JCRPE

Journal of Clinical Research in Pediatric Endocrinology

September 2018 | volume 10 | issue 3 | www.jcrpe.org | ISSN: 1308-5727

E-ISSN: 1308-5735

Official Journal of
Turkish Pediatric Endocrinology
and Diabetes Society

galenos yayınevi

JCRPE Journal of Clinical Research in Pediatric Endocrinology
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Printing at:
Özgün Ofset Ticaret Ltd. Şti.
Yeşilce Mah. Aytekin Sok.
No: 21, 34418 4. Levent,
İstanbul-Turkey
Date of printing: August 2018
ISSN: 1308-5727
E-ISSN: 1308-5735

JCRPE Journal of Clinical Research in Pediatric Endocrinology
www.jcrpe.org

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Book Chapters: Darendeliler F. Growth Hormone Treatment in Rare Disorders: The KIGS Experience. In: Ranke MB, Price DA, Reiter EO (eds). Growth Hormone Therapy in Pediatrics: 20 Years of KIGS. Basel, Karger, 2007;213-239.

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REVIEW

DOI: 10.4274/jcrpe.0014

J Clin Res Pediatr Endocrinol 2018;10(3):191-197

Abnormal Uterine Bleeding in Adolescents

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Abstract

Abnormal uterine bleeding (AUB) is the most common gynecologic complaint of adolescents admitted to hospital. Heavy menstrual bleeding (HMB) is the most frequent clinical presentation of AUB. Anovulatory cycles, owing to immature hypothalamic-pituitary-ovarian axis, is the leading etiology of HMB and there is an accompanying bleeding disorder in almost 20% of patients with HMB. Additionally, endocrine disorders such as hypothyroidism, hyperprolactinemia and polycystic ovary syndrome are possible causes of AUB. Exclusion of bleeding disorders, especially of von Willebrand disease is important for diagnosis and treatment of HMB, particularly in cases with AUB, which has been present since menarche. Management of HMB is based on the underlying etiology and severity of the bleeding. After other causes are excluded, anovulatory heavy bleeding can be treated successfully with combined oral contraceptives and iron supplementation either as an outpatient or in hospital depending on the clinical findings and level of anemia. The epidemiology, clinical presentation, diagnostic approach and treatment of HMB is discussed and our clinical experience in this field is presented in this review.
Keywords: Abnormal uterine bleeding, heavy menstrual bleeding, adolescents

Introduction

Abnormal uterine bleeding (AUB) is defined as bleeding from the uterine corpus that is abnormal in duration, volume, frequency and/or regularity. AUB accounts for half of the gynecologic problems among adolescents (1,2). Also, some adolescents maybe unaware that their bleedings patterns are abnormal, as menstrual cycles are known to often be irregular during adolescence. The underlying factors that cause AUB and/or AUB itself may have potential for long term health consequences, decrease life quality and affect school attendance. Evaluation of the menstrual cycle should be an additional vital sign to be looked into in any female adolescent during all routine pediatrician visits (3). Beginning with definition of normal menstrual cycle, evaluation, approach to diagnosis and treatment of AUB will be discussed in this paper.

Normal Menstrual Cycles in Adolescents

Menarche usually occurs between the ages of 12-13 years (4,5). The normal cycle of an adolescent female occurs every 21-45 days with bleeding lasting between two and seven days (6,7,8). The frequency of cycles decreases at higher postmenarchal ages (7). Menstrual cycles are 21-34 days, similar to adults, in 60-80% of adolescents by the third year after menarche (8,9). The average blood loss during a normal menstrual cycle is 30-40 mL, requiring the use of 3-6 pads or tampons per day or 10-15 soaked pads or tampons per cycle (10). More than 50% of the total menstrual loss is an endometrial transudate and 30-50% consists of whole blood components (11). Chronic loss of ≥80 mL blood is associated with anemia (12).

Abnormal Uterine Bleeding

The International Federation of Gynecology and Obstetrics (FIGO) recommends the use of the term AUB to describe any aberration of menstrual volume, regulation, duration and/or frequency in a woman who is not pregnant (Table 1). FIGO also proposes to discard some definitions from accepted terminology, such as "menorrhagia", "metrorrhagia", "hyper/hypomenorrhea", "polymenorrhea" and

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Conflict of interest: None declared
Received: 05.01.2018
Accepted: 17.02.2018

"dysfunctional uterine bleeding" as they are controversial, confusing and poorly defined (13,14).

Heavy menstrual bleeding (HMB) is the most common clinical presentation of AUB. Formerly called "dysfunctional uterine bleeding", refers to AUB which is not caused by structural lesions of the uterus (15).

FIGO defines the etiology of AUB using the PALM-COEIN classification [Polyp, Adenomyosis, Leiomyoma, Malignancy, Hyperplasia (structural causes); Coagulopathy, Ovulatory dysfunction, Endometrial, Iatrogenic and Not yet classified (non-structural causes)] system (1). AUB is very rarely due to structural problems (1.3-1.7%) in adolescents (16,17). Anovulatory cycles, discussed separately below, which may manifest as amenorrhea, oligomenorrhea or HMB owing to immature hypothalamic-pituitary-ovarian axis are the most common cause of AUB among adolescents (18). As another leading etiology, coagulopathy prevalence is reported to vary between 5% and 28% among hospitalized adolescents with HMB in different studies (17,19,20,21,22). In a systematic review gathering data of 988 women (15-55 years) with HMB, the incidence of von Willebrand disease (vWD) was found to be 13% (23). Coagulopathy may also be due to other coagulation factor deficiencies, immune thrombocytopenia, platelet dysfunction, thrombocytopenia secondary to malignancy or due to treatments for malignancy (17,22,24,25). Coagulopathy may be an isolated or accompanying disorder. More than one cause may exacerbate or aggravate AUB. The differential diagnosis of HMB in adolescents is summarized in Table 2 (26).

Table 1. Abnormal uterine bleeding-The International Federation of Gynecology and Obstetrics recommendations for menstrual terminology

Category	Definition
Disorders in regularity	
Irregular menstrual bleeding	Variation >20 days over a period of one year
Absent menstrual bleeding (amenorrhea)	No bleeding in a 90-day period
Disorders in frequency	
Infrequent menstrual bleeding (oligomenorrhea)	One or two episodes in a 90-day period
Frequent menstrual bleeding	More than four episodes in a 90-day period
Disorders in amount of flow	
Heavy menstrual bleeding	Excessive blood loss which interferes with the woman's physical, emotional, social and material quality of life and which can occur alone or with other symptoms
Heavy and prolonged menstrual bleeding	Excessive blood loss exceeding eight days
Light menstrual bleeding	Bleeding less than 5 mL in a period
Disorders of duration of flow	
Prolonged menstrual bleeding	Menstrual periods that exceed eight days on a regular basis
Shortened menstrual bleeding	Menstrual bleeding lasting less than two days

Anovulatory Cycles

Occurrence of ovulatory menstrual cycles require the regular interaction of hypothalamus, hypophysis, ovary and endometrium. Gonadotropin releasing hormone (GnRH) pulses from the hypothalamus induce follicle stimulating hormone (FSH) and luteinizing hormone (LH) secretion from the hypophysis and these gonadotropins induce the development of a dominant follicle from one of the antral follicle candidates for ovulation. LH stimulates the thecal cells to divide and produce androgens. FSH stimulates the granulosa cells to divide and convert androgens to estradiol (E2) and E2 level continue to rise through the follicular phase. When E2 exceeds a critical level (>200 pg/mL for two days) GnRH rises with positive feedback and causes an LH surge. This LH surge activates proteolytic enzymes which leads to follicular rupture and causes luteinization of the granulosa and theca cells, resulting in a marked increase in progesterone production.

E2 induces endometrial epithelial cell proliferation, gland growth and vascularization and production of both E2 and progesterone receptors thus preparing the endometrium to respond to luteal production of progesterone. Progesterone stabilizes the thickening endometrium by influencing the production of key proteins such as matrix metalloproteinase

Table 2. Differential diagnosis of heavy menstrual bleeding in adolescents

Endocrine causes	**Infections**
Anovulatory bleeding	Cervicitis
PCOS	Adenomyosis
Thyroid disease	**Disorders of the uterus**
Other	Myoma
Bleeding disorders	Intrauterine device
von Willebrand disease	Polyps
Platelet dysfunction	Cancer
Thrombocytopenia	**Medications**
Clotting factor deficiency	Depot medroxyprogesterone
Pregnancy	Anticoagulants
Abortion	**Trauma**
Ectopic pregnancy	**Foreign body**
Gestational trophoblastic disease	**Hemorrhagic ovarian cysts**

PCOS: polycystic ovary syndrome

1, 3, and 9 which degrade extravascular and stromal matrix (27). Progesterone also stimulates production of tissue factor and plasminogen activator inhibitor 1, expediting coagulation and clot stabilization (28,29).

Pituitary potential to respond to GnRH stimulation and the positive feed-back effect of E2 progressively improve after menarche (30,31). During the first two postmenarchal years, approximately half of menstrual cycles are anovulatory. However, at five years post-menarche 75% of cycles are ovulatory and this increases further over the next several years, reaching an 80% rate (31). Delayed or absent ovulation, either physiological or due to polycystic ovary syndrome (PCOS), results in lack of progesterone and excessive E2 production from ovarian follicles, causing the endometrium to proliferate and to become prone to unpredictable menstrual bleeding in both timing and amount. For these reasons anovulatory cycles are the leading cause of HMB during adolescence.

Evaluation

The focus of initial evaluation of a patient with HMB is to determine whether the bleeding is acute and causing hemodynamic instability, through careful history taking, physical examination, laboratory testing and radiologic imaging.

History should be taken both with and without the parents being present because some of the questions asked would be difficult for patients to answer candidly in the presence of their parents, especially those relating to sexual activity, while asking with the parents present may help to clarify the details in some cases. History should include; menstrual history (age of menarche, regularity, duration, number of pads/tampons per day), sexual history, past medical history (systemic illness, current/recent medication), systemic review (symptoms associated with systemic causes of HMB such as obesity, PCOS, hypothyroidism, hyperprolactinemia, hypothalamic or adrenal disorder) and family history (coagulopathy, hormone sensitive cancers). A history of heavy menses since menarche, surgery related bleeding, bleeding associated with dental work, bruising or epistaxis with a frequency of at least once per month, frequent gum bleeding and bleeding symptoms in the family point to an underlying bleeding disorder (32).

Once hemodynamic stability is established, vital signs should be checked and systematic physical examination should be completed. Presence of goiter, pallor, bruising, petechiae and/or signs of androgen excess may clarify the underlying diagnosis (33). Pelvic examination with a speculum or transvaginal ultrasonography may not be possible in sexually inexperienced adolescents. It is possible to postpone this exam until a trial of medical therapy has been attempted, as structural lesions in adolescents are very rare. Pelvic ultrasonography provides non-invasive information about genital tract structural lesions, especially in adolescents in whom the physical examination is limited. It also gives additional information about endometrial thickness and PCOS.

Laboratory tests are aimed at determining the severity of the bleeding and to investigate potential etiologies of HMB. The minimum laboratory evaluation should include; human chorionic gonadotropin, complete blood count, peripheral blood smear, ferritin level, prothrombin time, activated partial thromboplastin time and fibrinogen. Adolescents at risk of bleeding disorders should undergo testing for vWD. The von Willebrand panel should include; plasma von Willebrand factor (vWF) antigen and functional tests for vWF and factor VIII activity (32,34). Those with a blood type O will have lower levels of vWF than those who have blood type A or B. So, vWF antigen reference values should be used which are appropriate for each patient's blood type (35). The Committee on Adolescent Health Care of the American College of Obstetricians and Gynecologists recommends obtaining a vWF panel either before or seven days after ceasing exogenous estrogen treatment (36). Estrogen replacement therapy has been shown to increase plasma vWF antigen (37) thus the rationale for the timing of the vWF panel which allows the levels to stabilize in respect to any medical estrogen therapy which may be being used. Additional tests include exclusion of infection in sexually active adolescents and evaluation of thyroid functions in patients with accompanying hypothyroid symptoms. Patients with a significant bleeding history and non-diagnostic initial testing should be referred to a hematologist for further investigation (38).

Treatment

Providing hemodynamic stability, correction of anemia and maintenance of normal cycles constitute the main goals in management of HMB. Treatment options include iron supplementation, combined oral contraceptives (COCs), progesterone, nonsteroidal anti-inflammatory drugs (NSAIDs), antifibrinolytics, desmopressin and GnRH analogues. Management is largely based on severity of the bleeding and anemia (39). If an underlying cause is identified, specific treatment is given additionally. As HMB in adolescents is mostly due to anovulatory cycles, treatment

is focused on anovulatory uterine bleeding. Classification of severity is given in Table 3.

Mild Anovulatory Uterine Bleeding: For girls with mild bleeding with normal hemoglobin, observation is enough, unless they report a negative change in their life quality. NSAIDs, such as ibuprofen and naproxen sodium, may help to decrease flow. If the hemoglobin is 10-12 g/dL, both observation and hormonal therapy are acceptable alternatives, as long as iron supplementation with 60 mg elemental iron per day is given. If hormonal therapy is decided on as the treatment choice, the possible regimens are the same as those for moderate anovulatory uterine bleeding, discussed below in detail. Re-evaluation should be made at three months or sooner if the bleeding persists or becomes more severe.

Moderate Anovulatory Uterine Bleeding: These patients can also be managed on an outpatient basis. In addition to iron supplementation, hormonal therapy is necessary to stabilize endometrial proliferation and shedding. There is no consensus on whether to treat with COCs or progestin-only regimens (40). In adolescents with moderate anemia who are actively bleeding, COCs are a better choice, as estrogen improves hemostasis (39). Monophasic COCs, containing at least 30 mcg of ethinyl E2, are preferred to prevent breakthrough bleeding. We recommend taking one pill every 8-12 hours until the bleeding stops, then to continue with one pill per day for a total of at least 21 days. If bleeding starts again dosing may be increased to twice a day for a total 21 days. 4-8 mg of ondansetron can be given if nausea occurs with high doses of E2 (30). At the end of 21 days, seven days of placebo or pause should be given. COCs treatment is continued for 3-6 months until the hemoglobin level reaches ≥12 g\dL. Different COCs regimens have been suggested in the literature (32,33,41).

Progestin-only hormone therapy can be an alternative to COCs for adolescents with moderate anemia who are not currently bleeding or have a contraindication for estrogen therapy, such as arterial/venous thromboembolic disease, hepatic dysfunction, migraine with aura and/or estrogen dependent tumors (42,43). Progestin-only options are; micronized oral progesterone (200 mg/day), medroxyorigesterone (10 mg/day), norethindrone acetate (2.5-5 mg/day), depot-medroxyprogesterone acetate (DMPA) or a levonorgestrel-releasing intrauterine device. The last two options are not suitable for acute management but can be preferable for those who need contraception or cannot take pills. Micronized oral progesterone contains peanut oil and there must be caution for allergy. Also, there is no sufficient evidence to date to state that it is safer to use this progesterone than using synthetic progestin (44). However, micronized oral progesterone is chemically identical to endogenous progestin and this is more physiological. In some studies, it was also shown to have fewer side effects than synthetic progestin pills (45,46). Oral progestin is given for 12 days every month and bleeding occurs 2-7 days after cessation. If bleeding does not start within one week the patient should be re-evaluated.

Table 3. Severity classification

Mild	Longer menses (> 7 days) or shorter cycles (< 3 weeks) for two months in succession, with slightly or moderately increased bleeding, a usually normal (≥12 g/dL) or mildly decreased (10-12 g/dL) hemoglobin value
Moderate	Moderately prolonged or frequent (every 1-3 weeks) menses, with moderate to heavy bleeding and a hemoglobin level of ≥10 g/dL
Severe	Heavy bleeding with a hemoglobin level of < 10 g/dL

Severe Anovulatory Uterine Bleeding: Patients with hemoglobin levels < 7 g/dL and those with hemoglobin levels < 10 g/dL but who have active heavy bleeding and hemodynamic instability (tachycardia, hypotension, orthostatic vital signs) must be hospitalized. They must be promptly evaluated in case blood transfusion is necessary. Patients with hemoglobin levels of 8-10 g/dL with parents who can reliably be contacted by telephone can be followed on an ambulatory basis (47). All patients with severe anemia due to menstrual bleeding must be assessed for bleeding disorders. Supplementation of 60-120 mg elemental iron must be started as soon as the patient is stable enough to take oral pills.

Hormone therapy recommended for patients with hemoglobin levels of 8-10 g/dL consists of monophasic COCs containing 30-50 mcg ethinyl E2, given once every six hours for 2-4 days, followed by the same dose given every eight hours for three days and then every 12 hours for the next 14 days. Additional anti-emetic treatment may be necessary. For patients with hemoglobin levels < 7 g/dL or < 10 g/dL with heavy bleeding, COCs are given every four hours until bleeding slows down, followed with one pill every six hours for 2-3 days, every eight hours for three days and then every 12 hours for 2 weeks and continue with one pill a day until a hemoglobin level of ≥10 g/dL is reached and at least for a total of 21 days. When the hemoglobin level exceeds 10 g/dL, COCs are used in a cyclic pattern for three to six months until a hemoglobin level of ≥12 g/dL is attained (47).

If bleeding continues heavily after 24-hour administration of COCs or the patient is unable to take oral pills, 25 mg IV conjugated estrogen is given every 4-6 hours up to 2-3 times until the bleeding lessens. Then treatment is continued with oral COCs as described above. Physicians should remember

the increased risk for thromboembolism with this therapy (48). Progestin-only therapies may be an option for patients who have a contraindication for COCs. For acute management, oral progestins are better than either DMPA or a levonorgestrel-releasing uterine device.

There may be a need for hemostatic agents such as tranexamic acid, aminocaproic acid and desmopressin, if bleeding exceeds 24 hours despite high dose COCs or there is a known platelet dysfunction. Tranexamic acid 3.9-4 g/day in three doses for 4-5 days is an effective treatment for HMB and is more effective than placebo (49). Although there is no evidence for increased incidence of thrombotic events associated with tranexamic acid, having a history of or active thromboembolic disease or an intrinsic risk for thrombosis are contraindications for tranexamic acid use. Concomitant usage of COCs increase the risk of thrombosis (49).

If hormonal and hemostatic treatment fail to lessen bleeding in 24-36 hours, examination under anesthesia, endometrial sampling and therapeutic curettage may be necessary (47,50).

Follow-up

If irregular menses or HMB persists under hormone therapy for three months or recurs after cessation of therapy, the patient should be assessed for possible problems of hypothalamic-pituitary-ovarian axis, PCOS and structural causes. Adolescents with a history of untreated anovulatory cycles for 2-3 years should be evaluated by endometrial biopsy, as there is an increased risk for endometrial carcinoma in such patients (32).

Experience of a Single Center

We evaluated the data of 22 patients with HMB referred to our adolescent outpatient clinic within a time period of 18 months. The mean age (range) of the patients was 13.9 years (11.3-16.9 years) and of menarche was 12.2 years (10.5-14.0 years) respectively, which is similar to the mean age of menarche in healthy Turkish girls. Half of the patients had been having heavy bleeding at each menses since menarche. Among those whose HMB began after menarche, the longest period between menarche and initiation of heavy bleeding was 3.3 years. Three of these patients had been given erythrocyte transfusions due to the degree of HMB prior to their admission to our hospital. The severity of bleeding was assessed as mild in five patients, moderate in three and severe in 14 patients. Seven of the severe patients had hemoglobin levels <8 g/dL. None of these patients were found to have platelet dysfunction or structural problems. One patient was diagnosed with hypothyroidism. Pelvic ultrasonography was compatible with PCOS in five patients. vWF antigen and activity was normal in all of the 18 patients who were assessed for vWF abnormalities.

All patients were given oral iron supplementation. Treatment with a monophasic COC containing 0.15 mg desogestrel and 30 mcg ethinyl E2 was initiated in all patients with moderate and severe anemia, in accordance with the guidelines given above, except for two patients who were treated with oral tranexamic acid initially and then switched to COCs because of recurrent bleeding. Among patients with mild anemia, two patients were also treated with COCs, one with a two years history of irregularity predicting a lengthened anovulatory phase and one with endometrial hyperplasia. Two patients with a hemoglobin level of 5.8 g/dL and 5.9 g/dL required erythrocyte transfusions. Treatment with iron supplementation and/or COCs was successful in all patients over the short term (Table 4).

Thus, AUB is one of the major problems of adolescent gynecology and anovulatory HMB is the commonest presentation of AUB. Anovulatory cycles are generally physiologic and resolve spontaneously in most adolescents as the hypothalamic-pituitary-ovarian axis matures. Additionally, HMB may be due to coagulopathy and each

Table 4. Clinical characteristics of our patients with heavy menstrual bleeding and their treatment

Parameter	
Number of patients	22
Mean age at admission (years)	13.9 ± 1.7
Mean age at menarche (years)	12.2 ± 0.9
Timing of HMB n (%)	
Since menarche	11 (50.0)
Initially normal menses	11 (50.0)
Severity of HMB n (%)	
Mild (hemoglobin ≥12 g/dL)	5 (22.7)
Moderate (hemoglobin 10-12 g/dL)	3 (13.6)
Severe (hemoglobin < 10 g/dL)	14 (63.7)
Hyperprolactinemia n (%)	0 (0.0)
Hypothyroidism n (%)	1 (4.5)
Low vWF antigen and/or activity n (%)	0 (0.0)
Treatment n (%)	
Iron supplementation	22 (100.0)
Hormonal therapy with COCs	19 (86.0)
Tranexamic acid	3 (13.6)
Erythrocyte transfusion	2 (9.1)

HBM: heavy menstrual bleeding, COC: combined oral contraceptives, vWF: von Willebrand factor

adolescent with HMB should be questioned for bleeding disorders. Once hemodynamic stability is controlled and provided, the patient must be evaluated for severity of anemia and possible causes of HMB. Severity of anemia and its underlying cause determines the treatment. Beside iron supplementation, COCs, progestin only drugs, intravenous estrogen and\or hemostatic agents can be used for treatment. After treatment is stopped the patient should be followed for persisting anovulation.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Selin Elmaoğulları, Zehra Aycan, Design: Selin Elmaoğulları, Zehra Aycan, Data Collection or Processing: Selin Elmaoğulları, Zehra Aycan, Analysis or Interpretation: Selin Elmaoğulları, Zehra Aycan, Literature Search: Selin Elmaoğulları, Zehra Aycan, Writing: Selin Elmaoğulları.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Munro MG, Critchley HO, Broder MS, Fraser IS; FIGO Working Group on Menstrual Disorders. FIGO classification system (PALM-COEIN) for causes of abnormal uterine bleeding in nongravid women of reproductive age. Int J Gynaecol Obstet 2011;113:3-13. Epub 2011 Feb 22
2. Caufriez A. Menstrual disorders in adolescence: pathophysiology and treatment. Horm Res 1991;36:156-159.
3. ACOG Committee on Adolescent Health Care. ACOG Committee Opinion No. 349, November 2006: Menstruation in girls and adolescents: using the menstrual cycle as a vital sign. Obstet Gynecol 2006;108:1323-1328.
4. Finer LB, Philbin JM. Trends in ages at key reproductive transitions in the United States, 1951-2010. Womens Health Issues 2014;24:271-279. Epub 2014 Apr 12
5. Esen İ, Oğuz B, Serin HM. Menstrual Characteristics of Pubertal Girls: A Questionnaire-Based Study in Turkey. J Clin Res Pediatr Endocrinol 2016;8:192-196. Epub 2015 Dec 18
6. No authors listed. World Health Organization multicenter study on menstrual and ovulatory patterns in adolescent girls. II. Longitudinal study of menstrual patterns in the early postmenarcheal period, duration of bleeding episodes and menstrual cycles. World Health Organization Task Force on Adolescent Reproductive Health. J Adolesc Health Care 1986;7:236-244.
7. Flug D, Largo RH, Prader A. Menstrual patterns in adolescent Swiss girls: a longitudinal study. Ann Hum Biol 1984;11:495-508.
8. Widholm O, Kantero RL. A statistical analysis of the menstrual patterns of 8,000 Finnish girls and their mothers. Acta Obstet Gynecol Scand Suppl 1971;14:(Suppl 14):1-36.
9. Hickey M, Balen A. Menstrual disorders in adolescence: investigation and management. Hum Reprod Update 2003;9:493-504.
10. Bennett AR, Gray SH. What to do when she's bleeding through: the recognition, evaluation, and management of abnormal uterine bleeding in adolescents. Curr Opin Pediatr 2014;26:413-419.
11. Fraser IS, Warner P, Marantos PA. Estimating menstrual blood loss in women with normal and excessive menstrual fluid volume. Obstet Gynecol 2001;98:806-814.
12. No authors listed. ACOG Committee Opinion No. 651: Menstruation in Girls and Adolescents: Using the Menstrual Cycle as a Vital Sign. Obstet Gynecol 2015;126:143-146.
13. Fraser IS, Critchley HO, Broder M, Munro MG. The FIGO recommendations on terminologies and definitions for normal and abnormal uterine bleeding. Semin Reprod Med 2011;29:383-390. Epub 2011 Nov 7
14. Fraser IS, Critchley HO, Munro MG, Broder M. Can we achieve international agreement on terminologies and definitions used to describe abnormalities of menstrual bleeding? Hum Reprod 2007;22:635-643. Epub 2007 Jan 4
15. Khrouf M, Terras K. Diagnosis and Management of Formerly Called "Dysfunctional Uterine Bleeding" According to PALM-COEIN FIGO Classification and the New Guidelines. J Obstet Gynaecol India 2014;64:388-393. Epub 2014 Nov 19
16. Pecchioli Y, Oyewumi L, Allen LM, Kives S. The Utility of Routine Ultrasound in the Diagnosis and Management of Adolescents with Abnormal Uterine Bleeding. J Pediatr Adolesc Gynecol 2017;30:239-242. Epub 2016 Oct 6
17. Claessens EA, Cowell CA. Acute adolescent menorrhagia. Am J Obstet Gynecol 1981;139:277-280.
18. Lemarchand-Beraud T, Zufferey MM, Reymond M, Rey I. Maturation of the hypothalamo-pituitary-ovarian axis in adolescent girls. J Clin Endocrinol Metab 1982;54:241-246.
19. Smith YR, Quint EH, Hertzberg RB. Menorrhagia in adolescents requiring hospitalization. J Pediatr Adolesc Gynecol 1998;11:13-15.
20. Başaran HO, Akgül S, Kanbur NO, Gümrük F, Cetin M, Derman O. Dysfunctional uterine bleeding in adolescent girls and evaluation of their response to treatment. Turk J Pediatr 2013;55:186-189.
21. Oral E, Cağdaş A, Gezer A, Kaleli S, Aydin Y, Oçer F. Hematological abnormalities in adolescent menorrhagia. Arch Gynecol Obstet 2002;266:72-74.
22. Falcone T, Desjardins C, Bourque J, Granger L, Hemmings R, Quiros E. Dysfunctional uterine bleeding in adolescents. J Reprod Med 1994;39:761-764.
23. Shankar M, Lee CA, Sabin CA, Economides DL, Kadir RA. von Willebrand disease in women with menorrhagia: a systematic review. BJOG 2004;111:734-740.
24. Kanbur NO, Derman O, Kutluk T, Gürgey A. Coagulation disorders as the cause of menorrhagia in adolescents. Int J Adolesc Med Health 2004;16:183-185.
25. Díaz R, Dietrich JE, Mahoney D Jr, Yee DL, Srivaths LV. Hemostatic abnormalities in young females with heavy menstrual bleeding. J Pediatr Adolesc Gynecol 2014;27:324-329. Epub 2014 Sep 23
26. Wilkinson JP, Kadir RA. Management of abnormal uterine bleeding in adolescents. J Pediatr Adolesc Gynecol 2010;23(6 Suppl):22-30. Epub 2010 Sep 24
27. Barbieri RL. The endocrinology of the menstrual cycle. Methods Mol Biol 2014;1154:145-169.
28. Liu X, Nie J, Guo SW. Elevated immunoreactivity to tissue factor and its association with dysmenorrhea severity and the amount of menses in adenomyosis. Hum Reprod 2011;26:337-345. Epub 2010 Nov 28
29. Nordengren J, Pilka R, Noskova V, Ehinger A, Domanski H, Andersson C, Høyer-Hansen G, Hansson SR, Casslén B. Differential localization and expression of urokinase plasminogen activator (uPA), its receptor (uPAR), and its inhibitor (PAI-1) mRNA and protein in endometrial tissue during the menstrual cycle. Mol Hum Reprod 2004;10:655-663. Epub 2004 Jul 8

30. Deligeoroglou E, Karountzos V, Creatsas G. Abnormal uterine bleeding and dysfunctional uterine bleeding in pediatric and adolescent gynecology. Gynecol Endocrinol 2013;29:74-78. Epub 2012 Sep 5
31. Metcalf MG, Skidmore DS, Lowry GF, Mackenzie JA. Incidence of ovulation in the years after the menarche. J Endocrinol 1983;97:213-219.
32. American College of Obstetricians and Gynecologists. ACOG committee opinion no. 557: Management of acute abnormal uterine bleeding in nonpregnant reproductive-aged women. Obstet Gynecol 2013;121:891-896.
33. Bradley LD, Gueye NA. The medical management of abnormal uterine bleeding in reproductive-aged women. Am J Obstet Gynecol 2016;214:31-44. Epub 2015 Aug 5
34. Demers C, Derzko C, David M, Douglas J; Society of Obstetricians and Gynaecologists of Canada. Gynaecological and obstetric management of women with inherited bleeding disorders. Int J Gynaecol Obstet 2006;95:75-87.
35. Klarmann D, Eggert C, Geisen C, Becker S, Seifried E, Klingebiel T, Kreuz W. Association of ABO(H) and I blood group system development with von Willebrand factor and Factor VIII plasma levels in children and adolescents. Transfusion 2010;50:1571-1580. Epub 2010 Feb 26
36. Committee on Adolescent Health Care; Committee on Gynecologic Practice. Committee Opinion No.580: von Willebrand disease in women. Obstet Gynecol 2013;122:1368-1373.
37. Rabbani LE, Seminario NA, Sciacca RR, Chen HJ, Giardina EG. Oral conjugated equine estrogen increases plasma von Willebrand factor in postmenopausal women. J Am Coll Cardiol 2002;40:1991-1999.
38. Rajpurkar M, O'Brien SH, Haamid FW, Cooper DL, Gunawardena S, Chitlur M. Heavy Menstrual Bleeding as a Common Presenting Symptom of Rare Platelet Disorders: Illustrative Case Examples. J Pediatr Adolesc Gynecol 2016;29:537-541. Epub 2016 Feb 11
39. Gray SH. Menstrual disorders. Pediatr Rev 2013;34:6-17.
40. Hickey M, Higham JM, Fraser I. Progestogens with or without oestrogen for irregular uterine bleeding associated with anovulation. Cochrane Database Syst Rev 2012:CD001895.
41. Rimsza ME. Dysfunctional uterine bleeding. Pediatr Rev 2002;23:227-233.
42. Santos M, Hendry D, Sangi-Haghpeykar H, Dietrich JE. Retrospective review of norethindrone use in adolescents. J Pediatr Adolesc Gynecol 2014;27:41-44. Epub 2013 Dec 4
43. Cowan BD, Morrison JC. Management of abnormal genital bleeding in girls and women. N Engl J Med 1991;324:1710-1715.
44. Files JA, Ko MG, Pruthi S. Bioidentical hormone therapy. Mayo Clin Proc 2011;86673-680. Epub 2011 Apr 29
45. de Lignieres B. Oral micronized progesterone. Clin Ther 1999;21:41-60.
46. Fournier A, Berrino F, Clavel-Chapelon F. Unequal risks for breast cancer associated with different hormone replacement therapies: results from the E3N cohort study. Breast Cancer Res Treat 2008;107:103-111. Epub 2007 Feb 27
47. Gray SH. Abnormal vaginal bleeding in the adolescent, in Emans, Laufer, Goldstein's Pediatric & Adolescent Gynecology. Emans SJ (ed) Lippincott Williams & Wilkins, Philadelphia, 2012:159.
48. Zreik TG, Odunsi K, Cass I, Olive DL, Sarrel P. A case of fatal pulmonary thromboembolism associated with the use of intravenous estrogen therapy. Fertil Steril 1999;71:373-375.
49. Leminen H, Hurskainen R. Tranexamic acid for the treatment of heavy menstrual bleeding: efficacy and safety. Int J Womens Health 2012;4:413-421. Epub 2012 Aug 17
50. No authors listed. Committee opinion no. 606: Options for prevention and management of heavy menstrual bleeding in adolescent patients undergoing cancer treatment. Obstet Gynecol 2014;124:397-402.

ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.0015

J Clin Res Pediatr Endocrinol 2018;10(3):198-205

Prevalence and Related Factors of Euthyroid Sick Syndrome in Children with Untreated Cancer According to Two Different Criteria

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What is already known on this topic?

Euthyroid sick syndrome is seen in patients with cancer. Euthyroid sick syndrome has been associated with a worse prognosis in cancer patients.

What this study adds?

Up to now, it has not been recognized that children with cancer might have contemporaneous euthyroid sick syndrome at the time of cancer diagnosis. To our knowledge, this is the first study to investigate euthyroid sick syndrome prevalence at the time of diagnosis of a number of different types of pediatric cancers. The prevalence of euthyroid sick syndrome in a range of different cancer types ranged from 11 to 17% depending on the definition of euthyroid sick syndrome used.

Abstract

Objective: In this study, we evaluated the frequency of euthyroid sick syndrome (ESS) among patients with childhood cancer and its association with the stage of disease, nutritional parameters and cytokines levels.
Methods: Eighty newly diagnosed children were included in the study. ESS was assessed in two different ways. According to criteria 1 ESS was present if free triiodothyronine (fT3) was below the lower limit and free thyroxine was within the normal or low limits, thyroid-stimulating hormone (TSH) was in the normal range. According to criteria 2, in addition to the above, it was required that reverse triiodothyronine (rT3) be performed and was higher than normal limits.
Results: Three of our pediatric patients had subclinical hypothyroidism and two had subclinical hyperthyroidism. Out of 75 patients, ESS was identified in 14 (17.3%) according to criteria 1 and in eight (10.6%) according to criteria 2. Only fT3 levels were significantly different in the ESS (+) and ESS (-) groups ($p < 0.05$) according to criteria 1. A significantly negative correlation between interleukin (IL)-6 and fT3 was found, according to both sets of criteria. tumor necrosis factor alpha was negatively correlated with fT3 levels only in the criteria 1 group. There were no correlations between IL-1β and fT3, free thyroxine, rT3 and TSH levels.
Conclusion: ESS may occur in childhood cancer and thyroid function testing should be performed routinely when cancer is diagnosed.
Keywords: Euthyroid sick syndrome, children, cancer, interleukin 6, interleukin 8, tumor necrosis factor alpha

Introduction

Euthyroid sick syndrome (ESS), also known as non-thyroidal illness syndrome or low triiodothyronine (T3) syndrome, is characterized by alterations in the levels of thyroid hormones due to non-thyroidal diseases in the absence of any disorder related to the hypothalamic-hypophysial axis or thyroid gland (1,2). An imbalance between the activities of types I and II deiodinase, decreased sensitivity of the hypothalamus and pituitary gland to thyroid hormones

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Conflict of interest: None declared
Received: 10.01.2018
Accepted: 06.03.2018

and reduced T4 protein binding and cellular uptake have been proposed for the pathogenesis of the syndrome, which is not well understood as yet (3,4). Oxidative stress and increased cytokines such as interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α), are among the factors possibly contributing to the development of the syndrome (5,6).

It has been much debated whether ESS represents a physiological adaptive response to systemic illness or conversely a maladaptive state at the tissue level (3). ESS has been described in liver disease, renal failure, after stress or surgery, in the sick elderly, in malnutrition and in malignancies (7). It is also reported that the presence of ESS is not associated with the type of the underlying disease but instead on its severity (7,8).

There is scant knowledge about cancer and ESS in adult patients (9,10,11,12,13). Mohn et al (14) have investigated ESS prevalence in seven children with Hodgkin disease. However, no research to date has focused on the incidence of ESS in childhood cancer.

In the present study, we aimed to determine the frequency of ESS, to identify its relation with hematological parameters, with body mass index (BMI) and with serum albumin levels. A further aim was to investigate its association with the stage of the disease and the relationship between cytokine levels, IL-6, TNF-α and IL-1β in childhood cancer patients.

Methods

Eighty consecutive patients with histologically diagnosed childhood cancer from three pediatric oncology centers presenting between January 2015 and December 2016 were enrolled in this study. Exclusion criteria were the following: intrinsic thyroid or pituitary-hypothalamic disease, use of special drugs known to affect serum thyroid hormone concentration such as glucocorticoids, amiodorone, β blockers, sucralfate, phenytoin, salicylates and rifampin, and presence of diseases such as secondary malignancy, diabetes mellitus, nephrotic syndrome, chronic hepatic or renal disease and other systemic infectious diseases associated with thyroid function anomalies. The subjects underwent thyroid function tests, nutritional evaluation and staging of the disease.

This study was approved by the Ethics Committee of Mersin University (grant no: 290-2015). Written informed consent was obtained from each patient/patient's family.

Blood samples were obtained between 08.00 and 10.00 am after overnight fasting and the serum samples were stored, frozen at -70 °C, until analysis. Free T3 (fT3), free thyroxine (fT4) and thyroid stimulating hormone (TSH) parameters were measured by electro chemiluminescence immunoassay kits (Modular Cobas 6000, Roche Diagnostics, GmbH, Mannheim, Germany). Serum IL-1β, IL-6, TNF-α and reverse T3 (rT3) was assayed by enzyme-linked immunosorbent assay (ELISA) (DSX Automated ELISA, Dynex Technologies, GmbH, Denkendorf, Germany). Reference ranges are 1.71-3.7 pg/mL for fT3, 0.7-1.48 ng/dL for fT4, and 0.34-5.6 mIU/mL for basal serum TSH. Normative value for rT3 was obtained from the manufacturers as being in the range 2.4-33.6 ng/dL. Patients with normal fT3, fT4 and TSH were considered euthyroid and those who did not have normal fT3, fT4 and TSH were diagnosed as cases of thyroid dysfunction. Thyroid function abnormalities were categorized according to serum TSH and free thyroid hormone levels as follows; i) euthyroidism: normal fT3, fT4 and TSH levels, ii): hypothyroidism: TSH elevation with decreased fT3 and fT4 levels, iii) hyperthyroidism: elevated fT3 and fT4 with suppressed TSH, iv): subclinical hypothyroidism: TSH elevation with normal fT3 and fT4, v): subclinical hyperthyroidism: low TSH with normal fT3 and fT4. Age specific reference values were used to assess fT3, fT4 and TSH levels (15). Thyroid hormone levels were reassessed in patients diagnosed with subclinical hypothyroidism in order to confirm the diagnosis and similar results were obtained.

ESS can be defined in two different ways. In order to be able to determine which of these two different definitions are more effective, we used two different ESS definitions; i): decreased fT3, normal or decreased fT4 and normal TSH [fT3 based definition (criteria 1)] (10,11), ii): decreased fT3, normal or decreased fT4, normal TSH and elevated rT3 [rT3 based definition (criteria 2)] (16).

Nutritional and Biochemical Evaluation

Anthropometric and biochemical measurements were performed to assess the nutritional and biochemical state of the cases. For anthropometric measurement, weight and height were measured and BMI [weight (kg)/ height (m^2)] was calculated. Weight, height and BMI data were expressed as standard deviation scores (SDS) and compared with age- and sex-matched charts for Turkish children (17). Biochemical measurements included albumin and hemoglobin concentrations and leukocyte and platelet count. Serum albumin levels were measured using Abbot-labeled kits (catalog no: 30-3050/R2) in a Beckman Coulter-Synchron LX-20 chemistry auto analyzer device in the biochemistry laboratory of our hospital.

Tumor Staging

Cases with cancer were staged according to their diagnosis. Hodgkin disease was staged according to the Ann–Arbor classification system (18). For non-Hodgkin lymphoma, the Murphy classification was used (19). International Neuroblastoma Staging System was used for patients with neuroblastoma (20). Patients with hepatoblastoma and hepatocellular cancer were staged using the PRETEXT system of the International Society of Pediatric Oncology (SIOP) (21). Wilms tumor was staged using the SIOP 2001 clinical staging system (22). Ewing sarcoma and osteosarcoma were staged according to the Enneking system (23). Patients with retinoblatoma were staged according to the international retinoblastoma staging system (24). Patients with nasopharyngeal carcinoma were staged by using the Tumor Node Metastasis system proposed by the "American Joint Committee on Cancer" (25).

Treatment Response

The response criteria were defined as follows:

Complete response: Disappearance of all clinical and radiological evidence of disease.

Very good partial response: Primary mass reduced by more than 90%, no evidence of distant disease.

Partial response: Reduction of at least 50% of tumor size without evidence of new lesions.

Stable disease: Decrease of tumor size less than 50% or increase of tumor size less than 25%.

Progressive disease: More than 25% increase in any tumor size and/or appearance of new lesions.

Statistical Analysis

The data were analyzed using the MedCalc Packet program. Normality of the data was checked by using the Shapiro-Wilk test. The data were evaluated using descriptive statistical methods (mean ± standard deviation, median, frequencies, and percentages). For intergroup comparison of categorical variables, a chi-square test was used. Comparisons between groups were done by Mann-Whitney U test or independent sample t test where appropriate. Spearman's and Pearson correlation coefficient was used to assess relationships between thyroid hormone levels and IL-1, IL-6 and TNF-α. Significant differences (two-tailed p values) of <0.05 were regarded as significant.

Results

A total of 80 children (47 boys, 33 girls) with a mean age of 7.07 ± 5.04 (range: 2 months-16 years) were included in the study. The patients had different types of childhood cancers of different stages. Patient characteristics and laboratory findings of the study group are shown in Table 1. No patient had an elevation in thyroid autoantibody levels (anti-thyroid peroxidase and anti-thyroglobulin antibodies) and all patients had normal thyroid ultrasonography findings. In this series, three children were diagnosed as subclinical hypothyroidism and two as subclinical hyperthyroidisms. Thyroid hormone levels of children with subclinical hypothyroidism and subclinical hyperthyroidism are presented in Table 2. The remaining 75 children were clinically euthyroid. In the total group, fT3 (pg/mL), fT4 (ng/dL), TSH (mIU/mL) and rT3 (ng/dL) levels were 2.81 ± 1.10, 1.24 ± 0.35, 3.13 ± 1.83 and 32.72 ± 11.97, respectively. Out

Table 1. Dermographic and laboratory features of the patients

Age [years, mean ± SD (range)]	7.07 ± 5.04 (2 months-17 years)
Gender [n, (%)]	
Male	47 (58.8%)
Female	33 (41.2%)
Primary disease [n, (%)]	
Non-Hodgkin lymphoma	19 (23.8%)
Neuroblastoma	12 (15%)
Hodgkin disease	11 (13.8%)
Wilms tumor	9 (11.3%)
Hepatoblastoma	9 (11.3%)
Osteosarcoma	6 (7.5%)
Ewing sarcoma	5 (6.2%)
Nasopharyngeal carcinoma	4 (5.0%)
Rhabdomyosarcoma	3 (3.7%)
Retinoblastoma	1 (1.2%)
Hepatocellular carcinoma	1 (1.2%)
Stage [n, (%)]	
I	8 (10.0%)
II	37 (46.3%)
III	25 (31.2%)
IV	10 (12.5%)
Height (cm)	113.97 ± 32.79
Height SDS	0.32 ± 0.81
Weight (kg)	24.17 ± 15.51
Weight SDS	-0.06 ± 1.09
Body mass index (kg/m$^{2)}$	17.16 ± 4.35
Body mass index SDS	0.41 ± 1.41
Hemoglobin level (g/dL, mean ± SD)	10.27 ± 1.63
White blood cell count (x10^9/L, mean ± SD)	8.74 ± 5.15
Platelet count (x10^9/L, mean ± SD)	283.76 ± 153.77
Albumin (g/dL)	3.53 ± 0.78

SD: standard deviation, SDS: standard deviation score

of 75 patients, ESS was identified in 14 (17.3%) according to criteria 1 and in eight (10.6%) according to criteria 2. It was found that there was no statistically difference between fT4 and TSH levels according to criteria 1 ($p > 0.05$) but fT3 level was a statistically lower in the ESS (+) group according to criteria 1 ($p < 0.05$) (Table 3). Also, there were no statistically significant differences in serum albumin and hemoglobin concentrations and in the white blood cell and platelet counts of patients with or without ESS in either group (Table 4). At the same time, there was no statistical difference in patients with or without ESS in terms of sex, stage of disease, weight, weight SDS, height, height SDS, BMI, and BMI SDS when comparing according to criteria (Table 4).

Thyroid hormone levels of nine out of 14 patients who were diagnosed as ESS by criteria 1 reverted to normal values after induction therapy. In five patients, recurrent/refractory disease was detected at follow-up. One of these patients was refractory Hodgkin's lymphoma and was evaluated as progressive disease after two cycles of treatment. The others were relapsed neuroblastoma, Ewing sarcoma, osteosarcoma and rhabdomyosarcoma, respectively. These patients were found to respond partially to treatment when assessed after induction therapy. Patients without ESS were found to have complete or very good partial response after induction therapy according to criteria 1. The only relapsed case was one patient without ESS, and this patient was followed up with a diagnosis of neuroblastoma. Seven of eight ESS patients identified according to criteria 2 had normal thyroid hormone levels after induction therapy but only one patient with neuroblastoma was diagnosed as ESS according to criteria 2 and did not have a normal level of thyroid hormone after induction therapy and had recurrent disease at follow-up. IL-6, IL-1β and TNF-α levels for each criteria are shown in Table 5. We observed a significant negative correlation, using Pearson' correlation coefficient between IL-6 and fT3 ($r = -0.733$, $p = 0.003$) in patients with ESS according to criteria 1. There was a similar correlation between IL-6 and fT3 ($r = -0.836$, $p = 0.01$) in patients with ESS according to criteria 2. Likewise, TNF-α was negatively and significantly correlated with lowered fT3 levels according to criteria 1 ($r = -0.744$, $p = 0.002$) but no significant correlation was found according to criteria 2 ($r = -0.195$ and $p = 0.64$). Also, there were no correlations between IL-1β and fT3, fT4, rT3 and TSH levels.

Discussion

ESS can be described as abnormal thyroid function test results that occur in the setting of a non-thyroidal illness and in the absence of pre-existing hypothalamo-pituitary and thyroid gland dysfunction. ESS may be regarded as an acute phase response of the organism that serves as one of the major mechanisms to restore homeostasis in severe illness. The most typical alterations are low T3, low or normal T4, or elevated rT3 in the presence of normal TSH levels. However,

Table 2. Thyroid function in patients with subclinical hypothyroidism and subclinical hyperthyroidism

Patient	Tumor type	Diagnosis	fT3 (pg/mL)	fT4 (ng/dL)	TSH (mIU/mL)
1	Neuroblastoma	Subclinical hypothyroidism	2.31	1.42	12.3
2	Hepatoblastoma	Subclinical hypothyroidism	1.83	0.90	14.1
3	Non-Hodgkin lymphoma	Subclinical hypothyroidism	3.10	1.21	10.6
4	Ewing sarcoma	Subclinical hyperthyroidism	1.98	1.3	0.12
5	Non-Hodgkin lymphoma	Subclinical hyperthyroidism	2.43	0.96	0.18

fT3: free triiodothyronine, fT4: free thyroxine, TSH: thyroid stimulating hormone

Table 3. Thyroid function in children according to groups

Criteria	fT3 (pg/mL)	fT4 (ng/dL)	TSH (mIU/mL)	rT3 (ng/dL)
Criteria 1				
ESS (+)	1.11 ± 0.28	1.11 ± 0.25	3.06 ± 1.38	38.29 ± 19.52
ESS (-)	3.15 ± 0.88[a]	1.26 ± 0.36	3.15 ± 1.92	31.53 ± 9.47
Criteria 2				
ESS (+)	1.25 ± 0.19	1.12 ± 0.24	2.81 ± 1.22	49.55 ± 18.58
ESS (-)	2.69 ± 1.04[a]	1.25 ± 0.36	3.17 ± 1.89	30.85 ± 9.48[b]

With euthyroid sick syndrome versus without euthyroid sick syndrome; [a]$p < 0.01$, [b]$p < 0.001$.

Values are expressed as mean ± standard deviation, independent samples t-test was used for free triiodothyronine, free thyroxine, thyroid stimulating hormone and reverse triiodothyronine. Criteria 1: free triiodothyronine-based euthyroid sick syndrome, Criteria 2: reverse triiodothyronine-based euthyroid sick syndrome.

ESS: euthyroid sick syndrome, fT3: free triiodothyronine, fT4: free thyroxine, TSH: thyroid stimulating hormone, rT3: reverse triiodothyronine

it has been noted that fT3-based definitions are used for ESS diagnosis rather than rT3-based definitions (16). While evaluating an entity about which there is no consensus on identification and even given name (ESS or non-thyroidal illness), we decided to assess two different diagnostic criteria that were present in our study. In the studies, the presence of ESS was reported as an adverse factor to prognosis in different diseases. There is no consensus on whether this disease should be treated. For this reason, we wanted to determine the differences between the two diagnostic criteria in the diagnosis, follow-up, and prognosis of the underlying disease, and which diagnostic criteria would be more appropriate for children.

ESS has been investigated in critically ill patients and also in adult cancer patients, especially those with lung cancer. Cengiz et al (13) found that 35% of patients had ESS in non-small cell lung cancer. Yasar et al (10) investigated 120 lung cancer patients and ESS was identified in 30 (42 %) of 71 non-small cell lung cancer patients and 22 (44 %) of 49 small-cell lung cancer patients. Tellini et al (26) investigated thyroid hormone levels in 220 cases with malignancy in different organs and found ESS in 58% of the patients. Gao et al (9) reported the incidence of ESS in 188 cases with diffuse large B cell lymphoma as 12.8%. ESS ratios throughout these studies were determined at the time of diagnosis. Knowledge pertaining to ESS in childhood cancer is scarce. To our knowledge, the frequency of ESS in

Table 4. Comparison of demographic and laboratory features of patients by two ESS criteria[a]

Criteria	Criteria 1		Criteria 2	
	ESS (+)	ESS (-)	ESS (+)	ESS (-)
Gender [n, (%)]				
Male	8	34	5	37
Female	6	27	3	30
Stage				
I	3	5	2	6
II	5	29	3	30
III	5	20	1	23
IV	1	7	2	8
Weight (kg)	20.84 ± 13.12	24.87 ± 15.97	20.65 ± 12.01	24.55 ± 16.38
Weight SDS	-0.14 ± 1.16	-0.05 ± 1.08	-0.18 ± 1.3	-0.04 ± 1.11
Height (cm)	107.38 ± 26.05	115.35 ± 34.06	108.36 ± 24.98	115.64 ± 33.96
Height SDS	0.11 ± 1.23	0.34 ± 0.78	0.47 ± 1.23	0.28 ± 0.84
BMI (kg/m^2)	16.61 ± 1.98	17.28 ± 4.7	16.01 ± 1.95	17.28 ± 4.61
BMI SDS	0.50 ± 1.40	0.42 ± 1.38	0.43 ± 1.10	0.45 ± 1.12
Serum albumin (g/dL)	3.6 ± 0.62	3.51 ± 0.81	3.72 ± 0.68	3.49 ± 0.81
Hemoglobin (g/dL)	10.34 ± 1.75	10.25 ± 1.62	10.05 ± 1.68	10.32 ± 1.61
White blood cell count (x10^9/L)	8.78 ± 4.57	8.72 ± 5.29	7.78 ± 2.73	8.86 ± 5.39
Platelet count (x10^9/L)	254.38 ± 154.16	289.92 ± 154.22	244.00 ± 162.67	89.18 ± 153.01

[a]: for all parameters $p > 0.05$, ESS: euthyroid sick syndrome, SDS: standard deviation score, BMI: body mass index

Table 5. Serum IL-6, IL-1β and TNF-α levels in patients with or without ESS according to groups

	IL-6 (pg/mL)	IL-1β (pg/mL)	TNF-α (pg/mL)
Criteria 1			
ESS (+)	15.34 ± 10.15[a]	9.40 ± 1.13	108.74 ± 56.53[a]
ESS (-)	7.21 ± 6.91	9.11 ± 2.06	26.80 ± 41.91
Criteria 2			
ESS (+)	15.14 ± 7.219.29	9.10 ± 0.62	91.38 ± 39.02[a]
ESS (-)	7.91 ± 7.77[a]	9.17 ± 2.02	35.55 ± 53.10

With euthyroid sick syndrome versus without euthyroid sick syndrome; [a]$p < 0.0001$.

Values are expressed as mean ± standard deviation, independent samples t-test was used for interleukin-6, interleukin-1β and tumor necrosis factor alpha. Criteria 1: free triiodothyronine-based euthyroid sick syndrome; Criteria 2: reverse triiodothyronine-based euthyroid sick syndrome.

ESS: euthyroid sick syndrome, IL: interleukin, TNF-α: tumor necrosis factor alpha

childhood cancers has only been investigated in one study on one type of cancer. Mohn et al (14) investigated ESS prevalence in a small group of seven children with Hodgkin disease and found that five had ESS at the time of diagnosis giving an incidence of 71.4%. ESS was identified in 17.3% of the cases in this study according to criteria 1 and 12% of cases according to criteria 2. Our findings on incidence of ESS was lower than those reported by Tellini et al (26), Cengiz et al (13) and Yasar et al (10) but comparable to results reported by Gao et al (9). While our results indicate a much smaller incidence than was found by Mohn et al (14), this may be due to differences in type of childhood cancer or small group sizes in both our and Mohn's studies.

Several mechanisms are responsible for ESS. The most important of these is the inhibition of the 5′-deiodinating process in peripheral conversion of T4 to T3 (27,28). ESS is thought to be a result of impaired or decreased peripheral conversion of T4 to T3. However, either an increased conversion of T4 to rT3, and/or a decrease in the ability to degrade rT3 could result in ESS. Since the formation of T3 from T4 and the degradation of rT3 both require 5′-deiodinase, an impairment in the function of this enzyme would result in a decreased ability to form T3 and a reduced ability to further deiodinate rT3 (28). The answer to the questions of what is responsible for low T3 syndrome, whether low T3 syndrome constitutes an adaptive, and thus beneficial response, or whether it aggravates a patient's condition, is still a matter of debate.

ESS has been reported in a limited number of studies in adult cancer patients and has been considered as an independent predictor of poor prognosis (9,10,13). As the stage of disease increased, ESS was diagnosed more frequently in patients with cancer (9,10,13). In this present study, we saw that thyroid hormone levels did not return to normal value after induction therapy in five patients with ESS who were diagnosed according to fT3 values (criteria 1) and these patients had resistant/relapsed disease. These data show that the event free survival of patients with ESS is worse than non-ESS patients. In addition, our results suggest that patients who did not show a reversion to normal thyroid hormone levels after induction therapy are prone to have relapsed disease. In our opinion patients who are diagnosed as ESS and who do not have normal thyroid hormone levels after induction therapy should be monitored more closely. We cannot comment on the effects of ESS on overall survival because of the short follow-up time in our study.

With only a few exceptions, notably uremia and human immunodeficiency virus (HIV) infection and the closely associated acquired immunodeficiency syndrome (AIDS), serum rT3 concentrations are elevated in ESS (29,30,31). This is thought to be due to a decrease in the conversion of T4 to T3 and rT3 in the catabolic process. We found that the rT3-based ESS was less frequent in our study compared to the group in which ESS was fT3-based. We think that this may be due to the catabolic state of patients diagnosed with cancer. Measurements of TNF levels vary in patients with AIDS and they often have low levels of TNF (32,33). It is thought that this may be related to low rT3 levels in ESS in HIV-positive patients. In our study, we found that TNF-α levels did not correlate with T3 levels in patients with ESS according to criteria 2 (rT3-based ESS). This result may suggest that a low TNF-α level prevents elevation, as in ESS in HIV positive patients. Also, in our study, more relapsing cases were observed in the ESS group based on fT3. These results suggest that fT3-based ESS may be more clinically helpful, as our study and other studies indicate.

Loss of appetite, increased catabolism, feeding disorders and nutritional insufficiencies are thought to be responsible for the development of ESS in cancer patients (9,10,13). Schulte et al (34), in their study on ESS incidence among bone marrow transplant patients, found that ESS cases had lower BMI and serum albumin levels. Tellini et al (26) reported a correlation between ESS and albumin level and degree of weight loss in oncology patients. Cengiz et al (13) showed significant correlations between ESS and nutritional parameters including BMI and serum albumin level. Gao et al (9) showed reduced albumin levels significantly correlated with low T3 syndrome. In contrast to these findings Dişel et al (12) reported that there was no significant difference in weight loss and BMI score between their groups. Reliance on weight measurement can be misleading because of the potential for large tumor mass (>10% body weight) in children with solid tumors (35). This can lead to misinterpretation of weight-based measurements, such as weight for age, weight for height and BMI. In our study, most patients had an intra-abdominal mass, so we could not demonstrate any significant difference in BMI between patients with ESS and those without ESS.

The etiology of ESS is multifactorial. Cytokines, especially pro-inflammatory cytokines such as IL-6, TNF-α and IL-1β, have been suggested as putative mediators of ESS (36,37). It has been shown that these cytokines inhibit the 5'-deiodinase enzyme responsible for the conversion of T4 into T3 in peripheral tissues (36). Investigation of the effects of administering TNF-α and IL-1β to experimental animals and humans confirmed a possible role in the pathogenesis of ESS, with each cytokine inducing critical illness and inducing low serum T3 (36,37,38). Both cytokines also induce IL-6 production. IL-6 is known to exert regulatory

effects upon many endocrine systems, either independently, or acting with other cytokines (36,37,38). Acute decreases in T3 and TSH after IL-6 administration have been demonstrated (39). During prolonged administration of IL-6, these effects seemed to be transient. These findings show that IL-6 may, at least in part, mediate the development of ESS, whereas factors other than IL-6 contribute to the persistence of changes in thyroid hormone levels during the chronic phase. TNF-α may also play an important role in pathogenesis of ESS. Administration of recombinant TNF-α to healthy individuals was reported to reproduce thyroid hormone profile resembling ESS (37).

