Clinical Profile and Molecular Genetic Analysis of Prader–Willi Syndrome: A Single Center Experience

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Abstract

Aim: The prevalence of childhood and adolescent obesity is increasing worldwide as well as in India. Prader–Willi syndrome (PWS) is one of the most common causes of syndromic obesity with varied clinical manifestations across different lifespan. Hereewith, we describe clinical and molecular characteristics of eight PWS who were diagnosed in an obesity clinic of tertiary care hospital.

Materials and Methods: Clinically suspected cases of PWS were screened between January 2014 and January 2022. Detailed history and clinical examination were done to look for typical features of PWS like characteristic facial appearance, short stature, obesity, hyperphagia, delayed puberty or hypogonadism, diabetes mellitus, developmental delay, cognitive dysfunction, learning disabilities or abnormal behavior. All were evaluated, with 75 g oral glucose tolerance tests (GTT), HbA1c, Free T4, TSH, LH, FSH, testosterone, and growth hormone level. Intelligent quotient (IQ) of each patient was assessed by a psychiatrist using Binet–Kamat test. Molecular confirmation of clinically suspected PWS was done by either Methylation-specific polymerase chain reaction (MS-PCR) or Fluorescence in situ Hybridization (FISH) methods.

Results: Based on clinical and molecular characteristics, eight were diagnosed as PWS. Except one, all were male with characteristic facies, mean age of study cohort was 12 years and mean BMI of 44.58. Obesity, short stature, hyperphagia, hypotonia, and mild to moderate mental retardation were noted in entire (100%) PWS study population. All male PWS patients had cryptorchidism, which was bilateral in six patients and unilateral (right undescended testes) in one. Apart from obesity, short stature, other endocrine associations noted were diabetes mellitus in 50% and subclinical hypothyroidism in 37% of PWS. Molecular characteristics of PWS were confirmed by Methylation-specific PCR in seven and by FISH method in one.

Conclusion: Prader–Willi syndrome should be kept in mind in case of childhood or adolescent obesity with short stature, hypotonia, cryptorchidism, and developmental delay or cognitive dysfunction. Judicious use of molecular diagnostic testing should be made in all clinically suspected cases. Early diagnosis and appropriate management of this complex disorder by a multidisciplinary team will improve the quality of life and treatment outcome.

Keywords: Cryptorchidism, hyperphagia, hypogonadism, hypotonia, India, obesity, Prader Willi syndrome

Introduction

The prevalence of childhood and adolescent obesity is increasing worldwide as well as in India. From the previously published literature from India, the prevalence of obesity ranges from 20% to 30%. Despite significant progress in understanding the molecular basis of obesity and body fat regulation, the genetic causes of obesity have not been fully elucidated. More than 95% of obesity is polygenic in nature and is determined by the aggregate effect of multiple common genetic variants. The monogenic or syndromic causes of obesity are recognized in less than 5% of obesity population due to their very low prevalence and lack of awareness among the treating physicians.

Obesity syndromes are generally due to genetic or chromosomal abnormalities that arise as a result of sporadic mutations or genomic imprinting. Prader–Willi Syndrome (PWS) and Bardet–Biedl Syndrome (BBS) are the two most frequently noted obesity syndromes in clinical practice.
PWS is characterized by short stature, hyperphagia, obesity, hypotonia, hypogonadism, sleep disturbances, cognitive, and behavior abnormalities. The reported incidence of PWS from world literature is one in 15,000 live births. It is an under recognized entity of childhood and adolescent obesity and very few case reports and studies are available regarding PWS from India.

Early diagnosis of PWS enables affected individuals to begin early intervention/special needs programs along with appropriate management of co-existing co-morbidities. Hence, it is important for clinicians to be familiar with this syndrome. Herewith, we describe a case series of PWS along with clinical and molecular characteristics.

Materials and Methods

Individuals with childhood and adolescent obesity who were referred for obesity evaluation to the Department of Endocrinology, screened between January 2014 and January 2022, over 8 years period. Body weight and height were measured, and BMI was calculated. Obesity is determined using an age- and sex-specific percentile for BMI than the BMI categories used for adults due to the differences in body composition between adults and children. Obesity is defined as a BMI at or above the 95th percentile for children and teens of the same age and sex.

Detailed history including the birth history and clinical examination were done to evaluate the causes of syndromic and nonsyndromic obesity. Fasting and postprandial blood glucose, 75 g oral glucose tolerance tests (GTT), HbA1c, blood urea and serum creatinine, Free T4, thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone levels. Serum growth hormone level was estimated at 0, 60, and 90 min following clonidine stimulation to diagnose coexisting growth hormone deficiency for short stature (Cobas e411 analyzer, Roche Diagnostics, Manheim, Germany). If the testis cannot be palpated in scrotum, then the patients underwent ultrasonogram and/or magnetic resonance imaging (MRI) of pelvis. Overnight dexamethasone suppression test was done when clinical suspicious of Cushing’s syndrome.

