypogonadism and **one** had hypergonadotropic hypogonadism (75%). In a cohort of 115 adult patients with PWS, all males and 93% of females had hypogonadism (42). In the French national PWS pediatric database of 142 patients, the frequency of hypogonadism was 49% (32). The number of patients whose gonadal function was evaluated was limited in our cohort. Therefore, it is unreliable to attempt to draw definite conclusion for frequency of hypogonadism in this cohort.

The body composition of patients with PWS, characterized by reduced lean body mass and increased fat mass, resembles that of individuals with GH deficiency (43). Diminished GH secretion in PWS is well documented and it has been reported to be present in 40% to 100% of the patients (6,7,32,44).

In our series, the GH/IGF axis was evaluated in 48% (n=25) of the cases and GH stimulation tests revealed GH deficiency in 23 patients. Growth hormone treatment was started in 40.3% of the patients. Short stature is a common finding in PWS patients and occurs because of linear growth retardation and lack of a pubertal growth spurt (3). In our series, 25% of the patients had short stature at presentation. Among the patients who received growth hormone treatment 28.5% had short stature at the initiation of the treatment. The rationale for treating PWS children with GH is to not only enhance linear growth but also to improve body composition, energy expenditure and muscle strength (45). In our study, height velocity increased with treatment and significant improvement in height SDS was observed at the end of the first year. However, there was no change in BMI SDS. Data for the second year of GH treatment was suboptimal due to a low number of patients (n=11). Compared to baseline, there was an improvement in height SDS, however there was no change in BMI SDS. Several studies conducted on PWS children treated with GH and followed longitudinally have shown that prolonged treatment with GH improves but does not normalize body composition (45-47). Although there is no consensus on this issue, it is recommended to start GH treatment before the onset of obesity, which often begins by two years of age (7). Carrel et al. (48) conducted a study with 21 patients with PWS, in whom GH treatment was started prior to **two** years of age and continued for **six** years. At the end of treatment, the anthropometric measurements of these children were compared with 27 PWS patients of similar age, who did not receive GH treatment. These authors reported that GH therapy begun early in life favorably altered the natural history of PWS by reducing body fat mass. In our series, the median age at onset of GH treatment was four years (range 1.6-9.4). One reason for the lack of improvement in BMI SDS in our patients may be the relatively late initiation of treatment; 66.5% were already obese or overweight. Baker et al. (49) conducted a study in 60 PWS patients who started GH therapy between **3-7** years of age and continued for **eight** years. In this study, lean body mass (LBM) significantly increased, and percent fat mass and BMI significantly decreased in the first year. However, in the subsequent **seven** years, LBM and BMI remained stable, but percent fat mass gradually increased. At the end of **eight** years, LBM SDS was higher than baseline, but percent fat mass and BMI SDS were not significantly different from baseline. In this study, BMI of patients was compared with reference values of untreated age and sex matched children with PWS and BMI_{PWS} SDS was calculated according to these reference values. BMI_{PWS} SDS decreased significantly in the first year of treatment and this effect persisted during the entire study period and at the end BMI_{PWS} SDS was significantly lower than at baseline. The authors concluded that GH treatment is a potent force for counteracting the clinical course of obesity in patients with PWS. In the present study, only change in BMI was evaluated while body composition was not. Thus, even though no change in BMI was observed, fat and lean mass ratio may have changed. Nevertheless, there was no improvement in mean BMI SDS. However, stabilization of BMI SDS may be an acceptable outcome of the treatment compared to increasing worsening of BMI. We do not have enough

data about the untreated PWS group to compare the anthropometric changes with the GH treated group. However, tendency to deterioration of auxological and body composition parameters over time in untreated patients is widely accepted. Nutritional management is the mainstay of treatment in PWS, even during GH therapy (7). The lack of nutrional data was another limitation of our study. In our cohort, the dose of GH treatment was not uniform so that the variation in dosages may have confounded the anthropometric results.

We found an increase in fasting insulin and HOMA-IR levels, with no change in fasting glucose levels after the first year of GH treatment. Carrel et al. (50) also reported similar results; although there was no change in glucose level, there was a statistically insignificant increase in insulin level. Bakker et al. (49) showed an increase in fasting glucose and insulin levels after one year of treatment. However, some studies reported that GH treatment was not associated with adverse effects on glucose and insulin parameters (48,51).