In the present study serum IL-6 and TNF-α levels were higher in patients with ESS. Our results thus support their possible role as endocrine cytokines with a regulatory effect on the thyroid gland. IL-6 and TNF-α levels negatively correlated with fT3 concentration in criteria 1 patients with ESS (fT3 based). However, there was no correlation between TSH and IL-6 and TNF-α. This was not surprising since TSH was maintained within the reference values. Boelen et al (40) measured IL-6 and soluble cytokine receptors for IL-1 and TNF-α in patients with ESS and noted a significant negative correlation between these and circulating T3 levels. Our results corroborate this finding. According to our results, these cytokines may have a role in the onset of ESS in childhood cancer. van der Poll et al (41) showed, even though injection of endotoxin in 18 healthy humans mimicked the thyroid hormone profile of ESS, that the co-infusion of an IL-1 receptor antagonist did not affect the endotoxin-induced changes. This result showed IL-1 may not contribute to the development of ESS. We could not find any correlation between IL-1β and thyroid hormone levels in ESS groups in our study. We therefore suggest that there is no contribution of IL-1β to the development of ESS.

Study Limitations

There are some limitations of our study that should be noted. The study had a cross-sectional design and had a small sample size. Also, diagnostic heterogeneity makes it difficult to make comparisons between studies and to generalize about the results of our study. Also, we used BMI and serum albumin levels to investigate nutritional status and these have limitations as noted previously. Despite these limitations, to our knowledge this is the first report of ESS in a range of childhood cancers.

Conclusion

ESS can be present at diagnosis in a range of childhood cancers and thyroid functions should be assessed routinely in pediatric cancer patients at diagnosis. Further studies with larger sample size are needed to confirm the incidence of ESS and the prognostic contribution in childhood cancer.

Acknowledgment

The statistical analysis was performed by Professor Bahar Taşdelen (Mersin University Faculty of Medicine, Department of Biostatistics).

Ethics

Ethics Committee Approval: This study was approved by the Mersin University Faculty of Medicine Ethics Committee, (grant no: 290-2015).

Informed Consent: Written informed consent was obtained from all individuals above 12 years of age and from the parents of all individuals below 12 years of age.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Elvan Çağlar Çıtak, Gülçin Eskendari, Kerem Sezer, Serhan Küpeli, Begül Yağcı Küpeli, Design: Elvan Çağlar Çıtak, Serhan Küpeli, Begül Yağcı Küpeli, Data Collection and Processing: Ali Duyu, Erdem Ak, Begül Yağcı Küpeli, Serhan Küpeli, İbrahim Bayram, Gülay Sezgin, Analysis and Interpretation: Elvan Çağlar Çıtak, Kerem Sezer, Literature Research: Elvan Çağlar Çıtak, Erdem Ak, Ali Duyu, Writing: Elvan Çağlar Çıtak, Kerem Sezer, Gülçin Eskendari.

Financial Disclosure: This project was supported by Mersin University Department of Scientific Research Projects (project No: 2016-1-TP3-1432).

References

1. Wartofsky L, Burman KD. Alteration in thyroid function in patients with systemic illness: The "euthyroid sick syndrome". Endocr Rev 1982;3:164-217.
2. Marks SD. Nonthyroidal illness syndrome in children. Endocrine 2009;36:355-367. Epub 2009 Sep 25
3. De Groot LJ. Non-thyroidal illness syndrome is a manifestation of hypothalamicpituitary dysfunction, and in view of current evidence, should be treated with appropriate replacement therapies. Crit Care Clin 2006;22:57-86.
4. Yasar Z, Kirakli C, Cimen P, Ucar ZZ, Talay F, Tibet G. Is non-thyroidal illness syndrome a predictor for prolonged weaning in intubated chronic obstructive pulmonary disease patients? Int J Clin Exp Med 2015;8:10114-10121.
5. Xu G, Yan W, Li J. An update for the controversies and hypotheses of regulating nonthyroidal illness syndrome in chronic kidney diseases. Clin Exp Nephrol 2014;18:837-843. Epub 2014 Apr 22
6. de Vries EM, Fliers E, Boelen A. The molecular basis of the non-thyroidal illness syndrome. J Endocrinol 2015;225:67-81. Epub 2015 May 13
7. Zargar AH, Ganie MA, Masoodi SR, Laway BA, Bashir MI, Wani AI, Salahuddin M. Prevalence and pattern of sick euthyroid syndrome in

acute and chronic non-thyroidal illness. Its relationship with severity and outcome of the disorder. J Assoc Physicians India 2004;52:27-31.

8. Maldonaldo LS, Murata GH, Hersheman JM, Braunstein GD. Do thyroid function tests independently predict survival in the critically ill? Thyroid 1992;2:119-123.
9. Gao R, Liang JH, Wang L, Zhu HY, Wu W, Wu JZ, Xia Y, Cao L, Fan L, Yang T, Li JY, Xu W. Low T3 syndrome is a strong prognostic predictor in diffuse large B cell lymphoma. Br J Haematol 2017;177:95-105. Epub 2017 Feb 1
10. Yasar ZA, Kirakli C, Yilmaz U, Ucar ZZ, Talay F. Can non-thyroid illness syndrome predict mortality in lung cancer patients? A prospective cohort study. Horm Cancer 2014;5:240-246. Epub 2014 May 16
11. Huang J, Jin L, Ji G, Xing L, Xu C, Xiong X, Li H, Wu K, Ren G, Kong L. Implication from thyroid function decreasing during chemotherapy in breast cancer patients: chemosensitization role of triiodothyronine. BMC Cancer 2013;13:334.
12. Dişel U, Beşen A, Karadeniz C, Mertsoylu H, Sezer A, Köse F, TanerSümbül A, Gürkut O, Muallaoğlu S, Abali H, Ozyilkan O. Prevalence of thyroid dysfunction in untreated cancer patients: a cross-sectional study. Med Oncol 2012;29:3608-3613. Epub 2012 May 22
13. Cengiz SE, Cetinkaya E, Altin S, Gunluoglu Z, Demir A, Gunluoglu G, Epozturk K. Nutritional and prognostic significance of sick euthyroid syndrome in non-small cell lung cancer patients. Intern Med 2008;47:211-216. Epub 2008 Feb 15
14. Mohn A, Di Marzio A, Cerruto M, Angrilli F, Fioritoni C, Chiarelli F. Euthyroid sick syndrome in children with Hodgkin disease. Pediatr Hematol Oncol 2001;18:211-215
15. Cinaz P, Darendeliler F, Akinci A, Ozkan B, Dundar BN, Abaci A, Akcay T. Temel Çocuk Endokrinolojisi. Nobel Tıp Kitapevleri, 2013.
16. Van den Berghe G. Non-thyroidal illness in the ICU: a syndrome with different faces. Thyroid 2014;24:1456-1465. Epub 2014 Jun 19
17. Neyzi O, Bundak R, Gökçay G, Günöz H, Furman A, Darendeliler F, Baş F. Reference Values for Weight, Height, Head Circumference, and Body Mass Index in Turkish Children. J Clin Res Pediatr Endocrinol 2015;7:280-293.
18. Carbone PP, Kaplan HS, Musshoff K, Smithers DW, Tubiana M. Report of the committee on Hodgkin's disease staging classification. Cancer Res 1971;31:1860-1861.
19. Murphy SB, Fairclough DL, Hutchinson RE, Berard CW. Non-Hodgkin's lymphoma of childhood: an analysis of the histology, staging and response to treatment of 388 cases at single institution. J Clin Oncol 1989;7:186-193.
20. Brodeur GM, Pritchard J, Berthold F, Carlsen NL, Castel V, Castelberry RP, De Bernardi B, Evans AE, Favrot M, Hedborg F, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. J Clin Oncol 1993;11:1466-1477.
21. Aronson DC, Schnater JM, Staalman CR, Weverling GJ, Plaschkes J, Perilongo G, Brown J, Phillips A, Otte JB, Czauderna P, MacKinlay G, Vos A. Predictive value of the pretreatment extent of disease system in hepatoblastoma: results from the International Society of Pediatric Oncology Liver Tumor Study Group SIOPEL-1 study. J Clin Oncol 2005;23:1245-1252.
22. Godzinski J. The current status of treatment of Wilm's tumor as per the SIOP trails. J Indian Assoc Pediatr Surg 2015;20:16-20.
23. Enneking WF, Spanier SS, Goodman MA. A system for the surgical staging of musculoskeletal sarcoma. Clin Orthop Relat Res 1980;153:106-120.
24. Chantada G, Doz F, Antoneli CB, Grundy R, Clare Stannard FF, Dunkel IJ, Grabowski E, Leal-Leal C, Rodríguez-Galindo C, Schvartzman E, Popovic MB, Kremens B, Meadows AT, Zucker JM. A proposal for an international retinoblastoma staging system. Pediatr Blood Cancer 2006;47:801-805.
25. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A. AJCC cancer staging manual (7th ed). New York, NY: Springer, 2010.
26. Tellini U, Pellizzari L, Pravadelli B. Thyroid function in elderly with neoplasms. Minerva Med 1999;90:111-121.
27. Bello G, Ceaichisciuc I, Silva S, Antonelli M. The role of thyroid dysfunction in the critically ill:a review of the literature. Minerva Anestesiol 2010;76:919-928. Epub 2010 Oct 8
28. Farwell AP. Nonthyroidal illness syndrome. Curr Opin Endocrinol Diabetes Obes 2013;20:478-484.
29. Hommes MJ, Romijn JA, Endert E, Adriaanse R, Brabant G, Eeftinck Schattenkerk JK, Wiersinga WM, Sauerwein HP. Hypothyroid-like regulation of the pituitary-thyroid axis in stable human immunodeficiency virus infection. Metabolism 1993;42:556-561.
30. Parsa AA, Bhangoo A. HIV and thyroid dysfunction. Rev Endocr Metab Disord 2013;14:127-131.
31. Moura Neto A, Zantut-Wittmann DE. Abnormalities of Thyroid Hormone Metabolism during Systemic Illness: The Low T3 Syndrome in Different Clinical Settings. Int J Endocrinol 2016;2016:2157583. Epub 2016 Oct 10
32. Hommes MJ, Romijn JA, Godfried MH, Schattenkerk JK, Buurman WA, Endert E, Sauerwein HP. Increased resting energy expenditure in human immunodeficiency virus-infected men. Metabolism 1990;39:1186-1190.
33. Grunfeld C, Pang M, Doerrler W, Shigenaga JK, Jensen P, Feingold KR. Lipids, lipoproteins, triglyceride clearance, and cytokines in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. J Clin Endocrinol Metab 1992;74:1045-1052.
34. Schulte C, ReinhardtW, Beelen D, Mann K, Schaefer U. Low T3-syndrome and nutritional status as prognostic factors in patients undergoing bone marrow transplantation. Bone Marrow Transplant 1998;22:1171-1178.
35. Oğuz A, Karadeniz C, Pelit M, Hasanoğlu A. Arm anthropometry in evaluation of malnutrition in children with cancer. Pediatr Hematol Oncol 1999;16:35-41.
36. Papanicolaou DA. Euthyroid Sick Syndrome and the role of cytokines. Rev Endocr Metab Disord 2000;1:43-48.
37. Pappa TA, Vagenakis AG, Alevizaki M. The nonthyroidal illness syndrome in the non-critically ill patient. Eur J Clin Invest 2011;41:212-220. Epub 2010 Oct 21
38. Rasmussen AK. Cytokine actions on the thyroid gland. Dan Med Bull 2000;47:94-114.
39. Stouthard JM, van der Poll T, Endert E, Bakker PJ, Veenhof CHN, Sauerwein HP, Romijn JA. Effects of acute and chronic interleukin-6 administration on thyroid hormone metabolism in humans. J Clin Endocrinol Metab 1994;79:1342-1346.
40. Boelen A, Kwakkel J, Alkemade A, Renckens R, Kaptein E, Kuiper G, Wiersinga WM, Visser TJ. Induction of type 3 deiodinase activity in inflammatory cells of mice with chronic local inflammation. Endocrinology 2005;146:5128-5134. Epub 2005 Sep 8
41. van der Poll T, van Zee KJ, Endert E, Coyle SM, Stiles DM, Pribble JP, Catalano MA, Moldawer LL, Lowry SF. Interleukin-1 receptor blockade does not affect endotoxin-induced changes in plasma thyroid hormone and thyrotropin concentrations in man. J Clin Endocrinol Metab 1995;80:1341-1346.

ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.0032

J Clin Res Pediatr Endocrinol 2018;10(3):206-215

A Rare Cause of Congenital Adrenal Hyperplasia: Clinical and Genetic Findings and Follow-up Characteristics of Six Patients with 17-Hydroxylase Deficiency Including Two Novel Mutations

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What is already known on this topic?

17-hydroxylase deficiency is a rare cause of congenital adrenal hyperplasia that presents with hypergonadotropic hypogonadism, primary amenorrhea, hypertension and hypokalemia. Data on long term follow-up of patients with 17-hydroxylase deficiency is scarce. Whether genotype and phenotype are correlated is unclear.

What this study adds?

This study provides information about long term follow-up of patients with 17-hydroxylase deficiency and their therapeutic outcomes. We describe two novel mutations which both abolish CYP17A1 protein expression. Neither a significant similarity nor a significant difference was found between the genotype and phenotype of the study group.

Abstract

Objective: 17α-hydroxylase/17,20 lyase deficiency (17OHD) is a rare form of congenital adrenal hyperplasia (CAH), characterized by hypertension and varying degrees of ambiguous genitalia and delayed puberty. The disease is associated with bi-allelic mutations in the *CYP17A1* gene located on chromosome 10q24.3. We aimed to present clinical and genetic findings and follow-up and treatment outcomes of 17OHD patients.

Methods: We evaluated six patients with 17OHD from five families at presentation and at follow up. Standard deviation score of all auxological measurements was calculated according to national data and karyotype status. *CYP17A1* gene sequence alterations were investigated in all patients.

Results: The mean (±standard deviation) age of patients at presentation and follow-up time was 14.6±4.2 and 5.0±2.7 years respectively. Five patients were referred to us because of delayed puberty and primary amenorrhea and four for hypertension. One novel single nucleotide insertion leading to frame shift and another novel variant occurring at an ultra rare position, leading to a missense change, are reported, both of which caused 17OHD deficiency. Steroid replacement was started. The three patients with 46,XY karyotype who were raised as females underwent gonadectomy. Osteoporosis was detected in five patients. Four patients needed antihypertensive treatment. Improvement in osteoporosis was noted with gonadal steroid replacement and supportive therapy.

Conclusion: 17OHD, a rare cause of CAH, should be kept in mind in patients with pubertal delay and/or hypertension. Patients with 46,XY who are raised as females require gonadectomy. Due to late diagnosis, psychological problems, gender selection, hypertension and osteoporosis are important health problems affecting a high proportion of these patients.

Keywords: Congenital adrenal hyperplasia, *CYP17A1*, disorder of sex development, hypertension, primary amenorrhea

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Conflict of interest: None declared
Received: 19.01.2018
Accepted: 28.03.2018

Introduction

17-hydroxylase deficiency (17OHD) is a rare form of congenital adrenal hyperplasia (CAH). It has an estimated incidence of 1 in 50 000 newborns and accounts for 1% of all CAH cases. It is the second most common form of CAH in Brazil (1), Japan (2) and China (3,4). The disease is caused by biallelic mutations in the *CYP17A1* gene, encoding the enzyme 17α-hydroxyprogesterone (OHP) aldolase (Cytochrome P450c17) catalyzing the conversion of pregnenolone and progesterone to their 17-alpha-hydroxylated products and subsequently to dehydroepiandrosterone (DHEA) and androstenedione (Δ4A) via 17α-hydroxylation and the 17,20-lyase reaction. It is the key enzyme for cortisol synthesis that is essential for sex steroid production. Absence of enzyme activity drives overproduction of pregnenolone, progesterone, 11-deoxycorticosterone (DOC) and corticosterone that leads to the mineralocorticoid effect, resulting in hypertension and hypokalemia. Excess DOC with low renin concentration causes hypertension and the lack of androgens and estrogens cause sexual infantilism and pubertal failure. The absence of 17,20 lyase activity in the adrenal gland also results in deficiency of DHEA sulfate (DHEA-S), causing failure of adrenarche and pubic and axillary hair development (5,6). Although 46,XY patients have impaired steroid synthesis, they have normal Sertoli cell function producing anti-Mullerian hormone and causing regression of Mullerian structures (7).

Complete deficiency typically causes female external genitalia, delayed puberty, hypergonadotrophic hypogonadism, primary amenorrhea, hypertension, hypokalemia and absence of pubic hair. Differential diagnosis must include cytochrome P450 oxidoreductase deficiency and androgen insensitivity syndrome in complete deficiency (5).

The *CYP17A1* gene is located on chromosome 10q24.3, reference transcript (NM_000102) and contains eight exons with a length of 1870 bp, translating into a 508 amino acid polypeptide (NP_000093) containing a cytochrome p450 domain encoded between codons 28 to 493. The disease is inherited in an autosomal recessive manner and hence parental consanguinity becomes an important risk factor (8).

Currently over 100 mutations in the *CYP17A1* gene have been identified (including point mutations, small deletions/insertions, duplications, frame shift mutations and, rarely, large deletions). Founder effects may also contribute to the high prevalence of the disease in some countries. In Brazil c.1084C>T (p.R362C) and c.1216T>C (p.W406R) mutations had the founder effect (1) while in China nonsense mutation, c.987C>A (p.Y329*), and in frame deletion of p.(D487_F489) due to c.1459_1467delGACTCTTTC, affected more than 80% of the patients (4). In spite of this founder effect, phenotype and genotype correlation is still unknown and a remarkable variation in the severity of the disorder was noted even with the same mutation (8). Interestingly, large gene deletion, namely complete exon 1-6 deletion has been identified in two families from South East Turkey which suggests that this mutation may have a founder effect for Turkey (9,10).

We report the genotypes and phenotypes of six Turkish patients with 17OHD. We aimed to identify the clinical manifestations, genetic findings and follow-up and treatment results of 17-OHD patients.

Methods

Six Turkish patients, from five families, were included in the study. We followed these patients from 1999 to 2016 in the Pediatric Endocrinology Unit of İstanbul Faculty of Medicine. In most patients, clinical diagnosis was suspected because of the absence of secondary sex characteristics, mostly associated with the presence of hypertension and hypokalemia. The diagnosis of complete 17-OHD was established based on typical laboratory findings of reduced cortisol, plasma renin activity (PRA) and aldosterone and elevated gonadotropins and progesterone with almost absent sex steroids. All patients had been genotyped and specific CYP17A1 mutations were identified. The data, collected retrospectively, consisted of physical examination, auxological findings, hormone assays, biochemical and radiological findings, additional features at follow-up and surgical and medical treatment. Height and weight measurements of the patients were taken by the same auxologist. The parental heights were measured and an estimated target height, in keeping with the karyotype was calculated. Body mass index was calculated. At presentation for evaluation of hypertension, ambulatory blood pressure measurement was performed in all patients. At follow-up blood pressure was evaluated by a manual sphygmomanometer in the seated position or digital measurements at home. Hypertension in children and adolescents was defined as systolic and/or diastolic blood pressure, that is, on repeated measurement, ≥95th percentile (11). All patients were evaluated for peripheral effects of hypertension including fundus evaluation, echocardiographic evaluation and microalbuminuria. Bone age was evaluated using the Greulich-Pyle method according to karyotype. Predicted adult height was calculated according to Bayley Pinneau method. Standard deviation score (SDS) of all auxological measurements were calculated according to national data (12) and karyotype status.

The blood samples were collected in the morning after eight hours of fasting. Hormonal evaluation included baseline 17OHP, progesterone, cortisol, Δ4A, DHEA-S, adrenocorticotropic hormone (ACTH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), testosterone, PRA, aldosterone levels. Δ4A, and 17OHP levels were analyzed by radioimmnoassay. ACTH, PRA, aldosterone, LH, FSH, E2, progesterone, cortisol, DHEA-S were measured using the IMMULITE 2000 system (Siemens AG, Berlin and Munich, Germany), immunochemiluminescence assay; ICMA, Siemens. Baseline and stimulated progesterone and cortisol levels with ACTH (0.25 mg, IV Synacthen, Ciba-Geigy, Basel, Switzerland), were measured to diagnose the adrenal enzyme defect.

Pelvic ultrasonography (USG) was performed by a pediatric radiologist using a SonoSite Titan ultrasound machine (Bothell, WA, USA) with a 5 MHz probe.

Bone mineral density (BMD) was evaluated using dual-energy X-ray absorptiometry (DXA) (Hologic QDR 4500A Fan Beam X-ray Bone Densitometer, Hologic, Bedford, MA, USA) and analyzed using software version 12.3. BMD was measured in the spine (L1-L4). Volumetric measurements were done according to national data (13). Lumbar spine BMD z-scores between -1.0 and -2.0 define osteopenia and less than -2.0 define osteoporosis (14).

High resolution banding technique was used for karyotyping in the blood lymphocytes of all patients. Twenty metaphases were analyzed for each patient. DNA samples isolated from venous blood were investigated for disease causing mutations in all of the eight exons and exon-intron boundaries of the *CYP17A1* gene by Sanger sequencing (ABI 3500). Pathogenicity of the novel nonsynonymous mutation was analysed via *in silico* prediction programs (MutationTaster, Polyphen, SIFT). qPCR was performed in one patient because of failure to obtain PCR product for exon 1-6 of the *CYP17A1* gene. Primer pairs for inhouse validated control gene (*CENPJ* exon 3, 6, 12) and *CYP17A1* gene (exon 1, 6 and 7) were designed to produce a minimum of 150 bp and maximum of 250 bp products with identical melting point temperature. Performance of the designed primers were first tested by cold PCR, before the qPCR reaction with EvaGreen fluorescent dye was operated in three parallel runs concurrently for index, parental and control samples for each region on CFX96 Thermal Cylcler (Bio-Rad Laboratories, Inc., CA, USA). Double delta Ct method was used for the analysis of the quantification.

Written informed consent was obtained from all patients. The study protocol was approved by the Clinical Research Ethics Committe of İstanbul University (approval number: 2016/728).

Statistical Analysis

Statistical analysis was performed by using the SPSS 22 (IBM, Chicago, ILL, USA).

Results

Clinical Findings

All patients presented as phenotypical females and all had complete enzyme deficiency. Parents of Patient 4 had second degree, while others had first degree consanguineous marriage. Patients were referred to our clinic at a mean ± standard deviation (SD) (range) age of 14.6 ± 4.2 (6.2-17.8) years. Except for one, all of them were in adolescence. Mean ± SD (range) follow-up period was 5.0 ± 2.7 (2.2-8.9) years. Five patients presented with lack of pubertal development and primary amenorrhea (83.3%) and four patients with hypertension (66.7%). Three patients (50%) had short stature and Patient 1 and Patient 3 had severe short stature. Bone age was retarded in all patients. All patients were prepubertal, only Patient 5 was Tanner 2, because of previous use of estrogen. All patients, regardless of chromosomal sex, were raised as female. Three patients were 46,XX and three were 46,XY. 46,XY patients had a female appearance with palpable testes. The clinical findings of 17OHD patients at presentation are summarized in Table 1.

LH and FSH levels were significantly elevated, whereas T or E2 production were blunted. Patients' baseline progesterone and ACTH levels were high while cortisol, 17OHP and PRA were supressed. The DOC levels of three patients were measured and all were above the normal ranges. Three patients had hypokalemia during presentation (Table 1). All patients had pelvic and gonadal USG and the 46,XY patients lacked Mullerian structures. Response to ACTH stimulation test were consistent with the diagnosis of 17OHD (Table 1).

During follow-up hydrocortisone replacement therapy was started. Three 46,XY patients raised as females underwent gonadectomy. Mean (± SD) age at gonadectomy was 13.5 ± 5.7 years. Transdermal E2 replacement was started to induce puberty in all patients. Subsequently, oral contraceptives were given to 46,XX patients to achieve regular menstrual cycles. Breast development reached stage 4-5 at 18.7 ± 1.8 age (duration of estrogen replacement: 2.2 ± 1.4 years). Menarche occurred at age 17.6 ± 0.7 years in 46,XX patients. DHEAS and Δ4A levels were low in all patients and two of them did not have adrenarche (Patient 1 and Patient 2), although two patients (Patient 3 and Patient

Table 1. Clinical and laboratory findings of patients at presentation. Biochemical findings are given with (units) or (units) followed by (normal range)

	Patient 1 (Family 1) (V:6)	Patient 2 (Family 2) (IV:2)	Patient 3 (Family 2) (IV:4)	Patient 4 (Family 3) (V:8)	Patient 5 (Family 4) (index)	Patient 6 (Family 5) (index)
Age (years)	15.3	14.9	16.7	16.6	17.8	6.2
Presenting features	**Primary amenorrhea**	**Primary amenorrhea, hypertension**	**Primary amenorrhea, hypertension**	**Primary amenorrhea, hypertension**	**Primary amenorrhea**	**Hypertension**
Consanguinity (degree)	1	1	1	2	1	1
Birth weight SDS	1.1	-2.3	0.1	-1.2	-	-0.5
Weight SDS	-2.8	-	-3.3	-2.3	-3.5	0.7
Height SDS	-3.1	-	-3.2	-2.2	-0.7	1.9
Blood pressure mmHg	115/70	150/100	160/100	190/110	110/70	160/100
External genitalia appearance	**Female**	**Female**	**Female (palpable testes)**	**Female (palpable testes)**	**Female**	**Female (palpable testes)**
Pubertal stage (Tanner)	B1/1PH1	B1/1PH1	B1/1PH2	B1/1PH1	B2/2PH1	B1/1PH1
Bone age (years)	10-11	8.8	11.5	13	14	6
CA-BA	4.7	6.1	5.1	3.6	3.8	0.2
Target height SDS	-1.5	1	0.8	-1.2	-1.6	-1.8
PAH SDS	-0.2	-	1.8	1.0	-0.1	-
Karyotype	46,XX	46,XX	46,XY	46,XY	46,XX	46,XY
ACTH (pg/mL) (0-60)	385	1619	354	-	375	176
Cortisol (µg/dL) (6.7-22.6)	-	-	-	-	-	-
Baseline	1.8	4.3	5.4	1.3	1.4	1.1
Peak#	2.0	4.8	3.6	1.7	2.0	3.0
Progesterone (ng/mL) (0.3-1.5)	-	-	-	-	-	-
Baseline	6.6	5.7	7.5	4.5	7.3	6.8
Peak#	7.9	6.3	9.1	10.5	8.4	12
11-DOC (pmol/mL) (0.12-0.6)	7.1	-	-	1.1	-	9.5
17-OHP (ng/mL) (0.2-1.3)	0.4	0.2	0.7	0.4	0.8	0.8
DHEA-S (µg/dL) (35-430)	1.8	0.4	1.1	31.2	1.7	3.3
Androstenedione (ng/mL) (0.75-3.1)	0.3	-	0.3	0.1	0.3	0.3
Na/K (mEq/L)	140/3.7	139/3.1	142/4.4	149/3.6	137/2.2	138/3.4
Aldosteron (pg/mL) (38-313)	219.7	278.5	426	95.7	84.6	836
PRA (ng/mL/hr) (1.5-6.5)	0.2	0.8	0.1	2.7	0.6	0.3
LH (mU/mL)	21.4	9.1	29.8	27.7	47.6	0.2
FSH (mU/mL)	65.4	51.8	111	14.8	48.2	14.7
E2 (pg/mL)	18.9	5	12.5	1.1	5	5
Testosterone (ng/mL) (0.1-0.75)	0.06	-	0.03	0.29	-	0.04
Mutations						
Zygosity	**Homozygous**	**Homozygous**		**Homozygous**	**Homozygous**	**Homozygous**
Nucleotide (NM_000102)	Ex.1-6 del	c.177_178insA		c.1226C > T	c.1085G > A	c.1306G > A
Protein (NP_000093)	NA	p.Y60Ifs*29		p.P409L	p.R362H	p.G436R
Reference	Turkkahraman et al. (9)	This study		This Study	Nájera et al. (31)	Küçükemre-Aydın et al. (32)

PAH: predicted adult height, SDS: standard deviation score, ACTH: adrenocorticotropic hormone, B: breast, PH: pubic hair, #: ACTH stimulation, CA: chronological age, BA: bone age, E2: estradiol, LH: luteinizing hormone, FSH: follicle stimulatinghormone, PRA: plasma renin activity, DHEA-S: dehydroepiandrosterone sulfate, OHP: hydroxyprogesterone, DOC: deoxycorticosterone

6) had sparse pubic hair and two patients (Patient 4 and Patient 5) had pubic hair consistent with Tanner stage 3-4.