Obese children and adolescent with specific clinical features of PWS, like characteristic facies, short stature, hyperphagia, hypotonia, features of hypogonadism like micropenis and cryptorchidism, sleep disturbances, cognitive, and behavior abnormalities were subsequently evaluated with genetic testing by Methylation-specific polymerase chain reaction (MS-PCR) or Fluorescence in situ Hybridization (FISH) methods to confirm the diagnosis of PWS. Intelligent quotient (IQ) of each patient was assessed by psychiatrist using Binet–Kamat test. The entire patients were recruited, and clinical photographs were taken after written informed consent from both their parents. The study was approved by Institutional Ethical Committee.

Results

Our Prader–Willi syndrome case cohorts were predominantly male, except one female, with age ranges from 7 to 16 years, mean age of 12 year. The characteristic facies of PWS such as narrow bifrontal diameter, almond-shaped eyes, short, upturned nose, and downturned corners of the mouth were noted in all cases and are compiled in Figure 1. All were short in stature with height less than the 3rd percentile for their chronological age with significant history of hyperphagia and hypotonia on clinical examination. The features of male hypogonadism in the form of gynecomastia and/or lipomastia, micropenis, and cryptorchidism were noted in all male study population. The external genital examination findings of micropenis, poorly developed scrotal sac, and cryptorchidism are highlighted in Figure 2. The hormonal profiles were suggestive of hypogonadotrophic hypogonadism, and two patients had coexisting growth hormone deficiency. All patients had mild to moderate cognitive dysfunction as assessed by a low IQ score and learning difficulties with poor scholastic performances. Diagnosis of PWS was confirmed by Methylation-specific PCR in seven and by FISH method in remaining one. The clinical findings reported in the study are summarized in Table 1.

One of our cases had perineal hypospadias, underwent urological repair, and seven of them were subjected to orchidopexy. Four of our adolescent PWS had type 2 DM requiring treatment and three had subclinical hypothyroidism on levo-thyroxine supplementation. One of our patients was treated with recombinant growth hormone therapy. Three of adolescent PWS received testosterone injection for the secondary sexual characteristics.

Discussion

PWS is originally described by Andrea Prader and Heinrich Willi in 1956 with estimated incidence of one in 15,000 live births. The diagnosis is primarily clinical, based on the presence of typical clinical features, like characteristic facies, short stature, hyperphagia, obesity, hypotonia, hypogonadism, sleep disturbances, cognitive, and behavior abnormalities. All ethnic groups may be affected in PWS, however, it is disproportionately high in Caucasians. From best of our knowledge, only three published case series are available regarding the PWS among Indian population indicating that despite of high prevalence of this syndrome it is less commonly recognized in India.

In our case series, all were diagnosed during late childhood or adolescent period in spite of all the classical features of PWS. This reflects a need for higher awareness about PWS among the different specialists like Pediatrician, Endocrinologist, Pediatric surgeon, Psychiatrist where PWS may seek advice with different manifestations to anyone of the above specialists. Mean age at diagnosis in the present study was 12 years which is similar to the other published case series in India. However, worldwide, the age of diagnosis has fallen significantly, and the majority of cases are now diagnosed during the first
months of life. PWS may be suspected in newborn when severe hypotonia with poor suck followed by hyperphagia and features of hypogonadism like hypospadias, micropenis and cryptorchidism. This could lead to earlier diagnosis and initiation of treatment, resulting in improved outcome.

All of our patients had hyperphagia with obesity, short stature, developmental delay, cognitive impairment, and hypogonadism that should prompt genetic testing according to newer guidelines and consensus criteria. Two most important differential diagnosis of short stature with obesity are primary hypothyroidism and Cushing’s syndrome. Obese children are relatively taller compared with age- and sex-matched normal weight children. Hence any childhood obesity with short stature need detailed endocrine and hormonal evaluation to assess the cause of obesity. The other most important cause of syndromic obesity is Bardet–Biedl syndrome (BBS), the presence of normal to tall stature, polydactyly and retinitis pigmentosa will differentiate BBS from PWS. Cohen syndrome is rare and manifested as obesity, microcephaly, developmental delay and hypotonia, distinguished from PWS by molecular testing.

Figure 1: The characteristic facies of PWS such as narrow bifrontal diameter, almond-shaped eyes, short, upturned nose, and downturned corners of the mouth