Previous studies have shown improvements in patients' lipid profiles with GH treatment (48-50). However, in our study, despite a significant increase in HDL levels, there was also a significant increase in LDL and total cholesterol levels. In our series, the change in serum lipid levels may not be due to GH therapy and may be part of the natural course of the disease.

Children with PWS have a high incidence of both central and obstructive apnea (52). In the patients who underwent polysomnography, abnormalities were detected in 70%. This finding shows the importance of polysomnography in the follow up of PWS, especially in the patients in whom GH treatment is planned, because GH treatment can hypothetically lead to expansion of airways-associated lymphoid tissue in PWS children, due to increased IGF-I effects, and thus exacerbate obstructive apnea (53). Severe sleep apnea is a contraindication for GH treatment (7). In our cohort, GH treatment was started in **three** patients with sleep apnea who also received BIPAP/CPAP support and in three patients after adenotonsilectomy. Due to exacerbation of apnea, two patients underwent adenotonsilectomy and treatment was discontinued in one patient after the second year. In one patient, sleep apnea was detected during GH treatment and treatment was continued under BiPAP support. Deaths have been reported associated with GH treatment, especially in the early phase of GH treatment (52-54). In our cohort, no death was reported during GH treatment. However, GH treatment increased the severity of apnea in four patients. Therefore, during GH treatment in PWS, close follow-up of patients with ENT and/or polysomnography is recommended.

Study Limitations

The most important limitation of this study is its retrospective design. Data was collected from different centers with a web-based national data system and the clinical follow up protocols were heterogenous. We did not have data on neuromotor development involving all patients. Additionally, data was collected only from pediatric endocrinology clinics and thus patient characteristics could be different in those who were admitted to genetic or other clinics. As noted earlier, data regarding body composition and nutritional status are incomplete. Also, there is insufficient anthropometric data in the untreated PWS group to compare the changes with those observed in the rGH treated group. Here, we report short-term results of GH treatment in a small group. Prospective studies in larger populations with long term follow-up are needed to assess the effect of GH treatment and to draw definite conclusions.

Conclusion

The present study provides data on the demographic characteristics and frequency of associated problems in PWS during childhood, based on the experience of pediatric endocrinology centers in Turkey. This study has highlighted the lack of a national protocol for follow up and GH treatment in pediatric patients with PWS. The most frequent complaint was hypotonia followed by feeding difficulties. Obesity was the initial finding in 44.2% of the patients**.** Growth hormone treatment was started in less than half of the patients. While GH treatment significantly increased the height SDS, BMI SDS remained unchanged, which might be due the relatively late onset of GH treatment. National programs to increase awareness of PWS to improve diagnosis and guidelines for standardized follow up to improve clinical care should be instituted.

Authorship Contributions

Surgical and Medical Practices: Aydilek Dağdeviren Çakır, Olcay Evliyaoğlu, Beyhan Tüysüz, Oya Ercan
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Literature Search: Aydilek Dağdeviren Çakır, Olcay Evliyaoğlu
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Table 1: Clinical features of the patients at presentation

Characteristics	n (%)
Hypotonia	29 (55.7)
Feeding problems	19 (36.5)
Cryptorchidism*	18 (69.2)
Obesity	16 (30.7)
Developmental delay	14 (26.9)
Short stature	13 (25)
Typical dysmorphic facies	7 (13.4)
Mental retardation	5 (9.6)
Micropenis*	4 (15.3)
Failure to thrive	4 (7.6)
Sleep disorders	4 (7.6)
Small hands and feet	3 (5.7)

*In male patients

Table 2: Clinical Characteristics of the patients at presentation

	Mean +SD /n(%)	Median	Min-Max
Age (years)	2.7±3.2	1.5	0.08 - 15.4
Gender, female	26 (50)		
Height SDS	-1.25±1.23	-1.25	-4.9 - 0.9
Target height SDS	-0.66 ±0.73	-0.69	-2.5 - 1.2
Weight SDS	0.25±2.16	0.05	-4.7 - 4.85
BMI (kg/m^2)	19.8±7.01	19.2	8.8 - 41.6
BMI SDS	0.96±2.56	1.64	-4.7 - 4.7
BMI percentile			
< 5th	9 (17.3)		
≥5th to <85th	15(28.8)		
≥ 85th to < 95th	5 (9.6)		
≥ 95th	23 (44.2)		

Table 3: Clinical and laboratory evaluation of patients before and after one year of growth hormone treatment