Four patients had hypertension at presentation and were started on antihypertensive treatment (calcium channel blocker and aldosterone antagonist) (Table 2). In all patients, continuation of antihypertensive pharmacotherapy was needed, even after adrenal precursor levels decreased on hydrocortisone replacement. Echocardiography for hypertensive cardiomyopathy was normal in all patients and only Patient 2 had ASD at cardiac evaluation. Three patients had grade 1 hypertensive retinopathy (Patients 3, 4 and 6) and none of them had microalbuminuria. Antihypertensive doses were adjusted according to blood pressure monitoring. Despite attempts to wean the patients off antihypertensive therapy, this was not possible and therapy was continued.

BMD measurement revealed osteoporosis in five patients. In addition to sex steroid replacement, vitamin D and calcium treatment were initiated. Only Patient 4 needed bisphosphonate treatment, due to severe osteoporosis, and alendronate was given between the ages of 17 and 21 years. Improvement in osteoporosis was noted with sex steroid replacement and supportive therapy.

At final evaluation four patients had reached final height. Only Patient 3 with a 46,XY karyotype had short stature with a height SDS of -2.8 (final height 163.5 cm). Two patients who did not reach final height also had short stature (Patient 2 and Patient 6). Height SDS of Patient 6 was -2.0, while that of Patient 2 was -0.3. Patient 2 had short stature according to the difference between height SDS and target height SDS which was equal to -1.3. Currently these two patients are continuing to increase in height.

Genetic Findings

Molecular analysis revealed five different homozygous mutations, one of which is novel and was found in two patients from the same family, c.177_178insA (p.Y60Ifs*29). Another very rare alteration, 1 in 246.050 allele, c.1226C>T (p.P409L, rs367833709) was found in one further patient (see Figures 1, 2 and 3). Parental testing showed heterozygosity for the alterations, supporting autosomal recessive inheritance. *In silico* analyses findings predicted that the c.1226C>T alteration would be disease causing (Table 1) (Figure 3).

Table 2. Clinical findings of the patients at follow-up

	Patient 1 (Family 1) (V:6)	Patient 2 (Family 2) (IV:2)	Patient 3 (Family 2) (IV:4)	Patient 4 (Family 3) (V:8)	Patient 5 (Family 4) (index)	Patient 6 (Family 5) (index)
Gonadectomy age (years)	-	-	17.2	16.4	-	7
E2 replacement age (years)	16.3	15.2	17.8	16.6	14.7	14
Age of menarche	18.3	16.9	-	-	18.5	
BMD L1-L4 z score						
First (age-years)	-0.5 (15.3)	-5.3(14.8)	-3.4 (16.9)	-4.4 (16.7)	-3.2 (18.3)	-2.6 (11.3)
Recent (age-years)	-1.6 (24.6)	-2.6 (16.3)	-0.85 (22)	0.6 (31)	-2.5 (20.8)	-3.9 (15.7)
Recent evaluation						
Age (years)	19.2	17.1	21	23.7	20.4	15.1
Weight SDS	-1.4	-2.1	-1.0	-0.2	-1.5	0.5
Height SDS	-1.9	-0.3	-2.8	-1.3	0	-2.0
BMI SDS	-0.2	-2.4	0.5	0.5	-2.0	1.8
Bone age (years)	16	12	16-17	16	17	12.5
PAH SDS	-0.9	2.3	-0.3	-0.7	0.6	0.4
Height SDS-TH SDS	-0.4	-1.3	-3.6	-0.1	1.6	0.2
Pubertal stage	B5/5PH1	B3/3PH1	B4/4PH2	B5/5PH4	B5/5PH3	B3/3PH2-3
Treatment	HC + OCS	HC + OCS Ca channel blocker Ca + vitamin D	HC + E2 Ca channel blocker Ca + vitamin D	Dex + E2 Aldosteron antagonist Ca + vitamin D	HC + OCS Ca + vitamin D	HC + E2 Ca channel blocker Ca + vitamin D

BMI: body mass index, PAH: predicted adult height, BMD: bone mineral density, TH: target height, SDS: standard deviation score, B: breast, PH: pubic hair, HC: hydrocortisone, Dex: dexamethasone, E2: estradiol, OCS: oral contraceptive (E2 + Progesterone), Ca: calcium

Discussion

In this study we report the clinical and genetic findings and follow-up characteristics of 17OHD patients from a single center. Six patients from five families, all born to consanguineous parents, presented at adolescent ages, mostly with symptoms of pubertal delay and hypertension. 17OHD, a rare form of CAH, usually presents in adolescence (8). In this study, four patients (66.7%) had hypertension and three had hypokalemia (50%) while others had not developed the symptoms of mineralocorticoid excess at presentation. Patients who suffer from this condition may develop hypertension and hypokalemia at any age, which makes the diagnosis difficult.

All six patients reported here, who had absent sex steroid activity and pubertal development, presented with complete deficiency. All patients had hypergonadotropic hypogonadism. However the defect may be partial or complete. Partial 17OHD patients have some degree of estrogenic and androgenic function. In partial forms patients have normal 17α-hydroxylase activity, while 17,20 lyase activity is absent. Patients may have spontaneous breast and pubic hair development, oligomenorrhea or secondary amenorrhea while complete forms have absent pubarche and adrenarche. Furthermore, patients with partial forms may be normokalemic and normotensive (15).

At presentation four of our patients were hypertensive and three patients had hypokalemia. Hydrocortisone replacement at physiologic doses (8-10 mg/m^2/day) is required for treatment. Hydrocortisone supresses precursor concentrations and improves symptoms. The dose is titrated to normalise blood pressure and potassium levels. In this cohort, following hydrocortisone treatment, hypokalemia resolved while hypertensive patients needed

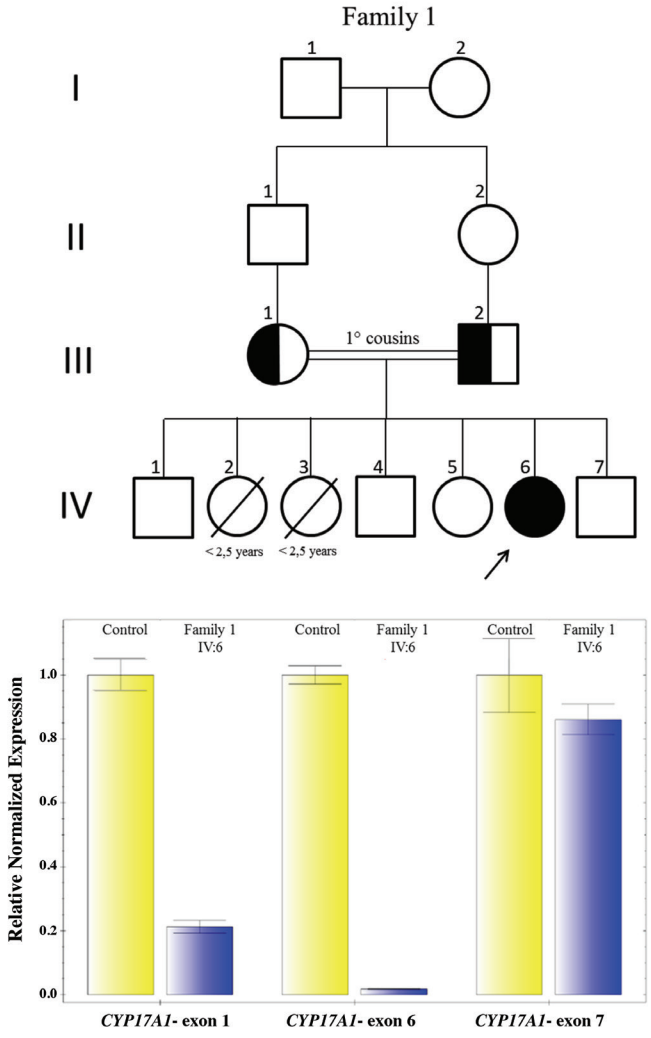

Figure 1. Pedigree of Family 1 and *CYP17A1* gene exon 1-6 deletion

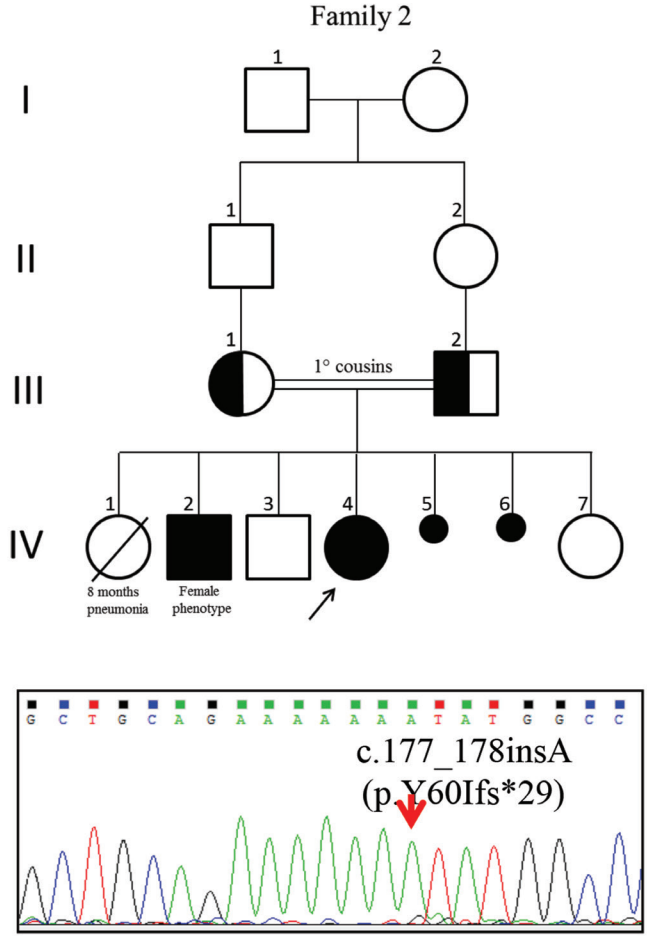

Figure 2. Pedigree of Family 2 and sequencing electropherograms of the *CYP17A1* gene. The upper lane shows the mutation of c.177_178insA (p.Y60Ifs*29)

antihypertensive medication in addition to hydrocortisone to achieve blood pressure control. Calcium channel blockers or spironolactone may be required for hypertension refractory to hydrocortisone. Reports suggest that 10-15% of 17OHD patients may be normotensive (16). Some patients have been diagnosed during investigation for hypertension (8). In a Chinese cohort with twenty six 17OHD patients, two patients with the complete form were normotensive (3). Varying degrees of hypertension in the 17OHD patients suggests that other factors other than the degree of P450c17 activity may be involved in the regulation of hypertension (17). Because high levels of circulating DOC saturate the mineralocorticoid receptor under most circumstances, the severity of clinical features and the age onset of hypertension and hypokalemia appear to vary, even among patients with the same mutation (5,8). Patient 1 (del exon 1-6) and Patient 5 (p.R362H) never had hypertension. It remains unclear whether this is due to the suppression of mineralocorticoid precursors by hydrocortisone, or whether it is simply because 10-15% of patients will be normotensive.

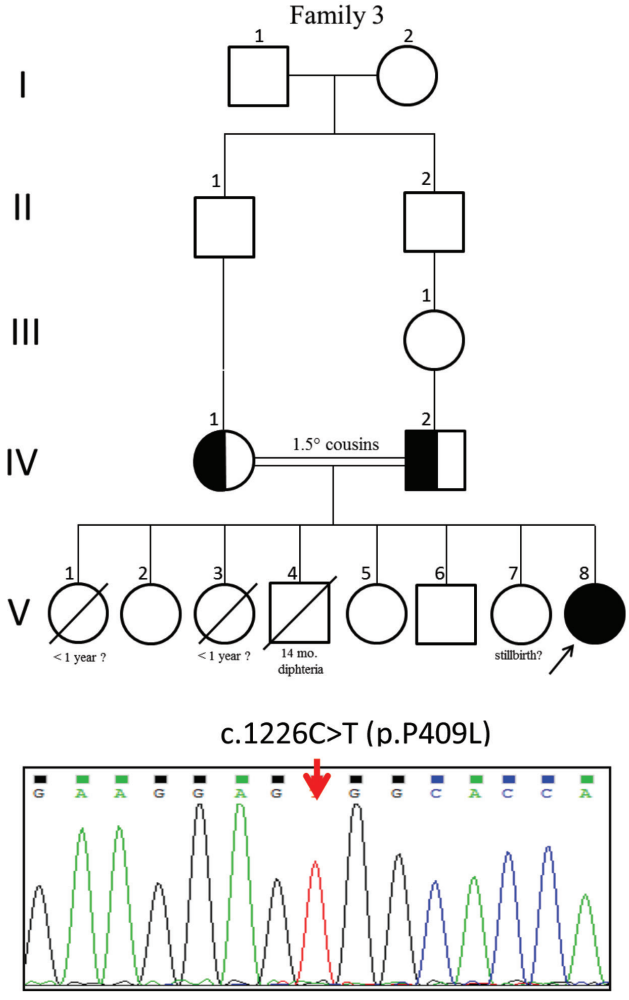

Figure 3. Pedigree of Family 3 and sequencing electropherograms of the *CYP17A1* gene. The upper lane shows the mutation of c.1226C > T(p.P409L)

Four of our patients reached adult height and, after sex steroid replacement, only one of these had short stature at final evaluation. The other two patients have not yet reached their final height. The short stature in our patients may be due to sex steroid deficiency. After sex steroid replacement height velocity and thus final height increased. Short stature is an unexpected finding in 17 OHD patients because sex steroid deficiency causes failure of epiphyseal fusion and bone age retardation, which is known to result in tall stature. 17OHD patients generally have normal or tall stature (6,8,18). Despite this, Ross et al (19) reported that low levels of estrogens in prepubertal Turner syndrome patients, have a positive impact on height. Sex steroids have beneficial effects on linear growth (19). While the patients in this study had normal target height SDS, 50% had short stature at presentation according to genotypic sex. All patients had delayed bone age. Although bone age retardation suggests that linear growth potential in 17OHD patients will lead to improved final height, there is no data in the literature on this issue. Interestingly Schwab et al (18) reported two German sisters, one of whom was 46,XX and the other 46,XY. At the age of ten years the 46,XY sibling had a predicted height of 203 cm. Treatment with high dose estrogens for a 13 month period resulted in a final height of 186.3 cm (18). Although tall stature may be seen in patients, especially in those with 46,XY karyotype, none of our patients had tall stature. Turkkahraman et al (9) reported three siblings with exon 1-6 deletion on the *CYP17A1* gene, the same variant found in Patient 1. One sibling had tall stature with a height SDS of 3.5 while the other two siblings had height SDS of -1.6 and -1.3 respectively (9). Patient 1 had short stature at presentation, but went on to have an improved height SDS of -1.9 on follow-up. Thus linear growth may differ even among patients with the same mutation.

We had three patients with severe mutations (exon 1-6 deletion and p.Y60Ifs*29 mutation). Height at presentation remains unknown for one of these patients, while the others had severe short stature. One patient with a point mutation (Patient 4) presented with milder short stature. Our remaining two patients had point mutations and they did not have short stature. Four of these patients had reached their adult height and two of them with the exon 1-6 deletion and one with p.Y60Ifs*29 mutation, were short while the other two patients had point mutations and normal heights. It was interesting that the heights of the patients with point mutations were less severely affected. Patient 6 was diagnosed at an early age and had a high SDS for height for age at presentation. However, the most recent examination revealed a retarded bone age and borderline

short stature although this patient has not yet reached adult height. Data on long term follow up of growth of patients with 17OHD is scarce in the literature.

Sex steroid replacement should be started at the time of puberty, in keeping with the patient's phenotypic sex. Estrogen replacement should be followed by oral contraceptives in 46,XX patients. High levels of progesterone have negative effects on endometrium and breast tissue. Thus, some patients show breast tissue unresponsiveness despite high dose E2 treatment (20,21). In this study, breast development of the patients was at Tanner stage 1 at presentation and progressed to Tanner stage 4-5 after sex steroid replacement. Due to genetic differences, some patients had less progesterone influence on breast tissue while other tissues, such as the endometrium, were affected more extensively. This area requires further study.

In this cohort, Patient 4 reached pubic hair stage 4 at final evaluation. This patient had a DHEA-S level just below normal, which was higher than the values of the other patients all of whom had very low DHEA-S concentrations. It is reported that extreme adrenarche may develop after E2 replacement despite low androgen and DHEA-S (21). Use of dermal ointments containing estrogen have been reported to cause the growth of pubic hair in both males and females ranging from four months to two years of age (22). In light of this finding, we can speculate that estrogens also may have a stimulatory effect on hair follicles, either directly or by increasing local androgen production. In some cases adrenarche can reach the adult stage.

Three 46,XY patients underwent gonadectomy shortly after diagnosis. These patients carry the risk of developing gonadal tumours, requiring gonadectomy at the appropriate age. Soveid and colleagues reported myelipoma of adrenal glands in addition to gonadal tumours (23). The etiology of myelipoma in CAH is still unclear, but exposure to high levels of ACTH may play a role (24). Screening of adrenals and gonadectomy at the appropriate age are important for the future health of 17OHD patients.

In this cohort, five patients had osteoporosis at presentation. After sex steroid replacement and hydrocortisone treatment most of them improved. Cortisol and estrogen deficiency during adolescence has been associated with osteoporosis (25,26). In contrast, Wu et al (27) reported patients with 17OHD to be osteoporotic and they suggested that this may be due to the negative effect of corticosteroid replacement on bone tissue. Osteoporosis worsened with hydrocortisone and sex steroid treatment in one of our patients (Patient 6), a finding which may be due to long-term corticosteroid replacement.

We present one novel and one ultra rare gene variant with 17-OH deficiency, both described for the first time, in this study. A single base insertion in Exon 1 led to frameshift and caused premature termination of translation in Patients 2 and 3 (p.Y60Ifs*29) expected to abolish the total activity of the 17-OH enzyme.

The missense variant (p.P409L) identified in Patient 4, affecting the Cytochrome P450 domain by altering a non-polar aliphatic amino acid proline to a non-polar amino acid leucine, resulting in an isobutyl side chain that may cause abnormal protein structure with a 3-carbon chain that loops to incorporate into the molecular backbone, is also novel. A different nucleotide alteration at the same position causing a missense change (c.1226C > G;p.P409R) has been previously reported in a few Chinese cases (15,28,29,30).

The other molecular findings, the exon 1-6 deletion in Patient 1 (9), p.R362H in Patient 5 (31) and p.G436R in Patient 6 (32) were previously reported mutations. MLPA testing is a reliable and convenient method for the diagnosis of gross deletion/duplications and is widely used when a large number of sample sets are required to be tested in a single run. qPCR is also reliable when a single patient needs to be tested for deletion and duplication and is also cost effective despite being a "boutique" test.

Our clinical findings, together with *CYP17A1* genotypes of the patients, did not correlate with biochemical and hormone results, reflecting the expressional variation of bi-allelic mutations. Clinical and hormonal findings of two siblings (one 46,XX, the other 46,XY) with the same mutation (c.177_178insA; p.Y60Ifs*29) were also somewhat different. Findings in siblings with the same mutation revealed that the 46,XX sibling had hypokalemia and breasts at Tanner stage 3, while the 46,XY sibling was normokalemic and was at Tanner stage 4 for breast development. Moreover, the 46,XX sibling had more severe osteoporosis. Bee et al (29) reported that phenotype may vary even amongst siblings with the same mutation. 46,XY individuals have more pronounced clinical symptoms than their 46,XX siblings (29).

Patient 1 was 46,XX and had the same mutation as three previously reported siblings (two 46,XY and one 46,XX) of Kurdish origin from Turkey. All four patients reported with this variant patients had gross partial deletion of the *CYP17A1* gene, encompassing exons 1-6 and all of them had the same hormonal profile of complete defect of 17OHD. However, severity of the disease was different. The previously reported index patient had hypertension and hypokalemia at presentation while our patient had neither. Siblings of the index patients did not develop hypertension

or hypokalemia either (9). This finding also supports the previous observations that the presence and onset of hypertension may be variable even in patients with the same genotype (8,9,29,33). In addition, exon 1-6 deletion was found in another patient of Kurdish origin from Turkey (10). The carriers of this mutation (exon 1-6 deletion) originated from Southeastern Turkey, a finding which suggests exon 1-6 deletion could be a founder mutation in Turkey.

The homozygous mutation of Patient 5 with XX chromosomal sex had the same homozygous mutation as a patient reported from Mexico (31). The Mexican case was a compound heterozygote with (R362H) and (p.K110*), presenting with severe hypertension and hypokalemia. While our patient did not have hypertension, hypokalemia was present. In our opinion it is hard to attribute hypertension to the truncating effect of the stop codon mutation. Further cases or functional analyses are needed to draw a conclusion on genotype-phenotype correlations of the mutations described thus far.

Study Limitations

Limitations of this study include the small sample size, however it is still one of the largest pediatric cohorts, supporting to the disease has being rare.

Conclusion

17-OHD is a rare cause of CAH and should be kept in mind in patients with pubertal delay/primary amenorrhea, hypertension and hypokalemia. Here we describe two novel mutations which both abolish *CYP17A1* protein expression in adrenal and gonadal tissue. In our experience short stature is particularly evident at presentation. Some patients may have full blown adrenarche and E2 replacement may increase the progression of adrenarche. Gonadectomy at the appropriate age is necessary in 46,XY patients who are raised as female because of the high risk of later malignancy. Due to late diagnosis, psychological problems, gender selection, hypertension and osteoporosis constitute important health problems among these patients.

Acknowledgment

We thank the patients and their parents and all the related physicians who contributed to the patient's samples and clinical data.

Ethics

Ethics Committee Approval: Clinical Research Ethics Committe of Istanbul University (approval number: 2016/728).

Informed Consent: Written informed consent was obtained from all patients.

Peer-review: Internal and external peer-reviewed.

Authorship Contributions

Concept: Aslı Derya Kardelen, Firdevs Baş, Şükran Poyrazoğlu, Feyza Darendeliler, Design: Aslı Derya Kardelen, Güven Toksoy, Firdevs Baş, Oya Uyguner, Feyza Darendeliler, Data Collection or Processing: Aslı Derya Kardelen, Firdevs Baş, Güven Toksoy, Zehra Yavaş Abalı, Genco Gençay, Rüveyde Bundak, Umut Altunoğlu, Şahin Avcı, Adam Najaflı, Oya Uyguner, Birsen Karaman, Seher Başaran, Analysis or Interpretation: Aslı Derya Kardelen, Firdevs Baş, Literature Search: Aslı Derya Kardelen, Firdevs Baş, Güven Toksoy, Oya Uyguner, Writing: Aslı Derya Kardelen, Firdevs Baş, Genco Gençay, Feyza Darendeliler.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Costa-Santos M, Kater CE, Auchus RJ; Brazilian Congenital Adrenal Hyperplasia Multicenter Study Group. Two Prevalent CYP17 Mutations and Genotype-Phenotype Correlations in 24 Brazilian Patients with 17-Hydroxylase Deficiency. J Clin Endocrinol Metab 2004;89:49-60.
2. Miura K, Yasuda K, Yanase T, Yamakita N, Sasano H, Nawata H, Inoue M, Fukaya T, Shizuta Y. Mutation of Cytochrome P-45017 a Gene (CYP17) in a Japanese Patient Previously Reported as Having Glucocorticoid-Responsive Hyperaldosteronism: With a Review of Japanese Patients with Mutations of CYP17. J Clin Endocrinol Metab 1996;81:3797-3801.
3. Zhang M, Sun S, Liu Y, Zhang H, Jiao Y, Wang W, Li X. New, recurrent, and prevalent mutations: Clinical and molecular characterization of 26 Chinese patients with 17 alpha hydroxylase/17,20-lyase deficiency. J Steroid Biochem Mol Biol 2015;150:11-16. Epub 2015 Feb 16
4. Han B, Xue L, Fan M, Zhao S, Liu W, Zhu H, Cheng T, Lu Y, Cheng K, Song H, Liu Y, Qiao J. Clinical and molecular manifestation of fifteen 17OHD patients: a novel mutation and a founder effect. Endocrine 2016;53:784-790. Epub 2016 May 5
5. Auchus RJ. Steroid 17-hydroxylase and 17,20-lyase deficiencies, genetic and pharmacologic. J Steroid Biochem Mol Biol 2017;165:71-78. Epub 2016 Feb 6
6. Goldsmith O, Solomon DH, Horton R. Hypogonadism and mineralocorticoid excess. The 17-hydroxylase deficiency syndrome. N Engl J Med 1967;277:673-677.
7. New MI. Male pseudohermaphroditism due to 17α-hydroxylase deficiency. J Clin Invest 1970;49:1930-1941.
8. Yanase T, Simpson ER, Waterman MR. 17α-Hydroxylase/17,20-Lyase Deficiency: From Clinical Investigation to Molecular Definition. Endocr Rev 1991;12:91-108.
9. Turkkahraman D, Guran T, Ivison H, Griffin A, Vijzelaar R, Krone N. Identification of a novel large CYP17A1 deletion by MLPA analysis in a family with classic 17α-hydroxylase deficiency. Sex Dev 2015;9:91-97.
10. Camats N, Üstyol A, Atabek ME, Dick B, Flück CE. A novel CYP17A1 deletion causes a functional knockout of the steroid enzyme 17-hydroxylase and 17,20-lyase in a Turkish family and illustrates

the precise role of the CYP17A1 gene. Clin Case Rep 2015;3:793-797. Epub 2015 Aug 26

11. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004;114(Suppl 4th Report):555-576.
12. Neyzi O, Bundak R, Gökçay G, Günöz H, Furman A, Darendeliler F, Baş F. Reference Values for Weight, Height, Head Circumference, and Body Mass Index in Turkish Children. J Clin Res Pediatr Endocrinol 2015;7:280-293.
13. Goksen D, Darcan S, Coker M, Kose T. Bone mineral density of healthy Turkish children and adolescents. J Clin Densitom 2006;9:84-90. Epub 2006 Mar 27
14. Gordon CM, Leonard MB, Zemel BS; International Society for Clinical Densitometry. 2013 Pediatric Position Development Conference: executive summary and reflections. J Clin Densitom 2014;17:219-224. Epub 2014 Mar 20
15. Yao F, Huang S, Kang X, Zhang W, Wang P, Tian Q. CYP17A1 mutations identified in 17 Chinese patients with 17α-hydroxylase/17,20-lyase deficiency. Gynecol Endocrinol 2013;29:10-15. Epub 2012 Sep 7
16. Kater CE, Biglieri EG. Disorders of steroid 17 alpha-hydroxylase deficiency. Endocrinol Metab Clin North Am 1994;23:341-357.
17. Wang YP, Zhao YJ, Zhou GY, He B. CYP17A1 gene mutations and hypertension variations found in 46,XY females with combined 17α-hydroxylase/17, 20-lyase deficiency. Gynecol Endocrinol 2014;30:456-460. Epub 2014 Mar 6
18. Schwab KO, Moisan AM, Homoki J, Peter M, Simard J. 17α-Hydroxylase/17,20-Lyase Deficiency due to Novel Compound Heterozygote Mutations: Treatment for Tall Stature in a Female with Male Pseudohermaphroditism and Spontaneous Puberty in her Affected Sister. J Pediatr Endocrinol Metab 2005;18:403-411.
19. Ross JL, Quigley CA, Cao D, Feuillan P, Kowal K, Chipman JJ, Cutler GB Jr. Growth hormone plus childhood low-dose estrogen in Turner's syndrome. N Engl J Med 2011;364:1230-1242.
20. Athanasoulia AP, Auer M, Riepe FG, Stalla GK. Rare Missense P450c17 (CYP17A1) Mutation in Exon 1 as a Cause of 46,XY Disorder of Sexual Development: Implications of Breast Tissue "Unresponsiveness" despite Adequate Estradiol Substitution. Sex Dev 2013;7:212-215. Epub 2013 Mar 1
21. Turan S, Bereket A, Guran T, Akcay T, Papari-Zareei M, Auchus RJ. Puberty in a case with novel 17-hydroxylase mutation and the putative role of estrogen in development of pubic hair. Eur J Endocrinol 2009;160:325-30. Epub 2008 Nov 7
22. Beas F, Vargas L, Spada RP, Merchak N. Pseudoprecocious puberty in infants caused by a dermal ointment containing estrogens. J Pediatr 1969;75:127-130.
23. Soveid M, Rais-Jalali GA Md. Seventeen Alpha-Hydroxylase Deficiency Associated with Absent Gonads and Myelolipoma: A Case Report and Review of Literature. Iran J Med Sci 2016;41:543-547.
24. German-Mena E, Zibari GB, Levine SN. Adrenal myelolipomas in patients with congenital adrenal hyperplasia: review of the literature and a case report. Endocr Pract 2011;17:441-447.
25. Weinstein RS. Clinical practice. Glucocorticoid-induced bone disease. N Engl J Med 2011;365:62-70.
26. Seibel MJ, Cooper MS, Zhou H. Glucocorticoid-induced osteoporosis: mechanisms, management, and future perspectives. Lancet Diabetes Endocrinol 2013;1:59-70. Epub 2013 Jun 28
27. Wu C, Fan S, Qian Y, Zhou Y, Jin J, Dai Z, Jiang L. 17α-hydroxylase/17, 20-lyase deficiency: Clinical and molecular characterization of eight Chinese patients. Endocr Pract 2017;23:576-582. Epub 2017 Feb 22
28. Lam CW, Arlt W, Chan CK, Honour JW, Lin CJ, Tong SF, Choy KW, Miller WL. Mutation of proline 409 to arginine in the meander region of cytochrome p450c17 causes severe 17 alpha-hydroxylase deficiency. Mol Genet Metab 2001;72:254-259.
29. Bee YM, Manju C, Papari-Zareei M, Auchus RJ. Phenotypic variation in a Chinese family with 46,XY and 46,XX 17α-hydroxylase deficiency. Gynecol Endocrinol 2012;28:322-325. Epub 2011 Nov 16
30. Li H, Qiao J, Guo H. 17-alpha-hydroxylase deficiency: a case report with clinical and molecular analysis. Gynecol Endocrinol 2010;26:521-523.
31. Nájera N, Garibay N, Pastrana Y, Palma I, Peña YR, Pérez J, Coyote N, Hidalgo A, Kofman-Alfaro S, Queipo G. Loss of Cytochrome P450 17A1 Protein Expression in a 17α-Hydroxylase/17,20-Lyase-Deficient 46,XY Female Caused by Two Novel Mutations in the CYP17A1 Gene. Endocr Pathol 2009;20:249-255.
32. Küçükemre-Aydın B, Öğrendil-Yanar Ö, Bilge I, Baş F, Poyrazoğlu Ş, Yılmaz A, Emre S, Bundak R, Saka N, Darendeliler F. An easily missed diagnosis: 17-alpha-hydroxylase/17,20-lyase deficiency. Turk J Pediatr 2015;57:277-281.
33. Sills IN, MacGillivray MH, Amrhein JA, Migeon CJ, Peterson RE. 17 alpha-hydroxylase deficiency in a genetic male and female sibling pair. Int J Gynaecol Obstet 1981;19:473-479.

ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.0007

J Clin Res Pediatr Endocrinol 2018;10(3):216-222

Parental Perception of Terminology of Disorders of Sex Development in Western Turkey

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What is already known on this topic?

Few studies have been conducted to explore the perspective of families on the terminology of disorders of sex development. While this terminology is in worldwide use among medical professionals' studies have shown that the new terminology is not well accepted by affected families. All studies to date have been conducted in western countries.

What this study adds?

This is one of the largest studies investigating parental perception of terminology about disorders of sex development (DSD). As such it reports the discontentment among parents in Turkey concerning DSD terminology. The importance of local studies reflecting linguistic and cultural differences about this complex topic are highlighted.

Abstract

Objective: Disorders of sex development (DSD) is a nomenclature intended to defeat the discomfort of families and patients and has found worldwide usage. The aim of this study was to address the perception and usage of terminology among the parents of DSD patients in a tertiary center in western Turkey.

Methods: The records of the DSD council (multidisciplinary team where each patient with DSD is discussed) between years 2008-2015 were reviewed retrospectively. Data including details of the management process, patient characteristics and follow-up details were noted. Then inquiries reflecting parental perception about terminology were implemented during clinical visits.

Results: In total, 121 patients were evaluated in monthly meetings of the DSD council and 79 inquiries were completed. Fifty-one percent of the families admitted knowing the terms DSD, ambiguous genitalia, "dubious genitals" and intersex. However, only 2% preferred using DSD, 6% intersex and 14% ambiguous genitalia. Fifty-two percent of the parents used a disease name in Latin (mostly hypospadias) addressing the disorder. The offspring of 69% of the parents who were familiar with the name "dubious genitals" were diagnosed in the neonatal period. The preferred terminology used by parents was strongly associated with the terminology used most commonly in the medical speciality their child most often attended.

Conclusion: Each country has its own social norms. We suggest therefore that local committees including medical professionals, patients and families should be employed to develop proper terminology.

Keywords: Disorders of sex development, intersex conditions, ambiguous genitalia, terminology

Introduction

Disorders of sex development (DSD) are "congenital conditions in which development of chromosomal, gonadal, or anatomic sex is atypical" (1). Assigning an appropriate name to this condition has always been controversial (2) and medical professionals are not the only group with an interest in getting this right for everyone. The confusing nature of the disease draws the attention of health professionals, sociologists and activists. All these groups

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Conflict of interest: None declared
Received: 07.01.2018
Accepted: 28.03.2018

have published many papers (3,4,5,6,7), solely debating nomenclature usage. However, few have focused on patients' families wishes and understanding. In our opinion families' perception of the disease affects their child in two ways; by affecting their decision-making and through the environment the child will grow-up in. In the era of patient-centered medicine, questioning families' opinion is important and necessary in order to conduct a responsible and ethical management of the condition. The aim of this study was to address the perception and use of terminology among the parents of DSD patients attending a tertiary center in western Turkey.

Methods

In our centre the evaluation and management of DSD patients is conducted by a multidisciplinary team. Each patient with DSD is discussed at the monthly joint meetings. The department mostly involved in the management changes according to the primary diagnosis of the patient although in most cases this is pediatric endocrinology. Every critical decision influencing the management process is also taken in these meetings. Our core team consists of a pediatric urologist, a pediatric endocrinologist, a child psychiatrist, and a geneticist. Adult endocrinologists and psychiatrists, as well as pathologists, neonatologists, gynecologists and social workers are consulted when necessary.

After approval of the Ege University, Clinical Research Ethics Committee (2016; decision number: 16-2.1/1), the records of the DSD council of our institution between years 2008-2015 were reviewed. After much thought, we decided to exclude families who were presumably unaware of the terminology with regard to their child's diagnosis (patients with Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome, severe hypospadias, bilateral undescended testes). The reasoning for this was that it was felt that inclusion of these cases would risk unnecessary bias into the study. A retrospective analysis of the data including details of the management process, demographics, patient history and follow-up details was performed. Then parents were contacted to obtain their consent and to conduct inquiries focusing on the terminology the parents knew and tend to use (Appendix 1). The questionnaire consisted of closed questions and short answer open questions concerning their knowledge and preference about the terms, their first contact with the disorder and details about the management. To understand their knowledge about the terms, families were first questioned about the terms they know regarding their child's condition. The interviewer was careful not to use any disorder names, simply calling it "the illness" during the entire interview in order not to influence their answers. At the end of the interview, if they did not recall, they were told the commonly used terms in Turkey (DSD, intersex, ambiguous genitals, dubious genitals) and asked which ones they had ever heard of. To evaluate which terms they were comfortable with, they were asked which names they use while talking to others, such as their spouses, their doctors and their relatives. They were also asked the term their doctors use and if different doctors use different names.

There were some difficulties while translating the study into English. In Turkish, there is no term as an exact translation of "intersex". Instead, *"çift cinsiyet"* is in use (which can be translated as *double sex* in English). As *"çift cinsiyet"* is used as a translation of intersex in Turkish, implementing a meaning close to a third sex, the word *intersex* will be used in the text for ease of reading. Another interesting term in Turkish is *"kuşkulu genital yapı"* which is a distorted translation of "ambiguous genitals". It will be used with its exact translation which is *"dubious genitals"* in the text. Our perinatologists tend to use this term, which is an acceptable catch-all phrase which avoids naming a specific diagnosis before consulting the DSD council. The term "ambiguous genitals" is included in the study as a different heading because it is used in Turkey in Latin form without being translated and therefore generates a different perception. The families mostly use it as *ambiguous* solely without understanding the meaning. In daily Turkish language, most medical terms are used in Latin, French or English, either in the original or slightly corrupted forms. Therefore, unlike parents in English-speaking countries, the word ambiguous probably appears as another disease name in Latin for them. Besides the evaluation of terminology, the parents knew and tend to use, the results were analyzed to assess the effects of different parameters on the terminology families used. These parameters included primary diagnosis, age at diagnosis (in the newborn period or later), duration of follow-up (less or more than five years), year of diagnosis (before or after 2006-year of the Chicago consensus meeting), appearance of external genitals, need for sex reassignment, need for name change, having a sibling with the disease, family history, history of admission to different hospitals and the department mostly involved in the management.

To evaluate if the appearance of external genitals had an impact on families' preference for the terminology, we divided patients into two groups; those who have atypical genitals and those that do not (8). Atypicality of genitals was defined as relative to the gender of rearing before reconstructive surgery. For patients reared as female, normal female and Prader Stage 1 were considered typical; Prader Stage 5 and normal male atypical. Likewise, for patients

reared as males, Prader 5 and normal male were considered typical; normal female and Prader 1 atypical. Prader 2, 3 and 4 were grouped as atypical for both.

Statistical Analysis

It was carried out using the SPSS statistical package (SPSS for windows V.16, SPSS, Chicago, IL, USA). To evaluate the effect of different variables on the terminology families used, comparisons were made using logistic regression analysis after transforming the data into dichotomous variables. Hosmer-Lemeshow goodness of fit test was used to assess model fit. A 5% type-1 error level was used to infer statistical significance.

Results

In total 121 patients were evaluated at monthly meetings of the DSD council during the study period. Twenty-five patients with diagnoses such as MRKH, severe hypospadias and bilateral undescended testes (whose families were presumably not familiar with any of the DSD terminology) were excluded from the study. Among the rest, nine families could not be reached and four families had two affected offspring, both followed in our institution. Therefore, 79 inquiries were completed.

Median (range) age at diagnosis was 1 year (0-16 years) and 41% of the patients were diagnosed in the newborn period. Median (range) follow-up was 5 years (1-19 years). Follow up period was longer than five years in 56%. Reasons for admission at the time of diagnosis were atypical genital appearance in 55 (70%), delayed puberty in 12 (15%), inguinal hernia in seven (8%), short stature in three (4%), symptoms of salt depletion in one (1%) and incidentally during the evaluation of a syndromic child in one (1%).

Seven patients (9%) had a history of sex reassignment and six of these also had their names changed. Four families had more than one affected child and nine parents, including these, had a family history. Forty-seven parents (41%) had been admitted to another center before referral to our institution. The majority of the parents (73%) indicated that endocrinology was the department mostly involved in the management of their children. Fifty-six (71%) children had atypical external genitalia for the gender they were reared as.

Sixty (75%) parents stated that they thought they had enough knowledge about the disease and 27 (34%) parents thought that their child knew what his/her disease was. It was noted that parents were comfortable while using the terms hypospadias or congenital adrenal hyperplasia (CAH). However, they avoided using the word "sex" during the questionnaire. An interesting observation was that some parents only said CAH when they were asked the names they know and used only CAH during the entire questionnaire. At the end, when they were asked about their knowledge of the remaining nomenclature, they first explained the pathology in the adrenal gland in detail and that the genital abnormality was secondary to it.

When asked about the terms that they could recall about the disease, 40 parents said specific disease names, mostly with Latin origin (which were hypospadias, adrenal insufficiency, testicular feminization, androgen insensitivity, CAH and 5-alpha reductase deficiency), 14 said chromosomal abnormality, 11 used the word *ambiguous*, seven referred to the name of the syndrome their child had, five said intersex and only two parents mentioned DSD. Seven parents said that they did not know any terms related to their child's condition (Figure 1). The parents who referred to the disease as a chromosomal abnormality were the parents of children who had chromosome-gender mismatch. One parent mentioned an old Turkish word of Arabic origin *(hünsâ; khunsa)* which is not in daily use (9). When they were asked if they ever heard of the terms commonly used; 42 mentioned intersex, 40 DSD, 39 dubious genitals and 36 *ambigious* (Figure 2).

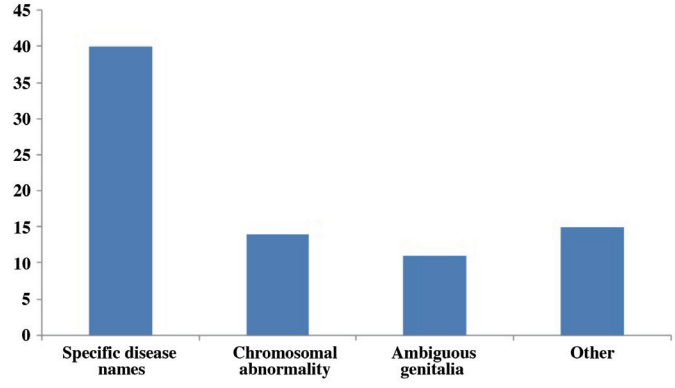

Figure 1. The terms parents expressed (answer to question #8)

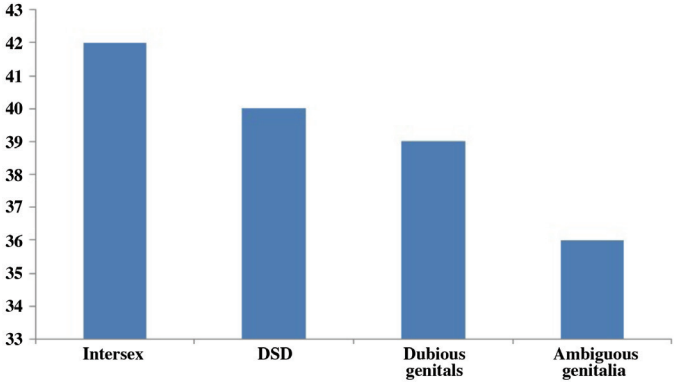

Figure 2. The terms parents were familiar with (answers to question #15)

DSD: disorders of sex development

There were also questions from which we were not able to collect any comparable data. Parents were asked which terms they use when talking to their spouse, to the doctor and to relatives. The majority replied that they did not use any terms while talking to their spouse or with the doctor because everybody knew what the issue was. They also stated that they do not talk to their relatives or friends about the disease at all. They were also asked for any ideas for a new terminology, but none of the parents made any suggestions.

When the state of knowledge about each term was evaluated using independent variables, statistically significant differences were revealed between the following pairs: the term "chromosomal abnormality" or "expression of a specific disease name" and the department mostly involved in the management; the term "dubious genitals" and the diagnosis in the newborn period.

Fourteen parents (24%) whose children were mainly followed by the endocrinology department stated the disease was a chromosomal abnormality while none of the parents who were followed by pediatric urology did ($p = 0.024$). Expression of a specific disease name was also found relevant to the department mostly involved in the management ($p = 0.048$). Twenty-three parents (41%) whose children were mainly followed by the endocrinology department used a specific disease name while 16 parents (80%) who were followed by pediatric urology did so.

Twenty-two of 32 (69%) parents whose children were diagnosed in the newborn period knew the term dubious genitals versus 17 of 47 (36%) who were diagnosed later ($p = 0.046$). No statistically significant difference was found between the remaining parameters.

Discussion

Gender is one of the major aspects of personality. Construction of a scientific terminology about a disease that interferes with gender, which is not pejorative but definitive is difficult. As Feder and Karkazis (2) perfectly describe, there is probably no terminology that can eradicate the stigma and no nomenclature that can position this group of conditions in the usual medical way. Unfortunately, parents' perception of the terminology has a direct impact on their perception of the disease which affects how they and their child cope with the disease.

Changing the terminology to DSD with the consensus statement in 2006 received widespread acceptance among clinicians (3). However, its perception was not the same for everyone. Linguistic, religious and cultural factors influence how the lexicon is understood. One major criticism about DSD was the disturbing effect of the word "disorder" (4). Besides the worldwide debates around it, as in German the Turkish equivalent of the word "disorder" in the phrase DSD is probably more disturbing than the English version (10). It has a meaning closer to failure or defect than disorder. It also does not have a widespread use in medical terminology. Not only the nomenclature, but also the perception and management of DSD are prone to intercultural differences (11). Some cultures do not allocate sex at birth with the belief that it can change later (12). However, gender is the major determinant of a human's entire life in many Eastern countries. Islam has a comprehensive attitude towards DSD including prayers, obligations and gender roles in society (9). Turkey is a multicultural country where the majority of inhabitants have a social life influenced both by modern European society and Islamic beliefs. In our country, any problem related to sex will cause shame, can hinder a marriage and even affect one's work life. Therefore, nomenclature of DSD is perhaps even more important to prevent stigmatization. Our study confirmed the importance of this issue by revealing the parents' tendency to avoid the word "sex" during the interview.

Doctors and activists play the main roles in constituting the terminology (1,2,4,13). Affected people (children with the disease and their families) who are at the center of the arguments are mostly not a part of decision-making. Few studies have been conducted to consider the perspective of families (3,5,14,6).

JH Davies, who is one of the proponents of the new terminology, evaluated the acceptability of the new terminology among 19 parents of children with DSD. The majority stated they preferred DSD over intersex although few found it an adequate term (3). Lin-su et al (14) interviewed a larger group (128 CAH patients, 408 parents) and stated that the majority of the patients did not like the new terminology and that it caused negative connotations. An activist, Davis, conducted interviews with patients and clinicians and argued that the patients do not like the term and the doctors' insistence on the DSD terminology was a reassertion of their medical authority (6,7). She says the patients who embrace the new terminology are the ones who are not contented with themselves and who find themselves abnormal (7). Ellie Magritte (5), the mother of a child with DSD, used the acronym DSD when referring to the disease writing both forms (disorder/difference of sex development) and emphasized how disturbing the ambiguity was.

Our study was consistent with earlier ones showing a lack of acceptance of the term DSD by the families despite the

worldwide use of it among clinicians (6,7,14,15). Half of the families admitted they knew the term and only two parents recalled it before being reminded by the interviewer.

Lin-Su et al (14) thought that health professionals did not use the term in their daily routine with their patients. This probably has an effect but even the families who admitted knowing it did not use the term. Most of the families in our study tended to use specific disease names mostly of Latin origin. This supports Karzakis' ideas (16) which emphasize the importance of recasting diverse diagnoses rather than keeping them as types of people whose care is directed at correcting sexual difference. Davies et al (3) and Dreger et al (17) also recommended temporary usage of the term DSD until specific diagnoses have been made.

There is no consensus on the terminology for DSD in Turkey. International Classification of Diseases-10 still refers to the disease as hermaphroditism. Doctors use intersex or DSD while talking to each other, prefer to use *çift cinsiyet (double sex)* while talking to media, and mostly avoid using any specific term while communicating with their patients. They can also use specific disease names or some jargon such as *kuşkulu genital yapı (ambiguous genitalia)*. There is no common patient-oriented language. We have discussed the issue in our multidisciplinary council and decided to use DSD. However, our study revealed probable lack of compliance with this decision and reflected the effect of doctors' use of terminology on parents. When asked, the parents stated that their doctors did not use any names for the disease. The findings show that the department mostly involved in the management was a factor affecting their preferences. Eighty percent of patients whose parents named the disease as "hypospadias" were mainly followed by pediatric urology, and all the parents who referred to the disease as a "chromosomal abnormality" were mainly followed by endocrinologists. This can be attributed to the need for fewer endocrinology consultations for patients without chromosome to gender mismatch but also reflects the preferences of the doctors.

We believe that specific disease names of Latin origin enhance acceptance of the subject as a medical problem, not a social one. We understand that this may not be acceptable to an adult with DSD, however families' perception and therefore attitude towards the disease designates the adult that the child would become. None of the current terms are adequate and a terminology covering the will of both patients and families has yet to be developed.

Another interesting finding of our study is the significant difference in those knowing the term *dubious genitals (kuşkulu genital yapı)* if the condition was diagnosed as a newborn. After referral to the DSD council, families probably do not hear this term again. However, half of the families who were diagnosed in the newborn period recalled it. This not only shows the effect of doctors' preferences but also emphasizes the persisting impact from their first contact with the disease.

Genital atypicality, sex of rearing and the gender reassignment process were reported to cause more stigmatization of parents (8). Therefore, the effect of these variables on parents' choice of terminology was also analyzed although no relationship was found.

Unlike other studies (3), parents stated they were satisfied with their level of knowledge about the condition. This may be due to close communication with their doctors or less expectation due to cultural motivations. DSD is a subject that is very hard to discuss in our country. Long explanations of the DSD council given by each department individually may be more than enough for the families who have never heard of and may even be trying to ignore the subject.

Study Limitations

This study tried to evaluate the parental perception about the nomenclature of DSD; however, it was performed as a single center study in Western Turkey. Therefore, it may not reflect the opinion of all population. Also, there were semantic losses while translating the study to English. The authors tried to cover these shortcomings with a detailed methods section.

Conclusion

Introduced with the hope of defeating the discomfort of patients and families, the term DSD does not seem to find use among the parents of patients. Parents of our DSD patients avoid using any word containing "sex" and prefer the specific disease names mostly with Latin origin instead. Their preferences were also found to be influenced by their doctors. Each country has its own social norms; therefore, local studies reflecting the linguistic and cultural differences and their uniform usage by doctors are mandatory to avoid negative connotations in the families' minds.

Acknowledgment

We would like to thank Şeref Etker for sharing his expertise and valuable comments that greatly improved our manuscript.

Ethics

Ethics Committee Approval: Ethical approval was taken from the Ege University, Clinical Research Ethics Committee (decision number: 16-2.1/1).

Informed Consent: It was taken from each parent.

Peer-review: Internal and external peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Sibel Tiryaki, Ali Tekin, İsmail Yağmur, Samim Özen, Burcu Özbaran, Damla Gökşen, Şükran Darcan, İbrahim Ulman, Ali Avanoğlu, Concept: Sibel Tiryaki, Ali Avanoğlu, Design: Sibel Tiryaki, Ali Tekin, İsmail Yağmur, Samim Özen, Burcu Özbaran, Damla Gökşen, Şükran Darcan, İbrahim Ulman, Ali Avanoğlu, Data Collection or Processing: Sibel Tiryaki, Analysis or Interpretation: Sibel Tiryaki, Ali Tekin, İsmail Yağmur, Samim Özen, Burcu Özbaran, Damla Gökşen, Şükran Darcan, İbrahim Ulman, Ali Avanoğlu, Literature Search: Sibel Tiryaki, Ali Tekin, İsmail Yağmur, Samim Özen, Burcu Özbaran, Damla Gökşen, Şükran Darcan, İbrahim Ulman, Ali Avanoğlu, Writing: Sibel Tiryaki, Ali Tekin, İsmail Yağmur, Samim Özen, Burcu Özbaran, Damla Gökşen, Şükran Darcan, İbrahim Ulman, Ali Avanoğlu.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Hughes IA, Houk C, Ahmed SF, Lee PA; Lawson Wilkins Pediatric Endocrine Society/European Society for Paediatric Endocrinology Consensus Group. Consensus statement on management of intersex disorders. J Pediatr Urol 2006;2:148-162. Epub 2006 May 23
2. Feder EK, Karkazis K. What's in a name? The controversy over "disorders of sex development." Hastings Cent Rep 2008;38:33-36.
3. Davies JH, Knight EJ, Savage A, Brown J, Malone PS. Evaluation of terminology used to describe disorders of sex development. J Pediatr Urol 2011;7:412-415. Epub 2010 Aug 14
4. Topp SS. Against the quiet revolution: The rhetorical construction of intersex individuals as disordered. Sexualities. 2013;16:180-194.
5. Magritte E. Working together in placing the long term interests of the child at the heart of the DSD evaluation. J Pediatr Urol 2012;8:571-575. Epub 2012 Sep 12
6. Davis G. "DSD is a perfectly fine term": Reasserting mediacal authority through a shift in intersex terminology. In: McGann PJ, Hutson DJ (eds). Advances in Medical Sociology - Sociology of Diagnosis. Emerald Group Publishing Ltd, 2011;155-182.
7. Davis G. The power in a name: diagnostic terminology and diverse experiences. Psychol Sex 2013;1:15-27.
8. Rolston AM, Gardner M, Vilain E, Sandberg DE. Parental reports of stigma associated with child's disorder of sex development. Int J Endocrinol 2015;2015:980121.
9. Zainuddin AA, Mahdy ZA. The Islamic Perspectives of Gender-Related Issues in the Management of Patients With Disorders of Sex Development. Arch Sex Behav 2017;46:353-360. Epub 2016 Apr 21
10. Hughes IA, Nihoul-Fékété C, Thomas B, Cohen-Kettenis PT. Consequences of the ESPE/LWPES guidelines for diagnosis and treatment of disorders of sex development. Best Pract Res Clin Endocrinol Metab 2007;21:351-365.
11. Warne GL, Raza J. Disorders of sex development (DSDs), their presentation and management in different cultures. Rev Endocr Metab Disord 2008;9:227-236. Epub 2008 Jul 17
12. Affairs SSC on C. Community Affairs References Committee [Internet]. Involuntary or coerced sterilisation of intersex people in Australia. 2013. p. 14. Available from: http://parlinfo.aph.gov.au/parlInfo/ search/display/display.w3p;query = Id % 3A % 22 publications % 2Ftabledpapers % 2F70936 % 22
13. Lee PA, Wisniewski AB, Baskin L, Vogiatzi MG, Vilain E, Rosenthal SM, Houk C. Advances in diagnosis and care of persons with DSD over the last decade. Int J Pediatr Endocrinol 2014;2014:19.
14. Lin-Su K, Lekarev O, Poppas DP, Vogiatzi MG. Congenital adrenal hyperplasia patient perception of "disorders of sex development" nomenclature. Int J Pediatr Endocrinol 2015;2015:9. Epub 2015 Mar 16
15. Pasterski V, Prentice P, Hughes IA. Consequences of the Chicago Consensus on Disorders of Sex Development (DSD): Current practices in Europe. Arch Dis Child 2010;95:618-623. Epub 2009 Sep 22
16. Karkazis K, Feder EK. Naming the problem: disorders and their meanings. Lancet 2008;372:2016-2017.
17. Dreger AD, Chase C, Sousa A, Gruppuso PA, Frader J. Changing the Nomenculature/Taxonomy for Intersex: A Scientific and Clinical Rationale. Journal of Pediatric Endocrinology and Metabolism 2005;18:729-723.

Appendix 1: Questionnaire

Name: ..... **DOB:** .....

Hospital ID#: ..... **Phone Number:** .....

Diagnosis: .....

1. When and how was your child diagnosed?

.....

2. With what gender your child was raised before admission? Was it changed? Was also the name changed? When?

.....

3. Do you have any other child affected? Anyone else in your family?

.....

4. Which institutions were you referred to for your child's condition? If you changed your doctor in the process, was it your choice?

.....

5. Do you think you have enough knowledge about your child's disease?

.....

6. Does your child know about his/her condition?

.....

7. Do you talk to your child about the condition?

.....

8. There are many terms used to refer to this condition. Which ones do you know?

.....

9. Which department is the one most involved in your child's management? Do you know the terms your doctors use?

.....

10. Do different doctors use different terms?

.....

11. Which term do you prefer to use when talking to your husband/wife?

.....

12. Which term do you prefer to use when talking to your child's doctor?

.....

13. Which term do you prefer to use when talking to your relatives or friends?

.....

14. Do you have a suggestion for a more proper term?

.....

15. Have you ever heard of the terms listed below?

.....

DSD, intersex, dubious genitals, ambiguous genitals

ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.5506

J Clin Res Pediatr Endocrinol 2018;10(3):223-229

Investigation of MKRN3 Mutation in Patients with Familial Central Precocious Puberty

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What is already known on this topic?

Since 2013, the underlying aetiology of some cases of familial idiopathic central precocious puberty (iCPP) has been elucidated. However, data on the incidence of these new aetiologies of familial iCPP in Turkish populations is scarce.

What this study adds?

This study showed a low rate of MKRN3 mutation in cases of familial idiopathic central precocious puberty (iCPP) in Turkey. This case series highlights the importance of always obtaining a good family history when investigating cases of iCPP as this may hasten diagnosis and help identify gene targets for investigation.

Abstract

Objective: There have been recent advances in the understanding of the etiology of idiopathic central precocious puberty (iCPP) including new genetic associations. The aim of this clinical study was to determine the frequency of MKRN3 mutation in cases of familial iCPP.

Methods: Potential sequence variations in the maternally imprinted *MKRN3* gene were evaluated in 19 participants from 10 families using next-generation sequencing analysis.

Results: MKRN3 variation was found in only one of the 19 (5.3%) subjects. The male patient, who had a medical history of precocious puberty, had a heterozygous mutation, NM_005664.3:c.630_650delins GCTGGGC (p.P211Lfs*16). The father of this patient also had a history of precocious puberty and had the same mutation. p.P211Lfs*16 is a novel variant and it was identified as probably pathogenic by *in silico* analysis, consistent with the clinical findings.

Conclusion: Given that MKRN3 mutation was detected in only one patient, with a paternal history of precocious puberty, this reinforces the importance of accurate family history taking. The detected incidence of *MKRN3* variants in our case series was much lower than reported elsewhere which suggests a need for further studies in Turkish iCPP patients.