Figure 2: The external genital examination findings of male PWS—Micropenis, poorly developed scrotal sac and cryptorchidism (Sequence of order changed)
Table 1: Clinical characteristics of Prader-Willi Syndrome noted in present Series

| Case | Age & Sex | Hyperphagia | Cryptorchidism | Delayedpuberty/ hypo-gonadism | Mental retardation | T2DM | Molecular Diagnosis |
|------|-----------|-------------|---------------|-------------------------------|-------------------|------|---------------------|
| 1    | 16/M      | Yes         | Bilateral     | Yes                           | Yes               | Yes  | Yes                 | MS PCR |
| 2    | 12/M      | Yes         | Bilateral     | Yes                           | Yes               | No   | No                  | MS PCR |
| 3    | 10/M      | Yes         | Bilateral     | Yes                           | Yes               | No   | No                  | MS PCR |
| 4    | 7/M       | Yes         | Right         | Yes                           | Yes               | No   | No                  | FISH  |
| 5    | 16/M      | Yes         | Bilateral     | Yes                           | Yes               | Yes  | Yes                 | MS PCR |
| 6    | 11/F      | Yes         | Not Applicable| Not Applicable                | Yes               | Yes  | Yes                 | MS PCR |
| 7    | 10/M      | Yes         | Bilateral     | Yes                           | Yes               | No   | No                  | MS PCR |
| 8    | 12/M      | Yes         | Bilateral     | Yes                           | Yes               | Yes  | Yes                 | MS PCR |
| %    | 100       | 100         | 100           | 100                           | 100               | 100  | 100                 |

Three of our patients (37%) had subclinical hypothyroidism. This is similar to reports by previous studies which have shown an association of hypothyroidism with PWS,[15] with a hypothyroidism occurring in about 10% of the patients with PWS.[15,16] Four of our adolescent patients had DM requiring oral antidiabetic agents for glycemic control. As per the previously published literature of PWS, that about 25% of PWS patients develop type 2 DM by mean age of 20 years.[16] This reflects a need for earlier identification and aggressive diet, exercise therapy for weight reduction and insulin sensitizing agents like metformin and pioglitazone. All our male patients had cryptorchidism and micropenis, which is consistent with previous studies worldwide and in India.[6,9]

Well-defined consensus clinical diagnostic criteria were established by Holm et al.[17] as early as the 1993. However, genetic testing has now become mandatory to confirm the diagnosis.[6,13] PWS arises from lack of expression of paternally inherited imprint genes on chromosome 15q11–q13.[6] Definitive diagnosis is by genetic testing, using peripheral blood lymphocytes. Imprinted genes demonstrate differential DNA methylation dependent on parental origin and patients with PWS have a maternal-only imprint with lack of paternal contribution. DNA methylation analysis is the only technique, which can both confirm and reject the diagnosis of PWS and is most commonly done using DNA methylation-specific techniques at the SNURF-SNRPN locus. Methylation-specific multiple ligation PCR amplification (MS—PCR) is now the investigation of choice to confirm PWS.[6] The type of genetic defect correlates with severity of phenotype and can predict risk of recurrence in the family. Paternal deletion of 15q11–q13 is the most common genetic defect seen in 75% of PWS, followed by maternal uniparental disomy (UPD) and imprinting errors in 24% and 1% respectively.[6] Fluorescence in situ hybridization (FISH) analysis, although it requires only a sample from the proband to detect chromosome 15q11–q13 deletions and can detect chromosomal translocations or rearrangements, is less superior to MS—PCR as it detects only 60% of interstitial chromosome deletions. Hence negative FISH does not exclude the diagnosis of PWS. In the present case series except one all PWS were confirmed by MS PCR. The leptin–melanocortin pathway genes such as LEP, LEPR, SH2B1, POMC, PCSK1, MC4R, and MC3R are the miscellaneous genetic causes of monogenic obesity.[13]

Management is best approached by a multidisciplinary team and earlier the intervention, lesser the complications and better the outcome. Dietary therapy during 2–10 years of life can provide effective treatment of obesity that reduces the cardiovascular and respiratory risks. Growth hormone (GH) therapy may overcome slow growth and FDA has approved Prader–Willi syndrome as an indication of GH therapy even without GH levels. GH therapy in PWS improves the body composition, muscle tone and strength, quality of life, and reduces depression.[18] Sleep apnea or respiratory difficulties should be ruled out before initiating GH therapy. Early initiation of behavior modification therapy to control hyperphagia and hence obesity helps to reduce morbidity and mortality.[6,13] PWS are at risk for sudden death due to gastrointestinal, respiratory, or cardiac complications. For management of cryptorchidism, orchiopexy is initiated earlier age group of life, results in better results. Testosterone therapy should be considered in all adolescent male PWS for secondary sexual features development, bone health, improvement in muscle mass, emotional and physical well-being. Similarly, estrogen therapy is recommended for female PWS for initiation of puberty. Early management of cognitive and psychiatric manifestations including skill and vocational training significantly improves quality of life in these patients.

Strengths of our series are that we emphasized about when to suspect PWS clinically in a resource limited settings. All were confirmed with genetic analysis, and we highlighted about the various molecular diagnostic techniques. Limitation of this series is the small number of cases.

**Conclusion**

Prader–Willi syndrome should be kept in mind in all cases of childhood and adolescent obesity with short stature, hypotonia, and cryptorchidism. Judicious use of molecular diagnostic testing should be made in all clinically suspected cases. Early diagnosis and appropriate management of this complex disorder by a multidisciplinary team will improve the quality of life and treatment outcome.
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Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Ethical committee
Approval Letter (Attached).

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Conflicts of interest
There are no conflicts of interest.

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