	Baseline	First year	p
Height SDS (n=21)	**-1.4 (-2.0; -0.6)**	**-0.9 (- 1.3; -0.4)**	**<0.001**
Weight SDS (n=21)	**0.3 (-0.8; 2.5)**	**1.2 (-0.2; 2.6)**	**0.035**
BMI SDS (n=21)	**1.8 (0.6; 3.2)**	**2.0 (1; 3.3)**	**>0.05**
Glucose (mg/dL) (n=21)	**81.5 (67.7; 86.2)**	**85.5 (79.2; 91)**	**>0.05**
Insulin (µIU/mL) (n=15)	**7.6 (5.2; 9.6)**	**10 (8.1; 12.4)**	**0.047**
HOMA-IR (n=15)	**1.5 (1.0;2.0)**	**2.15 (1.6;2.6)**	**0.016**
Total Cholesterol (mg/dL) (n=16)	**153.5 (126.7;198.7)**	**197.5 (155.7-234)**	**0.004**
LDL Cholesterol (mg/dL) (n=15)	**101 (78; 143)**	**120 (83; 174)**	**0.047**
HDL Cholesterol (mg/dL) (n=15)	**44 (37; 63)**	**52 (45; 64)**	**0.021**
Triglyceride(mg/dL) (n=13)	**92 (59; 116)**	**82 (51; 107)**	**0.1**
IGF-1 SDS (n=17)	**-2.5 (-2.7; -2)**	**-0.6 (-1.0; 0.8)**	**<0.001**

Results are given as median (25;75p)

Table 4:Clinical and laboratory evaluation of patients before and after the first and second years of growth hormone treatment.

	Baseline	First year	Second year	p
Height SDS (n=11)	**-1.6 (-2.5; -1.2)**	**-1.0 (-1.4; -0.8)**	**-0.9 (-1.3; -0.2)**	**<0.001 ***
Weight SDS (n=11)	**0.2 (-0.8; 1.4)**	**1.1 (-0.5; 1.4)**	**0.8 (0.4; 1.9)**	**>0.05**
BMI SDS (n=11)	**1.6 (0.8; 2.8)**	**1.5 (0.3; 2.7)**	**1.7 (1.4; 2.2)**	**>0.05**
Glucose (mg/dl) (n=11)	**80 (71; 85)**	**83 (77; 90)**	**90 (78; 93)**	**0.023µ**
Insulin (µIU/mL) (n=9)	**7.7 (4.9; 8.8)**	**8.9 (4.7; 10.8)**	**8.8 (5.5; 15.2)**	**>0.05**
HOMA-IR (n=9)	**1.5 (1;1.8)**	**2.1 (0.9; 2.3)**	**1.7 (1.1;3.5)**	**>0.05**
IGF-1 SDS (n=10)	**-2.4 (-2.7; -2)**	**-0,6 (-1.2; 0.9)**	**0.9 (-0.6; 3.1)**	**0.002 ¶**

Results are given as median (25;75p)

*The difference was due to the baseline and second year comparison (p=0.001)

µ The difference was due to the baseline and second year comparison (p=0.023)

¶ The difference was due to the baseline and second year comparison (p=0.001)

Figure 1: The results of genetic analysis of patients

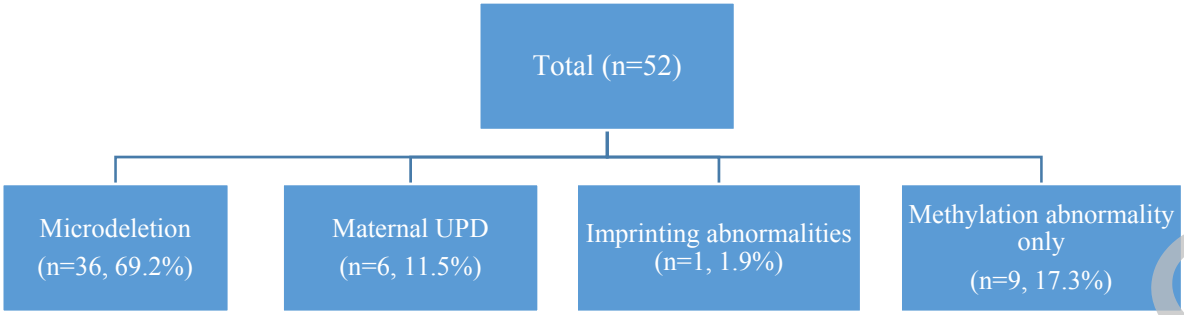

Figure 2: Flow chart of the patients whom GH treatment was started