Keywords: MKRN3 mutation, familial central precocious puberty, genetic analysis

Introduction

Central precocious puberty (CPP) is defined as the development of secondary sex characteristics before eight years of age in girls and nine years of age in boys, due to early activation of the hypothalamic-pituitary-gonadal (HPG) axis (1,2). Owing to recent advances in genetics, the underlying aetiology has been revealed in some cases of idiopathic CPP (iCPP). Gain-of-function mutations in the *KISS1* and *KISSR1* genes and loss-of-function mutations in the makorin ring finger protein 3 (*MKRN3*) gene were shown to result in CPP (3,4,5).

The *MKRN3* gene product exerts an inhibitory effect on gonadotropin releasing hormone (GnRH) neurons. It has been proposed that the HPG axis is reactivated by loss-of-

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Conflict of interest: None declared
Received: 22.11.2017
Accepted: 29.01.2018

function mutations in the *MKRN3* gene (6). It was reported that the frequency of *MKRN3* was higher in cases with familial iCPP compared with sporadic cases (7,8). However, the frequency can vary according to ethnicity (9). The aim of this clinical study was to determine the frequency of MKRN3 mutation in a group of Turkish families with familial iCPP.

Methods

The study included siblings diagnosed with iCPP and iCPP cases with a positive family history who presented to the Endocrinology Outpatient Clinic of Dr. Sami Ulus Obstetrics and Gynaecology, Children's Health and Disease Training and Research Hospital. All parents gave written informed consent before participation. The study was approved by the Ethics Committee of the Zekai Tahir Burak Maternity Teaching Hospital, Ankara, Turkey (46/2015). All children included in the study had at least one first or second degree relative with documented iCPP.

The Tanner and Marshall (10,11) criteria were used for puberty staging. Girls who had at least Tanner stage 2 breast development and stage 2 pubarche before eight years of age were assessed as cases of early puberty. Boys who had at least Tanner stage 2 testicular volume (>4 mL) or stage 2 pubarche before nine years of age were assessed as cases of early puberty.

In the girls luteinising hormone (LH), follicle-stimulating hormone (FSH) and 17β-estradiol (E2) were measured in a morning blood sample. A basal serum LH level ≥0.83 mIU/mL, with puberty precocious findings described above, was accepted as activation of the HPG axis (12). Cases with a basal LH level <0.83 mIU/mL underwent the standard stimulation test of 100 μg GnRH (Ferring Pharmaceuticals, Inc., Parsippany, NJ, USA) by intravenous injection between 8:00 and 8:30 am to assess early puberty. Blood samples were taken at 0, 30, 60, 90, and 120 min to measure serum LH and FSH levels. Peak LH ≥3.3 mIU/mL was accepted as the diagnostic criterion for activation of the HPG axis in girls (13). In boys, LH, FSH and testosterone were measured also in a morning blood sample. A basal serum LH level ≥0.83 mIU/mL, with puberty precocious findings described above, was accepted as activation of the HPG axis in boys (12). Cases with a basal LH level <0.3 mIU/mL underwent the standard stimulation test described above. Peak LH ≥4.1 mIU/mL was accepted as the diagnostic criterion for activation of the HPG axis (13).

Congenital adrenal hyperplasia was excluded by 17-hydroxyprogesterone (17-OHP) <1.5 ng/mL in early morning samples and/or peak 17-OHP <10 ng/mL following an ACTH stimulation test. Cranial magnetic resonance imaging (MRI) was performed to exclude any organic lesion in all cases diagnosed with CPP.

Standing height was measured to the nearest 0.1 cm with a Harpenden fixed stadiometer (Seritex, North America). Body weight was measured on a balance scale (SECA, North America) to the nearest 0.1 kg. Height and weight standard deviation score (SDS) were calculated by comparison with Turkish national reference data (www.ceddcozum.com) (14). Adult height prediction was calculated by dividing the height by the decimal fraction, using the table for predicting adult stature as described by Greulich and Pyle (15).

LH, FSH, and E2 levels were measured with an immunochemiluminometric assay using an Advia Centaur immunoanalyzer (Bayer Diagnostics, Tarrytown, NY, USA). Bone age (BA) was assessed according to the Greulich and Pyle (15) Atlas method.

Genetic Analysis

Genomic DNA was isolated from ethylenediamine tetraacetic acid blood sample by Magnesia DNA isolation Kit (Anatolia Geneworks, İstanbul, Turkey). Sequencing study was done by NGS technology and it was performed using the MiSeq next generation sequencing platform (Illumina Inc., San Diego, CA, USA). All coding exons of *MKRN3* and flanking regions were amplified using polymerase chain reaction (PCR) primers, designed with PRIMER-Primer Designer v.2.0 software (Scientific and Educational Software program). Amplicon libraries were prepared with the NexteraXT kit (Illumina Inc.). Sequences were aligned to the hg19 genome with MiSeq Reporter software (Illumina Inc.). Detection of variants was performed with IGV 2.3 (Board Institute) software. *In silico* analysis, database search and literature evaluations were done by Varsome, Polyphen2, HGMD-Public, PubMed, Google search, Clinvar, EXAC and 1,000 Genome studies.

Statistical Analysis

The data obtained were evaluated using the SPSS 16.0 software programme (SPSS Inc., Chicago, IL, USA).

Results

The study included 19 patients with CPP from 10 families. In the familial CPP group, there were 17 girls and two boys (one boy with a paternal history of precocious puberty) from 10 families. Clinical, anthropometric and biochemical data of the included patients and their parents are displayed in Tables 1 and 2. Among the 17 girls with familial iCPP, mean age at the onset of secondary sex characteristics

Table 1. Clinical, anthropometric, and biochemical characteristics of patients

Family/ patient no.	Current age, years	Puberty in parents	Sex	First clinical sign	Onset of puberty, years	Age at GnRH stimulation test, years	Tanner stage (T/P)	Height (cm)/ height SD	Weight (kg)/ weight SD	Bone age, years	Growth velocity (cm) year/ treatment	LH, mIU/mL basal/ stimulated	FSH, mIU/ mL basal/ stimulated
1.1	10.3	M: menarche 11 years F: 13 years	Female	T	7.4	7.8	3/2	130/0.8	29/0.8	10	7/+	<0.07/3.3	2.95/18
1.1	6.5		Female	T	2	3.3	2/1	94.6/-0.6	13/0.9	3.5	-/+	0.32/6.2	3.97/26.3
2.1	11.3	M: menarche 12.5 years F: onset at 14 years	Female	P	7	7.7	2/2	137.1/2.3	31/1.3	8.9	7/+	<0.07/8.2	<3/9.6
2.2	14.5		Female	P	5.9	6	2/2	122.1/1.4	23.9/0.9	7.9	-/+	<0.07/5.7	3.6/18.3
3.1	12.3	M: menarche 11.5 years F: unknown	Female	P	4.8	5	2/1	106.6/-0.6	16/-1.1	5	-/+	<0.1/5.3	1.48/24.5
3.2	16.3		Female	T	5.5	8.3	3/2	126.4/-0.3	26.8/0.1	11	5.4/+	<0.5/5.25	2.6/22.3
4.1	12.1	M: menarche 12 years F: onset at 14 years	Male	P	8.9	9.9	2**/3	142.4/1	35.3/0.6	11.6	6.5/+	0.87/18.3	<3/3.54
4.2	14.2		female	T	7.9	-	3/2	136.4/1.3	36.7/1.6	10	-/+	1.11/-	6.83/-
5.1	10.7	M: menarche 13.5 F: unknown	Female	T	6.3	7.1	3/1	123.1/0.2	24.5/0.3	8.9	6.7 /+	<0.07/4.67	<3/28.4
5.2	13.1		Female	T	7.9	9	3/1	143/1.9	45.4 /2.3	11	-/+	0.13/11	1.22/7.8
6.1	10.9	M: menarche 9.5 years F: onset at 13 years	Female	T	5.6	6.8	2/2	125.2/1	25.6/0.8	10	-/+	<0.07/23.1	<3/13.3
6.2	8.6		Female	T	6	6.1	2/2	122/1.4	27.8/1.8	8.9	-/+	<0.07/4.68	0.44/9.8
7.1	12.3	M: menarche 12 years F: unknown	Female	P	7	7.3	2/2	125.9/0.6	23.8/0	8.9	-/+	0.35/9.1	7.93/41
7.2	16		Female	P	7.5	7.5	2/2	129/1	26/0.4	8.9	-/+	0.1/6.2	3.7/19.4
8.1	11.5	M: menarche 12 years F: unknown	Female	T	7.5	7.6	2/2	117.6/-1.3	24.3/0	7.9	-/+	<0.07/5.87	5.56/16.6
8.2	14.9		Female	T	7	7.1	2/2	121.5/0	22.7/0	7	-/+	0.14/6.56	1.3/9.4
9.1	12	M: menarche 12 years F: onset at 12 years	Female	T	8	9.1	3/4	129.9/-0.5	25.3/-0.8	11	5/+	0.14/3.69	1.73/17.6
9.2	10.5		Female	T	7	7.4	2/1	115.4/-1.5	21.6/-0.7	8.9	-/+	0.16/6.5	1.79/19.8
10.1*/&	13.2	M: menarche 13.5 years F: onset at 9 years	Male	Acne, beard	9	-	4**/4	156.5/1.27	44/0.3	14	Near final/-	5.41/-	13.7/-

M: Mother, F: Father, T: Thelarche, P: Adrenarche, *: The father had a history of precocious puberty, **: Tanner stage for male, &: MKRN3-mutation-positive patient, SD: standard deviation, LH: luteinising hormone, FSH: follicle-stimulating hormone, GnRH: gonadotropin releasing hormone

was 6.5 ± 1.5 years, and mean age at treatment onset was 7.2 ± 1.4 years. In this group, the mean BA was 8.7 ± 2.0 years, and the BA:CA ratio was 1.2 ± 0.1. The 17-OHP level was normal in all cases with pubarche. Therefore, none of the patients proceeded to an ACTH stimulation test. Cranial MRI was normal in all cases.

Among the whole group, a novel heterozygous mutation, MRKN3:NM_005664.3:c.630_650delinsGCTGGGC (p.P211Lfs*16), was detected in only one boy with a paternal history of precocious puberty (Figure 1). A flow chart of patient and family recruitment into the study is shown in Figure 2. *MKRN3* gene analysis was performed only in this patient's father. We did not have the opportunity to study the genotype in his remaining family members. The patient with MKRN3 mutation presented with facial hair growth at 11 years and 7 months of age. Facial hair growth had appeared 1.5 years earlier. Family history revealed that facial hair growth had appeared at the same age in his father. The patient's brother is unaffected and was found to be pre-pubertal in the examination performed at 10 years of age. The patient's physical examination yielded the following findings: height, 156.5 cm [+1.27 standard deviation (SD)]; body weight, 44.6 kg (+0.3 SD); 15 mL testicular volume bilaterally; stage 5

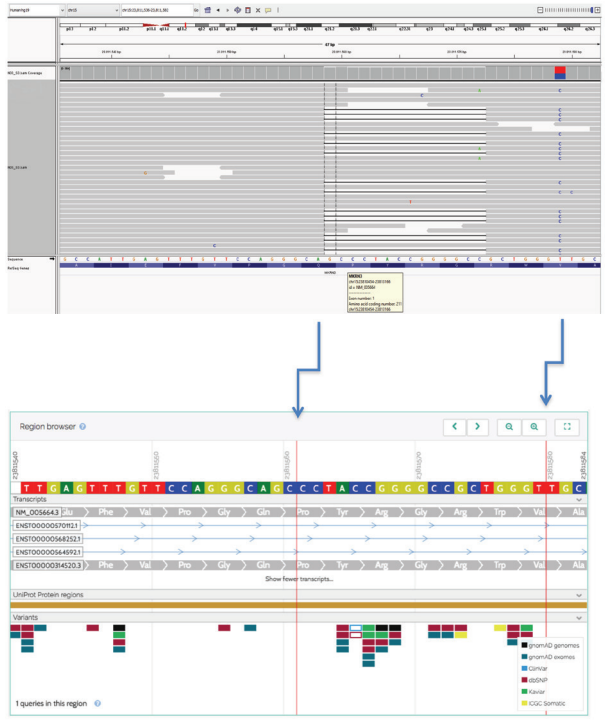

Figure 1. Mutation image of *MKRN3* gene of the patient with IGV2.3 software [NM_005664.3:c.630_650delins GCTGGGC (p.P211Lfs*16)] and Varsome software image

Table 2. Anthropometric characteristics of patients' parents, target and predicted height of patients

Family/ patient no.	Current age, years	Sex	Onset of puberty, years	Mother's height (cm)/ SD	Father's height (cm)/ SD	Target height cm/ SD	Predicted height (cm)/SD	Difference in height SD-target height SD	Difference in target height SD-predicted height SD
1.1	10.3	Female	7.4	151/-1.86	168/-1.17	153/-1.55	143.5/-3	2.4	1.45
1.1	6.5	Female	2	151/-1.86	168/-1.17	153/-1.55	-	1	-
2.1	11.3	Female	7	164.9/0.27	171/-0.76	161.5/-0.26	167 /0.6	2.6	0.86
2.2	14.5	Female	5.9	164.9/0.27	171/-0.76	161.5/-0.26	156.1/-1.1	1.7	0.84
3.1	12.3	Female	4.8	149.8/-2.04	164/-1.7	150.4/-1.95	-	1.4	-
3.2	16.3	Female	5.5	149.8/-2.04	164/-1.7	150.4/-1.95	139.5/-3.6	1.7	1.65
4.1	12.1	Male	8.9	149/-2.16	167/-1.3	164.5/-1.65	174 /-0.4	2.65	-1.25
4.2	14.2	Female	7.9	149/-2.16	167/-1.3	151.5/-1.78	158.2/-0.8	3.1	-0.98
5.1	10.7	Female	6.3	153.8/-1.43	173/-0.49	156.9/-0.96	150 /-2	1.2	1.04
5.2	13.1	Female	7.9	153.8/-1.43	173/-0.49	156.9/-0.96	158/-0.8	2.9	-0.16
6.1	10.9	Female	5.6	159/-0.64	167/-1.31	156.5/-1.02	145.2/-2.8	2	1.78
6.2	8.6	Female	6	159/-0.64	167/-1.31	156.5/-1.02	148.5/-2.2	2.4	1.18
7.1	12.3	Female	7	167/0.59	172/-0.6	163/0	153.3/-1.5	0.6	-0.6
7.2	16	Female	7.5	167/0.59	172/-0.6	163/0	157.1/-0.9	1	-1
8.1	11.5	Female	7.5	152.1/-1.7	169/-1	154/-1.4	150.3/-2	0.1	1.5
8.2	14.9	Female	7	152.1/-1.7	169/-1	154/-1.4	160.5/-0.4	1.4	-2.8
9.1	12	Female	8	152/-1.7	175/-0.2	157/-0.9	143.3/-3	0.4	-1.3
9.2	10.5	Female	7	152/-1.7	175/-0.2	157/-0.9	140.5/-3.5	-0.6	-0.3
10.1*/&	13.2	Male	9	167/0.6	159/-2.4	169.5/-1	168.8/-1.1	2.3	-3.3

*: The father had a history of precocious puberty, &: MKRN3-mutation-positive patient, SD: standard deviation

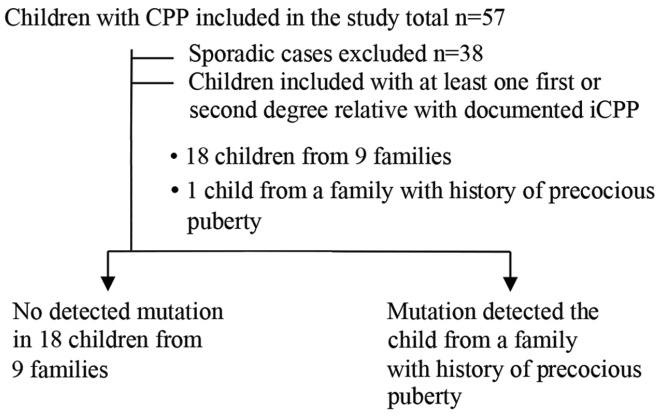

Figure 2. Flow chart of the study recruitment

CPP: central precocious puberty, iCPP: idiopathic central precocious puberty

pubarche; and axillary hair growth. The heights of mother and father were 167 (+0.6 SDS) and 159 cm (-2.4 SDS), respectively. The patient's target height and predicted height were estimated to be 169.5 cm (-1 SDS) and 168.8 cm (-1.1 SDS), respectively. Routine biochemistry tests and complete blood count were normal. The hormone test results were as follows: LH, 5.4 mIU/mL; FSH, 13.7 mIU/mL; total testosterone: 393.3 ng/dL; 17OHP: 0.6 ng/mL; and dehydroepiandrosterone sulphate, 60.4 mcg/dL. BA 14 years. The same mutation was also detected in his father. The physical examination of the other boy with no mutation showed height 142.4 cm (1 SDS), weight 35.3 kg (0.6 SDS); 6 mL testicular volume bilaterally; stage 3 pubarche; and axillary hair growth. The heights of mother and father were 149 cm (-2.16 SDS) and 167 cm (-1.3 SDS), respectively. The patient's target height and predicted height were estimated to be 164.5 cm (-1.65 SDS) and 174 cm (-0.4 SDS), respectively. The patient was followed-up without treatment due to slowly progressive puberty.

This mutation is a frame shift variant and causing production of a truncated protein with 226 amino acids while the wild type protein consists of 507 amino acids. Mutation taster predicts this variant as a disease-causing mutation, probably due to loss of function.

Discussion

MRKN3, which encodes the *MKRN3*, is an intronless gene located on chromosome 15q11.2 in the Prader–Willi syndrome critical region (16). The imprinted *MKRN3* gene is expressed only in the paternal allele, and it affects both sexes equally, in contrast to female preponderance in iCPP cases (16). The presence of a history of paternal precocious puberty, shorter final height and detection of *MRKN3* gene mutation confirm paternal inheritance. The MRKN3 protein, a product of this gene, includes two copies of a C3H motif in the N-terminal, a novel Cys–His configuration, a C3HC4 RING zinc finger, and a final C3H motif (6). A novel frameshift mutation (between C3H motifs in the N-terminal) in the imprinted *MKRN3* gene was identified in one male case and his affected father. *In silico* analysis suggested that this variant would be pathogenic. Scrutiny of human genetic variant databases revealed that this variant had not been previously reported.

In their study, Abreu et al (5) found a loss-of-function mutation in the *MKRN3* gene associated with familial iCPP. This work led to an investigation of the mechanism underlying familial iCPP, which has been important not only for understanding iCPP but also for a better understanding of the timing of normal puberty in humans. Since 2013, MKRN3 mutation has been the most frequently identified genetic cause of iCPP. The authors screened 40 individuals with familial iCPP from 15 families for MKRN3 mutations, and reported identifying MKRN3 mutation in 15 individuals from five families (37.5%) (5). In another study, MKRN3 mutation was detected in 13 of 28 cases (46%) with familial iCPP, and in only one of 18 cases with sporadic iCPP (7). In a study of 20 boys with iCPP from 17 families, Bessa et al (17) detected MKRN3 mutation in eight boys from five families. The authors emphasised the importance of investigating boys with MKRN3 mutation and a history of paternal precocious puberty. In a recent study from Turkey, Simsek et al (18) reported that two heterozygous frameshift mutations were identified in the *MKRN3* gene in two probands with familial iCPP and in seven patients with iCPP, as well as 11 unaffected family members. We investigated 19 individuals from 10 families with iCPP and found one novel frameshift (5.3%) mutation. Simsek et al (18) reported that due to the imprinted pattern of inheritance, the phenotype skipped one generation in one family because the proband's father and paternal uncle had inherited the mutated allele from their mothers. They also showed that in another family, because the proband's father and affected paternal cousin's father had inherited the mutated allele from the paternal grandfather, the phenotype was present in the second and third generations. A paternal aunt in the latter family also had iCPP, but her children were asymptomatic carriers of the same mutation. As those authors suggested, and as the history of our patient with MKRN3 mutation highlights, an accurate family history is extremely important, as it can reveal the paternal inheritance of familial iCPP due to a mutation in MRKN3. Physicians should consider this

type of inheritance in patients with iCPP thus allowing targeted *MKRN3* genetic analysis, thereby providing an additional tool for the diagnosis of children with iCPP.

In boys, there may be delay in recognising indicators of precocious puberty compared with those (thelarche, menarche) in girls (5,8,9,19,20). The findings of precocious puberty were not recognised by the family in our MKRN3 mutation case, and he presented at the hospital at a late pubertal stage, when he began to shave his facial hair. In the literature, the mean age at onset of puberty was reported as 8.2 years in 13 boys with MKRN3 mutation (5,17,19). Given that age at onset of puberty is approximately six years of age in girls with MKRN3 mutation (5,16,20), pubertal onset appears to be more precocious in affected girls (around two years) than in affected boys (around 0.8 years). In addition, the time from the onset of pubertal symptoms to diagnosis is longer in boys (5,21). It has been reported that puberty can be successfully suppressed by GnRH agonist treatment in cases with MKRN3 mutation and that menarche and other pubertal indicators show a normal course following treatment (5,7,22).

Study Limitations

The small number of patients and the wide range of criteria which were used to diagnose CPP were the limitations of this study.

Conclusion

MKRN3 mutation was detected in only one (5.3%) of 19 individuals from 10 families with familial CPP. Given the fact that the MKRN3 mutation was detected in only one patient with a paternal history of precocious puberty in our study, the importance of an accurate family history, which can reveal the paternal inheritance of familial iCPP due to a mutation in MKRN3, must be emphasized. Physicians should consider this type of inheritance in patients with iCPP thus facilitating targeted genetic analysis and providing an additional tool for the diagnosis of children with iCPP.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of the Zekai Tahir Burak Maternity Teaching Hospital, Ankara, Turkey (46/2015), and was conducted according to the principles of the Declaration of Helsinki.

Informed Consent: Written consent was obtained from all subjects and their parents before the study.

Peer-review: Internal and external peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Erdal Kurnaz, Şenay Savaş-Erdeve, Semra Çetinkaya, Zehra Aycan, Concept: Erdal Kurnaz, Şenay Savaş-Erdeve, Semra Çetinkaya, Zehra Aycan, Design: Zehra Aycan, Şenay Savaş-Erdeve, Gülay Ceylaner, Data Collection or Processing: Zehra Aycan, Şenay Savaş-Erdeve, Semra Çetinkaya, Erdal Kurnaz, Melikşah Keskin, Nursel Muratoğlu Şahin, Elvan Bayramoğlu, Analysis or Interpretation: Erdal Kurnaz, Şenay Savaş-Erdeve, Gülay Ceylaner, Literature Search: Erdal Kurnaz, Şenay Savaş-Erdeve, Writing: Erdal Kurnaz, Şenay Savaş-Erdeve, Zehra Aycan.

Financial Disclosure: The authors declare that this study received no financial support.

References

1. Diamantopoulos S, Bao Y. Gynecomastia and premature thelarche: a guide for practitioners. Pediatr Rev 2007;28:57-68.
2. Ibanez L, Virdis R, Potau N, Zampolli M, Ghizzoni L, Albisu MA, Carrascosa A, Bernasconi S, Vicens-Calvet E. Natural history of premature pubarche: an auxological study. J Clin Endocrinol Metab 1992;74:254-257.
3. Teles MG, Bianco SD, Brito VN, Trarbach EB, Kuohung W, Xu S, Seminara SB, Mendonca BB, Kaiser UB, Latronico AC. A GPR54-activating mutation in a patient with central precocious puberty. N Engl J Med 2008;358:709-715.
4. Silveira LG, Noel SD, Silveira-Neto AP, Abreu AP, Brito VN, Santos MG, Bianco SD, Kuohung W, Xu S, Gryngarten M, Escobar ME, Arnhold IJ, Mendonca BB, Kaiser UB, Latronico AC. Mutations of the KISS1 gene in disorders of puberty. J Clin Endocrinol Metab 2010;95:2276-2280. Epub 2010 Mar 17
5. Abreu AP, Dauber A, Macedo DB, Noel SD, Brito VN, Gill JC, Cukier P, Thompson IR, Navarro VM, Gagliardi PC, Rodrigues T, Kochi C, Longui CA, Beckers D, de Zegher F, Montenegro LR, Mendonca BB, Carroll RS, Hirschhorn JN, Latronico AC, Kaiser UB. Central precocious puberty caused by mutations in the imprinted gene MKRN3. N Engl J Med 2013;368:2467-2475. Epub 2013 Jun 5
6. Abreu AP, Macedo DB, Brito VN, Kaiser UB, Latronico AC. A new pathway in the control of the initiation of puberty: the MKRN3 gene. J Mol Endocrinol 2015;54:131-139.
7. Simon D, Ba I, Mekhail N, Ecosse E, Paulsen A, Zenaty D, Houang M, Jesuran Perelroizen M, de Filippo GP, Salerno M, Simonin G, Reynaud R, Carel JC, Léger J, de Roux N. Mutations in the maternally imprinted gene MKRN3 are common in familial central precocious puberty. Eur J Endocrinol 2016;174:1-8. Epub 2015 Oct 1
8. de Vries L, Gat-Yablonski G, Dror N, Singer A, Phillip M. A novel MKRN3 missense mutation causing familial precocious puberty. Hum Reprod 2014;29:2838-2843. Epub 2014 Oct 14
9. Lee HS, Jin HS, Shim YS, Jeong HR, Kwon E, Choi V, Kim MC, Chung IS, Jeong SY, Hwang JS. Low Frequency of MKRN3 Mutations in Central Precocious Puberty Among Korean Girls. Horm Metab Res 2016;48:118-122. Epub 2015 May 4
10. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child 1969;44:291-303.
11. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child 1970;45:13-23.

12. Houk CP, Kunselman AR, Lee PA. Adequacy of a single unstimulated luteinizing hormone level to diagnose central precocious puberty in girls. Pediatrics 2009;123:1059-1063.

13. Resende EA, Lara BH, Reis JD, Ferreira BP, Pereira GA, Borges MF. Assessment of basal and gonadotropin-releasing hormone-stimulated gonadotropins by immunochemiluminometric and immunofluorometric assays in normal children. J Clin Endocrinol Metab 2007;92:1424-1429. Epub 2007 Feb 6

14. Neyzi O, Bundak R, Gökçay G, Günöz H, Furman A, Darendeliler F, Baş F. Reference Values for Weight, Height, Head Circumference, and Body Mass Index in Turkish Children. J Clin Res Pediatr Endocrinol 2015;7:280-293.

15. Radiographic Atlas of Skeletal Development of the Hand and Wrist. Greulich WW, Pyle SI. California, Stanford University Press, 1959.

16. Jong MT, Gray TA, Ji Y, Glenn CC, Saitoh S, Driscoll DJ, Nicholls RD. A novel imprinted gene, encoding a RING zinc-finger protein, and overlapping antisense transcript in the Prader-Willi syndrome critical region. Hum Mol Genet 1999;8:783-793.

17. Bessa DS, Macedo DB, Brito VN, França MM, Montenegro LR, Cunha-Silva M, Silveira LG, Hummel T, Bergadá I, Braslavsky D, Abreu AP, Dauber A, Mendonca BB, Kaiser UB, Latronico AC. High Frequency of MKRN3 Mutations in Male Central Precocious Puberty Previously Classified as Idiopathic. Neuroendocrinology 2017;105:17-25. Epub 2016 May 26

18. Simsek E, Demiral M, Ceylaner S, Kırel B. Two Frameshift Mutations in MKRN3 in Turkish Patients with Familial Central Precocious Puberty. Horm Res Paediatr 2017;87:405-411. Epub 2016 Nov 1

19. Settas N, Dacou-Voutetakis C, Karantza M, Kanaka-Gantenbein C, Chrousos GP, Voutetakis A. Central precocious puberty in a girl and early puberty in her brother caused by a novel mutation in the MKRN3 gene. J Clin Endocrinol Metab 2014;99:647-651. Epub 2014 Jan 17

20. Schreiner F, Gohlke B, Hamm M, Korsch E, Woelfle J. MKRN3 mutations in familial central precocious puberty. Horm Res Paediatr 2014;82:122-126. Epub 2014 Jul 5

21. Hagen CP, Sørensen K, Mieritz MG, Johannsen TH, Almstrup K, Juul A. Circulating MKRN3 levels decline prior to pubertal onset and through puberty: a longitudinal study of healthy girls. J Clin Endocrinol Metab 2015;100:1920-1926. Epub 2015 Feb 19

22. Macedo DB, Abreu AP, Reis AC, Montenegro LR, Dauber A, Beneduzzi D, Cukier P, Silveira LF, Teles MG, Carroll RS, Junior GG, Filho GG, Gucev Z, Arnhold IJ, de Castro M, Moreira AC, Martinelli CE Jr, Hirschhorn JN, Mendonca BB, Brito VN, Antonini SR, Kaiser UB, Latronico AC. Central precocious puberty that appears to be sporadic caused by paternally inherited mutations in the imprinted gene makorin ring finger 3. J Clin Endocrinol Metab 2014;99:1097-1103. Epub 2014 Mar 14

ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.0003

J Clin Res Pediatr Endocrinol 2018;10(3):230-238

A Synopsis of Current Practice in the Diagnosis and Management of Patients with Turner Syndrome in Turkey: A Survey of 18 Pediatric Endocrinology Centers

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What is already known on this topic?

International consensus guidelines concerning the diagnosis, treatment and follow-up of patients with Turner syndrome have been reviewed and updated in the last few years.

What this study adds?

This is the first study to document the shortcomings of current practice in diagnosis, treatment and follow-up of patients with Turner syndrome in Turkey.

Abstract

Objective: A comprehensive survey was conducted to evaluate the shortcomings of clinical care in patients with Turner syndrome (TS) in Turkey.

Methods: A structured questionnaire prepared by the Turner study group in Turkey, which covered relevant aspects of patient care in TS was sent to 44 pediatric endocrinology centers.

Results: Eighteen centers (41%) responded to the questionnaire. In the majority of the centers, diagnostic genetic testing, screening for Y chromosomal material, protocols regarding the timing and posology of growth hormone (GH) and estrogen, thrombophilia screening, fertility information and screening for glucose intolerance, thyroid, and coeliac diseases in patients with TS were in line with the current consensus. Thirteen centers (72.2%) performed GH stimulation tests. Only four centers (22.2%) used oxandrolone in patients with TS with very short stature. The majority of the centers relied on bone age and breast development to assess estrogen adequacy, though together with variable combinations of oestrogen surrogates. Two centers (11.1%) reported performing serum estradiol measurements. Eight centers (44.4%) routinely conducted cardiac/thoracic aorta magnetic resonance imaging. Screening for hearing, dental and ophthalmologic problems were performed by thirteen (72.2%), six (33.3%) and ten (55.6%) centers, respectively. Psychiatric assessments were made by four centers (22.2%) at diagnosis, with only one center (5.6%) requiring annual reassessments.

Conclusion: Although we found some conformity between the current consensus and practice of the participating centers in Turkey regarding TS, further improvements are mandatory in the multi-disciplinary approach to address co-morbidities, which if unrecognized, may be associated with reduced quality of life and even mortality.

Keywords: Turner syndrome, diagnosis, growth, puberty, oestrogen, oxandrolone, osteoporosis, adult transition, screening, cardiac magnetic resonance imaging, thoracic aorta

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Conflict of interest: None declared
Received: 17.01.2018
Accepted: 23.04.2018

Introduction

Turner syndrome (TS) is the most common female sex chromosome disorder with an incidence of 1 in 2000 to 1 in 2500 live female births (1). It is caused by the complete or partial loss of a second sex chromosome during embryonic development with or without cell line mosaicism.

Individuals may be diagnosed at any age from fetal through to adult. TS may be suspected *in utero* as a result of screening for fetal abnormalities, in infancy by the presence of lymphedema, often associated with webbed neck and coarctation of the aorta, in childhood as a result of growth failure, in adolescence as a result of short stature with pubertal delay or in adulthood as a result of premature ovarian failure (2). Optimal care of this population should include proactive screening for co-existing medical conditions, including imaging for cardiac and renal anomalies, monitoring for obesity and hypertension, evaluation of developmental or psychoeducational abnormalities, hearing loss, autoimmune diseases and short stature. Ovarian dysfunction and a high probability of infertility should be anticipated (2).

Owing to recent advances in the diagnosis and management of patients with TS (3,4), the Turner Study Group in Turkey set out to establish state-of-the art care for Turkish patients with TS in a new consensus statement to include these internatioanally endorsed recommendations and guidelines.

To this end, we sought to determine the current status in the diagnosis and management of patients with TS among Turkish endocrinologists.

Methods

All pediatric endocrinology centers in Turkey were invited, via email, to respond to a questionnaire. An experienced pediatric endocrinologist from each center was asked to complete the questionnaire. The questionnaire was constructed by an experienced pediatric endocrinologist (Atilla Büyükgebiz) in the Turkish Turner Study Group. The questionnaire included multiple choice questions (with the option to include a comment by the respondent) on the following issues in sequence: demographics of the participating center, the diagnosis of TS, treatment for short stature, hormone replacement therapy, cardiac imaging, osteoporosis, fertility, adult transition and screening for co-existent medical conditions. The questionnaire is available at request as a supplementary file. Ethical approval was not needed owing to lack of involvement of patients or patient data.

Statistical Analysis

The data were reported as frequency distributions when feasible.

Results

Eighteen centers (11 university hospitals, five government-based education and research hospitals, and one state hospital; 41.1%) returned the questionnaire. The approximate number of patients with newly diagnosed TS per year by the attending centers was <5 patients/year in ten centers (55.6%), 5 to 10 patients/year in seven centers (38.9%), and >10 patients/year in one center (5.6%). The total number of patients with TS followed up by the centers was >30 patients in nine centers (50%), <10 patients in four centers (22.2%), between 10 to 20 patients in three centers (16.7%) and between 20 to 30 patients in one center (5.6%).

All but one center reported further attempts to confirm the clinical suspicion of TS if standard karyotype analysis was reported as normal. The frequency distribution of the genetic tests used by the centers if the standard karyotype is normal is shown in Table 1. Thirteen centers (72.2%) asked the genetics lab for repeat karyotype analysis with 30 metaphase counts. Of these 13 centers, four (22.2%) also requested fluorescent *in situ* hybridization (FISH) analysis. One center (5.6%) ordered repeat karyotype analysis with 100 metaphase counts, and three centers (16.7%) exclusively ordered FISH analyses.

Ten centers (55.6%) screened for Y chromosomal material only in the presence of virilization. Two centers (11.1%) routinely screened for Y chromosomal material, whereas six centers (33.3%) did not screen for Y chromosomal material.

Eight centers (44.4%) performed gonadectomy when Y chromosomal material was detected (four centers at puberty, three centers in the postpubertal period and one center did not provide timing data). Three centers (16.7%)

Table 1. Frequencies of the genetic tests applied by the 18 pediatric endocrinology centers in patients clinically suspected as having Turner syndrome but with normal standard karyotype analyses

Tests	n (%)
Fluorescein *in situ* hybridization (FISH)	3 (16.6)
Repeat karyotype with 30 metaphase counts only	9 (50)
Repeat karyotype with 100 metaphase counts only	1 (5.6)
Either FISH or repeat karyotype with 30 metaphase counts	4 (22.2)
None	1 (5.6)

preferred cautioned follow-up in patients with TS with Y chromosomal material.

Thirteen centers (72.2%) reported that due to legal procedures, they performed two growth hormone (GH) stimulation tests to establish the eligibility of patients to receive GH therapy gratis. Six centers (33.3%) reported an additonal diagnosis of GH deficiency. Two centers (11.1%) also assessed nocturnal GH secretion.

The frequency distribution of the timing of GH treatment in patients with TS is depicted in Table 2. The majority of the participating centers (n=14, 77.8%) started GH when the height of the patient deviated from the growth curve. Four centers (22.2%) began treatment when height fell below the 3rd percentile. Two centers (11.1%) started GH at diagnosis if the patient was aged from 4-12 years or older.

The doses and dosing schedules of GH in patients with TS attending the participating centers are shown in Table 3. Nine centers (50%) started GH at a dose of 0.375 mg/kg/week and adjusted the dose depending on the clinical response of the patient. Of these 9 centers, three centers (16.7%) also indicated that they adopted a fixed dosing protocol. The other centers (n=9, 50%) adopted an initial GH dose of 0.045 mg/kg/day and adjusted the GH dose depending on the growth response and/or serum insulin-like growth factor 1 (IGF1) level.

The majority of centers (n=14, 77.8%) did not use oxandrolone to improve final height in patients with TS. Only four centers (22.2%) adopted oxandrolone use, one of which also emphasized variable availability of the medication in Turkey.

The frequency distribution of the timing of estrogen treatment for induction of pubertal development is shown in Table 4. Seven centers (38.9%) started estrogen therapy at the age of 12 to 13 years, regardless of the age at initiation of GH treatment. Of these, one center indicated that they also had adopted a protocol involving commencement of estrogen at the age of 15 years, if GH was started after the age of 11 years. Three centers (16.7%) unconditionally withheld estradiol replacement until the age of 15 years, if GH was started after the age of 11 years. The timing of the start of estrogen therapy was dependent on the timing of initiation of GH treatment in four centers (22.2%). Six centers (33.3%) waited until the age of 13 years just in case spontaneous puberty occurred before commencing estrogen. Four centers (22.2%) also indicated that they did not favor waiting until the age of 15 years to start estrogen.

Only three (17%) centers used a routine screening protocol for potential thrombophilia, prior to oestrogen treatment. The remainder stated that they had asked the health care providers of the patient to provide information regarding a family history of thrombophilia.

Eleven (61.1%) centers used transdermal estrogen. Of these, four centers also used oral estrogen. Five centers (33.3%) exclusively used oral estrogen and one center used ethinyl estradiol. None of the centers used conjugated estrogen. One center did not specify the type of estrogen used.

Table 2. Frequency distribution of the criteria to start growth hormone in treatment-naive patients with Turner syndrome among 18 pediatric endocrinology centers in Turkey

Time of initiation of GH therapy	n* (%)
When height trajectory shifts down on the growth curve	14 (77.8%)
When height falls below third percentile	4 (22.2%)
Immediately if the patient is diagnosed with Turner syndrome at 4 to 12 years of age	1 (5.6%)
Immediately if diagnosed at older ages	1 (5.6%)

*n refers to the number of times a choice was selected by the centres. Note that a single centre may have selected several choices at the same time

GH: growth hormone

Table 3. Frequencies of the initial dose and dosing schedule of GH in patients with Turner syndrome among 18 pediatric endocrinology centers in Turkey

Initial GH dose (mg/kg/week)	n (%)	Growth hormone dosing schedule	
		Fixed dose n (%)	Growth response or IGF1 dependent change in dose n (%)
0.375	12 (66.7%)	3 (16.7%)	9 (50%)
0.350	3 (16.6%)	-	3 (16.6%)
0.294	1 (5.6%)	-	1 (5.6%)
0.315	2 (11.1%)	-	2 (11.1%)

GH: growth hormone, IGF1: insulin-like growth factor 1

Table 4. Frequency of the timing of estrogen treatment in patients with Turner syndrome among 18 pediatric endocrinology centers in Turkey

	n* (%)
Age at initiation of GH treatment	5 (27.7)
Bone age of 10 years and high FSH	2 (11.1)
At 12-13 years in all patients	7 (38.9)
At 13 years in absence of puberty	6 (33.3)
At 15 years if GH is started later than 11 years	4 (22.2)
Never delay E2 replacement until 15 years	4 (22.2)

*n refers to the number of times a choice was selected by the centres, since some centres may have selected more than one choice

GH: growth hormone, FSH: follicle-stimulating hormone, E2: estradiol

The surrogates of estrogen adequacy adopted by the centers are shown in Table 5. A variable combination of the surrogates was adopted by 16 centers (89%) to monitor estrogen adequacy. Breast development, according to Tanner staging (n=17, 94%) and bone age (n=16, 89%) were the most frequently selected estrogen surrogates. Two centers (11%) relied exclusively on the degree of breast development to assess estrogen adequacy. However only two centers (11%) directly monitored serum estradiol levels in addition to monitoring several surrogates of estrogen effect.

Progesterone treatment by the centers was also subject to multiple responses, but the majority of centers awaited occurrence of withdrawal bleeding (n=11, 61%). Nine centers (50%) added progesterone when Tanner's breast stage of 3 or 4 was attained. Three centers (17%) added progesterone after either one (n=1) or two years (n=2) of estrogen treatment.

Eight centers (44.4%) routinely scheduled patients for cardiac/thoracic aorta MRI either at diagnosis of TS or when they reached an age when general anesthesia was not required. Three centers (17%) ordered cardiac MRI if there was a clinical suspicion of cardiac abnormality. Seven centers (39%) did not routinely order cardiac/thoracic aorta MRI in patients with TS.

All but two centers (89%) reported routine screening of patients with TS for osteoporosis. Ten centers (56%) started evaluation for osteoporosis at age 15 years and over.

Table 5. Frequency distribution of the parameters used by 18 paediatric endocrinology centers in Turkey to assess the adequacy of oestrogen replacement in patients with Turner syndrome

	n*
Clinical parameters	
Growth velocity	10 (55.6)
Breast development based on Tanner staging	17 (94.4)
Bone age	16 (88.9)
Pelvic ultrasonography based parameters	
Uterine size	12 (66.7)
Endometrial thickness	9 (50.0)
Biochemical and hormonal parameters	
Serum IGF1	1 (5.6)
Serum E2	2 (11.1)
Serum FSH	6 (33.3)
Plasma lipids	1 (5.6)

*n refers to the number of centres that marked that choice. Note that the majority of the centres marked more than one choice

IGF1: insulin-like growth factor 1, FSH: follicle-stimulating hormone, E2: estradiol

Five centers (39%) exclusively informed parents and later the patients that infertility in TS was an expected end-point. Six centers (33%) performed follow-up with serum anti-mullerian hormone (AMH) levels as a means of monitoring the ovarian reserves of the patients. Eleven centers (61%) informed the patients and parents about the possibility of ovarian cryopreservation.

Fifteen centers (83%) transferred patients with TS to adult endocrinology outpatient clinics with a medical report and/or a phone call to the adult endocrinologist. Only two centers (11%) conducted an adult transition outpatient clinic. One center (5.6%) sent a copy of the patient's medical file to the adult endocrinology clinic.

The distributions of the timing and screening frequencies of the parameters regarding co-existent complications in patients with TS are shown in Table 6.

Discussion

This survey of 18 pediatric endocrinology centers in Turkey confirmed the need to provide updates to physicians using structured protocols reflecting the current international consensus in order to provide optimal care to patients with TS. While conformity was found among the centers regarding genetic tests to diagnose TS, GH use and hormone replacement therapy in TS, there were apparent discrepancies between centers and the current consensus regarding healthcare-related surveillance issues. We believe that some of these discrepancies were probably due to the variable availability of medical resources/equipment in different parts of Turkey.

In line with the suggestions of the American College of Medical Genetics (5), almost all the centers performed either karyotype analyses with 30 metaphase counts or FISH analyses when the index of clinical suspicion for TS was high in a patient but was not supported by standard karyotype analyses. However, the mean age at diagnosis of TS was 10.2 years in a recent national study examining the clinical characteristics of patients with TS in Turkey (6). Early diagnosis of TS is pivotal for improving preventive measures and treatment. To this end, there is ongoing promising research for early diagnosis of TS via neonatal screening with whole exome sequencing (7) or assessment of X chromosome, inactivation-specific, differentially methylated CpG sites (8). Such a screening method would be extremely beneficial for earlier diagnosis of TS in Turkey given the current mean age at diagnosis. The feasibility of this form of screening is one of the current areas of focus for the Turner Syndrome Study Group in Turkey, along with

increasing awareness of TS among health care professionals, and to include TS in the differential diagnosis for girls with short stature, when baseline work-up does not yield definitive cause.

More than half of the centers in the current survey attempted further analysis to investigate for Y chromosomal material should they find evidence of virilization in patients with TS, whereas one third of the centers did not. Y chromosome sequences occur in approximately 6% to 11% of patients with TS, which is of concern because approximately 10% of these go on to develop gonadoblastoma (9). Due to the risk of malignancy, many TS specialists recommend prophylactic gonadectomy (10) and cryptic Y material should especially be assessed in TS patients with virilization, even in the absence of a marker or ring chromosome (11). A recent study reported comparable rates of gonadoblastoma between patients with cryptic Y chromosome and patients with overt Y chromosome and recommended routine molecular screening for Y chromosome material for all patients with TS (12). The current guidelines recommend prophylactic gonadectomy in all patients with TS with Y chromosome identified on standard karyotyping (4). Molecular screening detection of Y chromosome sequences is currently recommended in individuals with TS with virilization but negative cytogenetic analyses and negative FISH. Gravholt et al (9) reported that careful follow-up with close observation of the gonads using ultrasonography could be an option in some patients with TS who harbour Y chromosomal material emphasizing that in most of these patients, malignancy does not occur. Overall, these controversial reports indicate the need for further studies to reach hard end-point conclusions on gonadectomy in patients with TS who are positive for Y chromosomal material.

Despite the fact that current guidelines and reviews (3,4) do not recommend GH stimulation tests for patients with TS. However, 72% of the centers in the current survey conducted GH stimulation tests routinely because the high cost of GH treatment would then be covered by Turkish social security. Moreover, 33% of the patients were given GH with an inappropriate diagnosis of GH deficiency if they were found to have reduced response to GH stimulation tests in two tests. Although there is some evidence of heterogeneity regarding the GH IGF1 axis in patients with TS (13), current guidelines (3,4) suggest that GH should be started in patients with TS without the need for GH stimulation tests. There is ongoing

Table 6. The distribution of the timing and screening frequencies of the parameters regarding co-existent complications in patients with Turner syndrome as assessed by 18 pediatric endocrinology centers in Turkey

	With clinical indication n (%)	At diagnosis of TS n (%)	Quarterly n (%)	Biannually n (%)	Yearly n (%)
Blood pressure	-	18 (100)	10 (55.5)	4 (22.2)	4 (22.2)
24 h-ambulatory blood pressure monitoring	17 (94.4)	1 (5.6)	-	-	1 (5.6)
Complete blood count	3 (16.7)	18 (100)	1 (5.6)	3 (16.7)	7 (38.9)
Plasma lipids	3 (16.7)	11 (61.1)	-	3 (16.7)	11 (61.1)
Blood glucose	-	18 (100)	4 (22.2)	5 (27.8)	9 (50.0)
HbA1c	-	14 (77.8)	2 (11.1)	5 (27.8)	7 (38.9)
Thyroid function tests	-	18 (100)	3 (16.7)	7 (38.9)	8 (44.4)
Coeliac screening	-	17 (94.4)		1 (5.6)	17 (94.4)
Liver function tests	-	17 (94.4)	3 (16.7)	2 (11.2)	11 (61.1)
Renal USG	3 (16.7)	17 (94.4)	-	-	-
Serum creatinine, urea	-	17 (94.4)	2 (11.1)	5 (27.8)	10 (55.6)
Bone mineral density	10 (55.6)	-	-	-	7 (38.9)
Medical nutrition counselling	-	17 (94.4)	-	-	1 (5.6)
Consultations					
Audiology	-	13 (72.2)	-	-	4 (22.2)
Dental	12 (66.7)	6 (33.3)	-	-	-
Ophthalmology	-	10 (55.6)	-	-	2 (11.1)
Orthopedics	16 (88.9)	2 (11.1)	-	-	1(5.6)
Dermatology	18 (100)	1 (5.6)	-	-	-
Psychiatry	-	4 (22.2)	-	-	1 (5.6)

HbA1c: hemoglabin A1c, USG: ultrasonography, TS: Turner Syndrome

collaboration between the Turkish Pediatric Endocrinology and Diabetes Society and the Turkish Ministry of Health to solve this issue.

The majority of the centers in the current survey chose to start GH treatment when the patient with TS had evidence of growth failure, i.e., a height velocity $<50^{th}$ percentile observed over six months, the child was already short or had a high likelihood of short stature, which is in line with the recommendation from the current guidelines (3,4). The optimal age to start GH has yet to be established.

In the current survey, the dosage of GH practised by the centers was around 45 to 53 μg/kg/day, which is closer to the lower range of the doses suggested by current guidelines (3,4). The Nordinet International Outcome study, which was conducted between 2006 and 2016, described real-life dosing patterns in children using GH owing to various pathologies including TS. In the Nordinet study, GH doses in patients with TS were found to have a tendency to be at the lower end of the recommendations of the practice guidelines and label ranges, albeit factors associated with this tendency were unclear (14). The reasons for avoiding higher doses need to be explored because there is a limited time for potential efficacy of GH in patients with TS owing to the delayed diagnosis of TS in Turkey (6).

Only four centers (22.2%) used oxandrolone in patients with TS in the current survey. If the diagnosis of TS and subsequent GH treatment is delayed, and/or adult height outcome data is likely to be unsatisfactory with the standard GH dose alone, the current consensus (3) recommends treatment with oxandrolone. The uncommon use of oxandrolone in Turkey, despite considerably delayed diagnosis of TS, might be due to the intermittent availability of the drug in Turkey, as in other parts of Europe.

Regarding estrogen replacement for induction of puberty, almost 40% of the centers in the current survey started estrogen treatment at age 12-13 years, and 20% of the centers delayed estrogen until 15 years if GH was started after the age of 11. Although there are concerns for compromised height potential in patients with TS older than 11 years who are GH treatment-naive, if estrogen is started around the age of 12 to 13, delay in estrogen replacement was associated with very poor outcomes regarding bone mineral density (BMD) measurements (15). The current consensus (4) does not recommend delaying estrogen in patients with onset of GH treatment after the age of 11, and favors estrogen replacement initiation around 11 to 12 years. The optimal approach to feminization for patients with TS in terms of estrogen formulation and estrogen dose progression is not clear. Yet, the guideline supports the practice of incremental dose increases approximately every six months to mimic the normal pubertal tempo until adult dosing is reached over a two to three-year period. The use of low-dose estrogen in prepubertal ages is still under investigation and is discouraged in the current guidelines (4).

Apart from three centers (16.7%) in the current survey, profiling of the coagulation system prior to estrogen treatment was uncommon in our survey despite some evidence of abnormalities in coagulation in patients with TS (16). The current consensus does not recommend routine profiling of the coagulation system, but it advises investigating for a family history of coagulopathy.

The route of estrogen administration has been a hot topic of research. About 60% of the centers in the current survey preferred transdermal estrogen over the oral form, in accordance with the recent consensus (4). Transdermal estrogen has been preferred over the oral route owing to its more physiological form and avoidance of the liver first-pass metabolism of oral estrogen (17,18). In a 2006 survey among physicians from the United States of America, 78% were found to prescribe conjugated estrogens (19), whereas in a European survey, 39% of the physicians were using ethinyl estradiol, 32% used oral micronized estradiol, and only 12% used conjugated equine estrogen (20). Only 8% to 10% of the physicians were found to prescribe transdermal estradiol (20).

Clinical assessment, patient satisfaction, patient age and residual growth potential were considered as primary determinants of estrogen adequacy in the current consensus (4). Except for two centers, which exclusively relied on the degree of breast development as a criterion to assess estrogen adequacy, the centers in the current survey chose various combinations of surrogates of estrogen effect. The variables most commonly used by the centers were breast development in conjunction with bone age. Only two centers selected monitoring serum estradiol as a surrogate parameter to judge estrogen adequacy. In fact, serum estradiol measurement using an ultrasensitive assay may allow for titrating dosage. A protocol involving the use of transdermal estradiol (E2) and monitoring with an utrasensitive E2 assay does exist and is based on excellent studies (21,22), although estradiol concentrations that achieve maximal growth probably need further exploration.

The referral of patients with TS for baseline cardiac evaluation is well established in Turkey, as suggested in the guidelines. Patients with TS have a predilection for aortic dissection, which is almost six times more common than in the general population (15). Several indices of the aorta such as aortic size index as assessed through cardiac/thoracic MRI of the

aorta are commonly used to predict possible occurrence of such a risk (23). However, MRI studies are expensive and carry additional risk in patients aged less than 12 years with a frequent need for deep sedation. Accordingly, several surrogate markers for dilation of the aorta and vascular disease are currently under investigation (24). Unfortunately, the present survey showed that only 44% of centers routinely performed cardiac/thoracic aorta MRI, either at diagnosis or at an age when anesthesia was feasible, which is concordant with the current consensus. In our opinion, this is one of the aspects of care for girls with TS in Turkey that requires particular attention and improvement because aortic dissections have been reported as early as age 4 years (25). Our survey did not explore the reasons for the significant lack of referrals for cardiac/thoracic aorta MRI, but the high cost of the procedure and the lack of availability of the required device in many centers could possibly be potential causes.

More than half of centers in the current survey routinely evaluated patients with TS for osteoporosis at age 15 yrs and over. The current guidelines (4) suggest screening patients with TS with DEXA scan after adult hormone replacement has been instituted, with moderate levels of evidence. Moreover, beginning at age 9 to 11 years, and then repeating every 2 to 3 years, serum 25 hydroxyvitamin D level measurements are recommended, despite low levels of evidence. The degree of vitamin D sufficiency in Turkish girls with TS is unknown as levels were not investigated in the current survey. Endogeneous and exogeneous estrogen exposure is associated with improved BMD, although women with TS and normal BMD still have increased fracture risk compared with controls (24). Results regarding BMD in patients with TS are not consistent owing to differences in methodologies and small bone size (4). Most females with TS have normal BMD.

Fertility is one of the major concerns of patients and families affected by TS. In the current survey, around 60% of the centers informed the parents and the patients regarding the possibility of ovarian cryopreservation. Cryopreservation of mature oocytes and embryos is a proven fertility preservation approach, and cryopreservation of ovarian tissue is a promising technique with a growing number of live births, but is still at the investigation stage. Oocyte cryopreservation has been performed in children with TS aged as young as 13 years (3,4). However, the efficacy of the procedure needs to be proven on a larger scale. About 30% of the centers in the current survey reported that they regularly checked AMH levels in an effort to predict ovarian function. AMH was shown to be effective as a predictor of absent puberty, as AMH ≤2 SD for age predicted failure to enter puberty in young girls with TS and imminent primary ovarian insufficiency in adolescent and adult patients with TS (26,27). The use of AMH as a screening tool for ovarian function was not recommended in the current consensus. However, the use of AMH in conjunction with follicle-stimulating hormone in the context of fertility issues was discussed.

Unfortunately, only two centers (11.1%) in the current survey held an adult transition outpatient clinic and more than 80% of the centers in the survey sent young patients with TS to the adult clinic with only a medical review report. Failures during the transitional phase to adult care may result in moderate healthcare outcomes and decreased quality of life. To be of help in overcoming problems at transition, starting at the age of 11 to 13 years, physicians should repeat information directly to the girl about medical and health issues that were disclosed to the parents (28). The process of transition readiness needs to be followed by the health care provider, possibly with the use of questionnaires enquiring about healthcare autonomy, self-care and disease management as has been described previously (28). Unfortunately, it is difficult to find experts in adult care. In adult life, less than 4% of patients with TS undergo all the recommended medical investigations on a regular basis (29). Many reasons have been described for transition failures such as poor self-advocacy or self-management, little family support or unsatisfactory cooperation between healthcare professionals and organizational structures (30,31,32,33).

Our survey revealed that co-morbidity screening in patients with TS needs to be improved because there were significant deviations from what was suggested in the current consensus (4). The screening of the TS patients by blood pressure measurements, metabolic parameters such as fasting plasma glucose, hemoglobin A1c, lipids, thyroid function tests, coeliac antibodies, liver transaminases and renal ultrasound evaluation were somewhat in conformity with the current consensus. However, the survey results displayed significant discordance for audiology, dental, ophthalmology, orthopedic and psychiatry consultations.

Hearing loss is a well-known problem in patients with TS (34). Evaluation is indicated in all girls with TS at the time of diagnosis and at 2- to 5-year intervals

(3,4). However, although more than 70% of the centers referred the patients for audiometric evaluation at diagnosis, follow-up referrals decreased to as low as 22% of the centers. In the socially- and bone-disadvantaged TS population, addressing hearing problems is of paramount importance. Considering the well-established increase in accidental falls in people who require hearing aids and the increased incidence of bone mineral abnormalities in TS,

the importance of regular audiometric evaluation cannot be ignored.

Orthopedic, ophthalmologic, and dental evaluations were performed either at diagnosis in 11%, 56% and 33% of the centers, respectively, or as clinically indicated during the course of follow-up. It is recommended by TS experts that all these evaluations should be repeated at regular intervals (2).

In the current survey, it is unfortunate that psychiatric evaluation of patients with TS was found to be among one of the aspects of care that was most lacking. Only 22% of the centers in the current survey referred patients with TS to the psychiatry outpatient clinic and of these, only one center made annual referrals. A full discussion of the psychosocial development in patients with TS is beyond the scope of this manuscript, there are known to be deficits in mathematical abilities, visuospatial processing and verbal skills which may worsen over time so that annual developmental and behavioral scales are recommended (28). A genetic basis rather than phenotype-related shortcomings perceived by patients with TS seems to be emerging as a major cause of the psychiatric alterations (35).

Study Limitations

Our survey was not designed to delineate the factors associated with shortcomings of clinical care in patients with TS in Turkey. Further studies are needed to delineate these factors.

Conclusion

The current survey revealed that issues regarding the diagnosis of TS, treatment of short stature, pubertal management, and fertility-related questions by the patients/parents were addressed by many pediatric endocrinologists akin to those of Western countries. Yet, we also identified several shortcomings of care for patients with TS in Turkey when compared with developed countries, issues that are among the current areas of focus by the Turkish Turner Study Group.

Acknowledgment

The authors would like to express their gratitude to Mr. David Chapman for editing this manuscript.

*The authors would also like to thank the following pediatric endocrinology and diabetes centers for participating in the survey: Istanbul University, School of Medicine; Marmara University, School of Medicine; 9 Eylül University, School of Medicine; Uludağ University School of Medicine; Ege University, School of Medicine; Akdeniz University, School of Medicine; Erciyes University, School of Medicine; Ankara University, School of Medicine; Necmettin Erbakan University, School of Medicine; Eskişehir Osmangazi University, School of Medicine; Dicle University, School of Medicine; University of Health Sciences (Dr. Sami Ulus Obstetrics and Gynecology, Children's Health and Disease Education and Research Hospital, Şişli Hamidiye Etfal Education and Training Hospital, Kanuni Sultan Süleyman Education and Research Hospital, Zeynep Kamil Obstetrics and Gynecology, Children's Health and Disease Education and Research Hospital, Trabzon Kanuni Education and Research Hospital, Bakırköy Sadi Konuk Education and Research Hospital), Eskişehir State Hospital.

Ethics

Ethics Committee Approval: Ethical approval was not needed owing to lack of involvement of patients or patient data.

Informed Consent: It was not needed owing to lack of involvement of patients or patient data.

Peer-review: Externally peer reviewed.

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Financial Disclosure: The authors declared that this study received no financial support.

References

1. Stochholm K, Juul S, Juel K, Naeraa RW, Gravholt CH. Prevalence, incidence, diagnostic delay, and mortality in Turner syndrome. J Clin Endocrinol Metab 2006;91:3897-3902. Epub 2006 Jul 18
2. Bondy CA; Turner Syndrome Study Group. Care of girls and women with Turner syndrome: A guideline of the Turner Syndrome Study Group. J Clin Endocrinol Metab 2007;92:10-25. Epub 2006 Oct 17
3. Paolucci DG, Bamba V. Turner syndrome: care of the patient: Birth to late adolescence Pediatr Endocrinol Rev 2017;14(Suppl 2):454-461.
4. Gravholt CH, Andersen NH, Conway GS, Dekkers OM, Geffner ME, Klein KO, Lin AE, Mauras N, Quigley CA, Rubin K, Sandberg DE, Sas TCJ, Silberbach M, Söderström-Anttila V, Stochholm K, van Alfen-van derVelden JA, Woelfle J, Backeljauw PF; International Turner Syndrome Consensus Group. Clinical practice guidelines for the care of girls and

women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting. Eur J Endocrinol 2017;177:1-70.

5. Wolff DJ, Van Dyke DL, Powell CM; Working group of the ACMG Laboratory Quality Assurance Committee. Laboratory guideline for Turner syndrome. Genet Med 2010;12:52-55.
6. Yeşilkaya E, Bereket A, Darendeliler F, Baş F, Poyrazoğlu Ş, Küçükemre Aydın B, Darcan Ş, Dündar B, Büyükinan M, Kara C, Sarı E, Adal E, Akıncı A, Atabek ME, Demirel F, Çelik N, Özkan B, Özhan B, Orbak Z, Ersoy B, Doğan M, Ataş A, Turan S, Gökşen D, Tarım Ö, Yüksel B, Ercan O, Hatun Ş, Şimşek E, Ökten A, Abacı A, Döneray H, Özbek MN, Keskin M, Önal H, Akyürek N, Bulan K, Tepe D, Emeksiz HC, Demir K, Kızılay D, Topaloğlu AK, Eren E, Özen S, Abalı S, Akın L, Selver Eklioğlu B, Kaba S, Anık A, Baş S, Ünüvar T, Sağlam H, Bolu S, Özgen T, Doğan D, Deniz Çakır E, Şen Y, Andıran N, Çizmecioğlu F, Evliyaoğlu O, Karagüzel G, Pirgon Ö, Çatlı G, Can HD, Gürbüz F, Binay Ç, Baş VN, Fidancı K, Polat A, Gül D, Açıkel C, Demirbilek H, Cinaz P, Bondy C. Turner syndrome and associated problems in Turkish children: a multicenter study. J Clin Res Pediatr Endocrinol 2015;7:27-36.
7. Murdock DR, Donovan FX, Chandrasekharappa SC, Banks N, Bondy C, Muenke M, Kruszka P. Whole-Exome Sequencing for Diagnosis of Turner Syndrome: Toward Next-Generation Sequencing and Newborn Screening. J Clin Endocrinol Metab 2017;102:1529-1537.
8. Zhang Q, Guo X, Tian T, Wang T, Li Q, Wang L, Liu Y, Xing Q, He L, Zhao X. Detection of Turner syndrome using X-chromosome inactivation specific differentially methylated CpG sites: A pilot study. Clin Chim Acta 2017;468:174-179. Epub 2017 Mar 8
9. Gravholt CH, Fedder J, Naeraa RW, Müller J. Occurence of gonadoblastoma in females with Turner syndrome and Y chromosome material: a population study. J Clin Endocrinol Metab 2000;85:1399-3202.
10. Modi D, Bhartiya D. Y chromosome mosaicism and occurrence of gonadoblastoma in cases of Turner syndrome and amenorrhea. Reprod Biomed Online 2007;15:547-553.
11. Kanakatti Shankar R, Inge TH, Gutmark-Little I, Backeljauw PF. Oopherectomy versus salpingo-oophorectomy in Turner syndrome patients with Y-chromosome material: clinical experience and current practice patterns assessment. J Pediatr Surg 2014;49:1585-1588. Epub 2014 Jul 23
12. Kwon A, Hyun SE, Jung MK, Chae HW, Lee WJ, Kim TH, Kim DH, Kim HS. Risk of Gonadoblastoma Development in Patients with Turner Syndrome with Cryptic Y Chromosome Material. Horm Cancer 2017;8:166-173. Epub 2017 Mar 27
13. Mortensen KH, Andersen NH, Gravholt CH. Cardiovascular phenotype in Turner syndrome--integrating cardiology, genetics, and endocrinology. Endocr Rev 2012;33:677-714. Epub 2012 Jun 15
14. Blankenstein O, Snajderova M, Blair J, Pournara E, Pedersen BT, Petit IO. Real-life GH dosing patterns in children with GHD, TS or born SGA: a report from the NordiNet® International Outcome Study. Eur J Endocrinol 2017;177:145-155. Epub 2017 May 18
15. Nguyen HH, Wong P, Strauss BJ, Jones G, Ebeling PR, Milat F, Vincent A. Delay in estrogen commencement is associated with lower bone mineral density in Turner syndrome. Climacteric 2017;20:436-441. Epub 2017 May 19
16. Gravholt CH, Mortensen KH, Andersen NH, Ibsen L, Ingerslev J, Hjerrild BE. Coagulation and fibrinolytic disturbances are related to carotid intima thickness and arterial blood pressure in Turner syndrome. Clin Endocrinol (Oxf) 2012;76:649-656.
17. Zaiem F, Alahdab F, Nofal A, Hassan MH, Javed A. Oral versus transdermal estorogen in turner syndrome: A systematic rewiew and meta-analysis. Endocr Pract 2017;23:408-421. Epub 2017 Jan 17
18. Cintron D, Rodriguez-Gutierrez R, Serrano V, Latortue-Albino P, Erwin PJ, Murad MH. Effect of estrogen replacement therapy on bone and cardiovascular outcomes in women with turner syndrome: a systematic review and meta-analysis. Endocrine 2017;55:366-375. Epub 2016 Jul 29
19. Drobac S, Rubin K, Rogol AD, Rosenfield RL. A workshop on pubertal hormone replacement options in the United States. J Pediatr Endocrinol Metab 2006;19:55-64.
20. Kiess W, Conway G, Ritzen M, Rosenfield R, Bernasconi S, Juul A, van Pareren Y, de Muinck Keizer-Schrama SM, Bourguignon JP. Induction of Puberty in the Hypogonadal Girl--Practices and Attitudes of Pediatric Endocrinologists in Europe. Horm Res 2002;57:66-71.
21. Rosenfield RL, Perovic N, Devine N, Mauras N, Moshang T, Root AW, Sy JP. Optimizing Estrogen Replacement Treatment in Turner Syndrome. Pediatrics 1998;102):486-488.
22. Ankarberg-Lindgren C, Elfving M, Wikland KA, Norjavaara E. Nocturnal application of transdermal estradiol patches produces levels of estradiol that mimic those seen at the onset of spontaneous puberty in girls. J Clin Endocrinol Metab 2001;86:3039-3044.
23. Matura LA, Ho VB, Rosing DR, Bondy CA. Aortic dilatation and dissection in Turner syndrome. Circulation 2007;116:1663-1670. Epub 2007 Sep 17
24. Mavinkurve M, O'Gorman CS. Can brain natriuretic peptides and osteoprogerin serve as biochemical markers fort he detection of aortic pathology in children and adolescents with Turner syndrome? Front Endocrinol (Lausanne) 2017;8:142.
25. Sybert VP. Cardiovascular malformations and complications in Turner syndrome. Pediatrics 1998;101:11.
26. Cleemann L, Holm K, Kobbernagel H, Kristensen B, Skouby SO, Jensen AK, Gravholt CH. Dosage of estradiol, bone and body composition in Turner syndrome: a 5-year randomized controlled clinical trial. Eur J Endocrinol 2017;176:233-242. Epub 2016 Nov 23
27. Weintraub A, Eldar-Geva T. Anti-Mullerian Hormone (AMH) Determinations in the Pediatric and Adolescent Endocrine Practice. Pediatr Endocrinol Rev 2017;14:364-370.
28. Culen C, Ertl D, Schubert K, Bartha-Doering L, Haeusler G. Care of girls and women with Turner syndrome: beyond growth and hormones. Endocr Connect 2017;6:39-51. Epub 2017 Mar 23
29. Devernay M, Ecosse E, Coste J, Carel JC. Determinants of medical care for young women with Turner syndrome. J Clin Endocrinol Metab 2009;94:3408-3413. Epub 2009 May 26
30. White PH, Ardoin S. Transitioning wisely: improving the connection from pediatric to adult health care. Arthritis Rheumatol 2015;68:789-794.
31. Downing J, Gleeson HK, Clayton PE, Davies JR, Wales JK, Callery P. Transition in endocrinology: the challenge of maintaining continuity. Clin Endocrinol (Oxf) 2013;78:29-35.
32. Sakakibara H. Transition of Women with Turner Syndrome from Pediatrics to Adult Health Care: Current Situation and Associated Problems. Front Pediatr 2017;5:28.
33. Nishigaki S, Hamazaki T, Tsuruhara A, Yoshida T, Imamura T, Inada H, Fujita K, Shintaku H. Clinical features of women with Turner syndrome experiencing transition period in Japan. Endocr J 2017;64:499-505. Epub 2017 Mar 22.
34. Bonnard A, Hederstierna C, Bark R, Hultcrantz M. Audiometric features in young adults with Turner syndrome. Int J Audiol 2017;56:650-656. Epub 2017 Apr 19
35. Xie S, Yang J, Zhang Z, Zhao C, Bi Y, Zhao Q, Pan H, Gong G. The Effects of the X Chromosome on Intrinsic Functional Connectivity in the Human Brain: Evidence from Turner Syndrome Patients. Cereb Cortex 2017;27:474-484.

ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.0023

J Clin Res Pediatr Endocrinol 2018;10(3):239-246

Subnormal Growth Velocity and Related Factors During GnRH Analog Therapy for Idiopathic Central Precocious Puberty

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What is already known on this topic?

Data concerning subnormal growth velocity and factors that influence this during gonadotropin-releasing hormone analog therapy for idiopathic central precocious puberty are scarce.

What this study adds?

In girls with idiopathic central precocious puberty the risk of subnormal growth velocity appears highest at the 3rd year of gonadotropin-releasing hormone analog treatment in those patients with, at the time of diagnosis, pubic hair in conjunction with high baseline and peak luteinizing hormone (LH) and advanced bone age and excessive LH suppression on follow-up.

Abstract

Objective: Data concerning subnormal growth velocity (GV) and factors that influence this during gonadotropin-releasing hormone analog (GnRHa) therapy for idiopathic central precocious puberty (ICPP) are scarce. We investigated the incidence of subnormal GV and associated factors in patients receiving GnRHa therapy for ICPP.

Methods: In this retrospective cohort study, the records of 50 girls who had been diagnosed with ICPP and started on GnRHa treatment before the age of eight years were investigated. Subnormal GV frequency, related factors during GnRHa therapy and the effect on final height were examined.

Results: During the treatment, a significant decrease in the annual GV and GV standard deviation score (SDS) of the patients was observed. In 16 (32%) patients GV never declined below -1 SDS, while a decline was noted once and twice in 19 (38%) and 15 (30%) patients respectively. The median age of detection of subnormal GV was 9.9 (4.9-10.9) years. Patients with pubic hair at diagnosis were found to have an increased risk of subnormal GV ($p = 0.016$). There was a significant negative correlation between diagnostic basal luteinizing hormone (LH) level and the first and second year GV SDS ($p = 0.012$ and 0.017 respectively). A significant negative correlation between bone age at diagnosis and 3rd year GV SDS, and 4th year GV SDS ($p = 0.002$ and $p = 0.038$) was also observed. LH suppression significantly increased during treatment ($p = 0.001$).

Conclusions: In girls with ICPP the risk of subnormal GV appears highest at the 3rd year of GnRHa treatment, particularly in those patients with, at the time of diagnosis, pubic hair in conjunction with high baseline and peak LH and advanced BA and excessive LH suppression on follow-up.

Keywords: Central precocious puberty, growth velocity, GnRHa therapy

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Conflict of interest: None declared

Received: 26.01.2018

Accepted: 13.04.2018

J Clin Res Pediatr Endocrinol
2018;10(3):239-246

Introduction

Central precocious puberty (CPP) in girls is usually defined as the development of pubertal sex characteristics before the age of 8 years, usually as a consequence of the premature activation of the hypothalamic-pituitary-gonadal (HPG) axis. The pathogenesis of CPP includes early activation of pulsatile release of gonadotropin-releasing hormone (GnRH), leading to an increase in secretion of gonadotropins and gonadal steroids (1). In the majority of CPP cases, the etiology of the premature activation of the HPG axis is not clear. CPP in the absence of organic disease is known as idiopathic CPP (ICPP).

Children with precocious puberty tend to exhibit temporary accelerated growth due to increased sex hormones, but also a shorter growing period, which may ultimately lead to a shorter final height (FH). Early increased sex hormone concentrations shorten the growing period by promoting growth plate senescence, which refers to the structural and functional changes of the epiphyseal growth region including decline in chondrocyte proliferation and rate of longitudinal bone growth (2,3).

The mainstay of treatment for CPP is GnRH analogs (GnRHa). GnRHa bind to the GnRH receptors in the gonadotropic cells of anterior pituitary gland, resulting in desensitization of these receptors and eventual gonadal suppression due to down-regulation of the intrinsic pulsatile secretion of the LH and follicle-stimulating hormone (FSH) (4). This, in turn, reduces the growth velocity (GV), giving the long bones more time to lengthen before the growth plates fuse, thus increasing the FH that the child will achieve (5). However, in some patients, treatment with GnRHa does not just normalize GV but supresses it below the normal range (6,7,8,9). Previous studies have not revealed a clear hormonal cause for this phenomenon (10,11,12), raising the possibility that impaired growth during GnRHa therapy is due, at least in part, to premature growth plate senescence induced by prior estrogen exposure (13). Until now, during GnRHa treatment for ICPP, there is no cohort study for the evaluation of GV in which regular follow-up is performed from the beginning to the end of treatment. Therefore, during GnRH treatment the frequency of subnormal GV, time of occurrence, associated factors and the effect on FH are unclear.

In this retrospective cohort study, for the patients who had GnRHa treatment with ICPP diagnosis, GV records obtained at three month periods were investigated from the beginning until the end of treatment. The rate and time of occurrence of subnormal GV, factors associated with subnormal GV, and the effect on FH were investigated.

Methods

The records of 50 girls who had a diagnosis of ICPP in our clinic between July 2010 and July 2013, who had been started on GnRHa treatment before the age of eight years and who had completed the treatment with regular follow-up were investigated. Since the study was retrospective, ethics committee approval and informed consent were not taken.

All subjects experienced breast development, Tanner stage B2, as a first sign of puberty before eight years of age and all were premenarcheal at presentation. The girls were diagnosed with ICPP if chronological age (CA) at onset of breast development was <8 years and peak luteinizing hormone (LH) level was more than 5 IU/L (5 mIU/mL) in response to 2.5 µg/kg (maximum 0.1 mg) GnRH (0.1 mg Gonadorelin acetate, Ferring®) and if brain magnetic resonance imaging was normal. All subjects had recently experienced rapid growth in height and/or one or more than one year advance in bone age (BA) assessed by the method of Greulich and Pyle. Target height was calculated by mid parental height minus 6.5 cm. Target height range was calculated by target height ± 5 cm. Leuprolide acetate 1-month depot was started as 3.75 mg/dose administered intramuscularly every 28 days, at the time of ICPP diagnosis.

Follow-up assessments were performed every three months. Follow-up study visits included a physical examination with measurement of height and weight, assessment of Tanner stage. Determination of LH levels at 30 and 60 minutes after the GnRHa injection were performed every 6 months. Height and GV standard deviation score (SDS) were determined using anthropometric reference data for Turkish children (14). GV was considered subnormal if the GV was below -1 SDS. Patients with suboptimal pubertal suppression (clinical pubertal progression and peak LH response to the GnRHa >3.3 U/L) were excluded from the study. Treatment was discontinued in patients who had a CA of 11 or who had a CA of 10.5-11 and in conjunction with a BA of 12 years.

After drug withdrawal, visits were continued every six months until menarche, and then annually until the patient reached FH. Girls were considered to be at FH if they were growing less than 0.5 cm/year or if BA was greater than or equal to age 16 years.

Exclusion criteria included CPP caused by organic lesions, being born small for gestational age, thyroid disease, intake of any other medications, presence of chronic diseases or growth-affecting medical problems. A drop-out case was defined as one who did not complete the follow-up described in the protocol, which includes voluntary discontinuation of

treatment, irregularity of visits, treatment incompatibility, side effects of treatment, detection of additional diseases that may affect growth and suboptimal pubertal suppression.

Serum FSH and LH levels were measured by immunofluorometric assays (ARCHITECH System, Abbott Laboratory Diagnostics, USA) with detection limits of 0.05 mIU/mL and 0.07 mIU/mL for FSH and LH, respectively. The intraassay and interassay CV was 3.2 % in both gonadotropin assays.

Statistical Analysis

The data were entered into the SPSS 21.0 computer package program and analyzed. Qualitative data are presented as numbers/percentages, while quantitative data are given as means, medians and standard deviations. Nonparametric tests were used after the normal distribution conformity test. For the comparison of two groups Mann-Whitney U test was used and for three groups' comparison the Kruskal-Wallis test, where $p < 0.05$ was considered statistically significant.

Results

A total of data on 808 follow-up visits of 50 female patients who had received ICPP treatment before age eight years and had been evaluated at regular check-up visits, were evaluated. The median follow-up period was 48 ± 10.5 (33-72) months and 31 patients reached FH. The clinical, laboratory and radiological findings of patients are given in Table 1. Twenty-four patients were observed to have a FH compatible with midparental height (MPH) (MPH ± 5 cm). The FH of five patients was above MPH, and only two patients were below MPH.

During the treatment period, a significant decrease in the annual GV and GV SDS of the patients was observed ($p = 0.02$ and $p = 0.001$ respectively) (see Table 2 and Figure 1). Although GV of patients did not decline below -1 SDS in the first year of treatment, GV dropped below -1 SDS in 28.2 % (11 of 39) in the second year, 41.7 % (20 of 48) in the third year, 50 % (13 of 26) in the fourth year, 33.3 % (2 of 6) in the 5th year and in 75 % (3 of 4) in the 6th year of treatment. The median age of detection of subnormal GV was 9.9 (4.9-10.9) years. The median time of subnormal GV occurrence was the 3rd year of treatment (minimum-maximum 2-6 years). In the third and fourth years of treatment, CA of patients who showed a subnormal GV was 10.12 ± 0.67 and 10.28 ± 0.77, respectively. The CA of patients who showed a

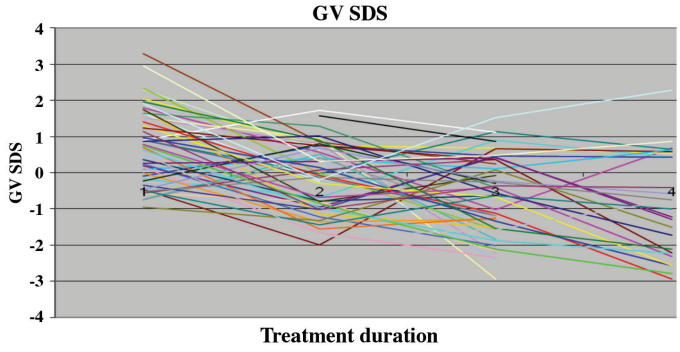

Figure 1. The annual growth velocity standard deviation score of patients

GV: growth velocity, SDS: standard deviation score

Table 1. The clinical, laboratory and radiological findings of the patients

	n	Mean	Median	SD	Minimum	Maximum
CA at diagnosis (years)	50	6.7	7.04	1.13	3.0	8.0
Height SDS at diagnosis	50	0.87	0.74	1.13	-1.0	3.5
Weight SDS at diagnosis	50	0.79	0.70	0.86	-0.7	2.8
BMI SDS at diagnosis	50	0.54	0.38	0.91	-1.07	2.83
Basal FSH (IU/L)	50	3.01	2.45	2.11	0.53	9.7
Stimulated FSH (IU/L)	42	19.08	29	7.54	7.29	36.4
Basal LH (IU/L)	50	0.35	0.11	0.59	0.07	3.1
Stimulated LH (IU/L)	42	6.95	5.7	3.12	4.14	18.4
Estradiol (pg/mL)	50	21.11	20	11.1	1.24	54.9
BA (years)	50	8.12	8.08	1.57	4.16	12
BA-CA (years)	50	1.36	1.33	0.92	-0.42	3.41
MPH (cm)	47	158.7	157.9	5.54	146	174.5
FH (cm)	31	159.8	159.3	6.33	144.5	170.3
FH-MPH	31	1.92	2.4	4.24	-9.0	13.2

CA: chronological age, SDS: standard deviation score, BMI: body mass index, FSH: follicle-stimulating hormone, LH: luteinizing hormone, BA: bone age, MPH: midparental height, FH: final height

subnormal GV was higher than that of patients who showed a normal GV (p = 0.005 and p = 0.045 respectively) (Table 3). During the treatment period GV never declined below -1 SDS in 16 (32%) of the patients, while in 19 patients' (38%) a decline below -1 SDS was noted once and in 15 patients (30%) twice. GV of patients who attained a FH below MPH never dropped below -1 SDS.

It was found that basal LH levels of patients (0.46 ± 0.69) who had at least one subnormal GV episode were higher than the basal LH levels of patients (0.12 ± 0.08) who did not have subnormal GV episodes (p = 0.044). It was found that peak LH levels of patients (mean 8.82 ± 3.63) who showed two episodes of subnormal GV were considerably higher than the peak LH levels of patients (mean 6.59 ± 3.43) who showed only one subnormal GV episode (p = 0.039).

When the patients were stratified according to age of diagnosis, as either between 3.0-6.9 years old group (group 1) or 7.0-8.0 years old (group 2), it was seen that group 2 had a lower 3rd and 4th year GV SDS compared to group 1 (p = 0.0001, p = 0.011 respectively). However, there was no difference in the frequency of declining of GV below -1 SDS.

When the relationship between initial diagnostic parameters and the annual GV SDS was examined (Table 4), it was found that the age of diagnosis was significantly negatively correlated with the 3rd year GV SDS, and 4th year GV SDS (p = 0.0001 and p = 0.009 respectively). There was also a significant negative correlation between height at diagnosis and 3rd year GV SDS (p = 0.019). There was a significant positive correlation only between basal FSH level at diagnosis and 3rd year GV SDS (p = 0.046). There was a significant negative correlation between basal LH level at diagnosis and GV SDS in the first and second years (p = 0.012 and p = 0.017 respectively). Basal estradiol at diagnosis was significantly negatively correlated with first year and 4th year GV SDS (p = 0.020 and p = 0.028). It was found that there was a significant negative correlation between the BA at diagnosis and the 3rd and 4th year GV SDS (p = 0.002 and p = 0.038). Advanced BA (BA-CA) at diagnosis was found to be negatively correlated only with first year GV SDS (p = 0.005). There was a significant positive correlation between MPH and 2nd year GV SDS and 3rd year GV SDS (p = 0.014 and p = 0.041), but no correlation was found between GV SDS, FH and FH-MPH. There was a significant positive correlation between the duration of treatment and 3rd and 4th year GV SDS (p = 0.001 and p = 0.008).

There was no correlation between breast stage at diagnosis and GV SDS. In addition, the incidence of subnormal GV was not different according to the breast stage at diagnosis. There was no correlation between the pubic hair stage at diagnosis and GV SDS. When the relation between pubic hair stage at diagnosis and subnormal GV was evaluated, in 85.7% (n = 18) of patients with no pubic hair no subnormal GV SDS occurred, whereas there was at least one episode of subnormal GV in 82.4% (n = 14) of the patients with pubic hair. Patients with pubic hair at diagnosis were found to have an increased risk of subnormal GV (p = 0.016).

The LH suppression level of all patients was evaluated in the first two years of treatment. This evaluation could be

Table 2. The annual growth velocity standard deviation score of the patients

	n	Mean	Median	SD	Minimum	Maximum
1st year GV SDS	50	0.75	0.75	1.02	-0.95	3.29
2nd year GV SDS	50	-0.12	-0.05	0.94	-2.00	1.73
3rd year GV SDS	48	-0.59	-0.48	1.11	-2.93	1.53
4th year GV SDS	26	-0.84	-0.87	1.41	-2.93	2.28
5th year GV SDS	8	-0.06	0.05	1.73	-2.54	2.40
6th year GV SDS	4	-0.86	-1.09	0.89	-1.68	0.41

GV: growth velocity, SDS: standard deviation score

Table 3. Chronological age of patients who had normal and subnormal growth velocity standard deviation score by year

CA (years)	Normal GV SDS						Subnormal GV SDS						p
	n	Mean	Median	SD	Minimum	Maximum	n	Mean	Median	SD	Minimum	Maximum	
2nd year	39	8.69	9.00	1.04	6.08	9.83	11	8.68	8.66	1.40	4.91	10.00	0.888
3rd year	28	9.21	9.45	1.25	5.91	10.66	20	10.12	10.25	0.67	8.00	11.00	**0.005**
4th year	13	9.44	9.83	1.26	6.90	10.90	13	10.28	10.6	0.77	8.41	11.00	**0.045**
5th year	6	9.67	9.74	1.18	7.90	11.00	2	10.21	10.21	0.54	9.83	10.60	0.739

GV: growth velocity, SDS: standard deviation score, CA: chronological age, SD: standard deviation

performed in only 47 patients in the third year of treatment, 26 patients in the fourth year of treatment and six patients in the fifth year of treatment. It was observed that LH suppression increased significantly over the treatment years ($p = 0.001$). The second year LH of the patients who showed a subnormal GV in the 3rd year of treatment was significantly more suppressed (mean = 0.87 ± 0.37 minimum-maximum: 0.49-1.70) than those with normal GV values (mean = 1.20 ± 0.53 minimum-maximum: 0.40-2.83) ($p = 0.030$), although no significant correlation was found between LH suppression level and GV SDS.

Discussion

Although some studies have reported subnormal GV in some GnRHa-treated patients with ICPP, factors associated with subnormal GV were not investigated in these studies (6-9) or only one year of treatment was evaluated (13). Our study is the first cohort study to investigate the frequency, time of occurrence, and factors associated with subnormal GV and its effect on FH during GnRHa treatment in patients with ICPP.

In this study, the GV and GV SDS significantly decreased over the years of GnRHa treatment. Although the GV

Table 4. The relationship between annual growth velocity standard deviation score and clinical features

	1st year GV SDS (n = 50) r/p	2nd year GV SDS (n = 50) r/p	3rd year GV SDS (n = 48) r/p	4th year GV SDS (n = 26) r/p
CA at diagnosis (years)	-0.055	0.075	-0.508	-0.503
	0.705	0.607	**0.0001**	**0.009**
Height at diagnosis (cm)	-0.136	0.126	-0.337	-0.381
	0.350	0.384	**0.019**	0.055
Height SDS at diagnosis	-0.152	0.051	0.180	0.159
	0.296	0.723	0.221	0.438
BMI SDS at diagnosis	0.039	-0.113	0.046	0.144
	0.788	0.433	0.758	0.484
Basal FSH at diagnosis (IU/L)	-0.271	-0.169	0.290	0.066
	0.06	0.241	**0.046**	0.748
Basal LH at diagnosis (IU/L)	-0.356	-0.335	0.130	-0.073
	0.012	**0.017**	0.379	0.721
Stimulated FSH (IU/L)	0.239	0.020	0.142	0.224
	0.132	0.900	0.376	0.328
Stimulated LH (IU/L)	-0.004	0.132	-0.243	-0.274
	0.981	0.404	0.125	0.229
Basal estradiol at diagnosis (pg/mL)	-0.332	-0.048	-0.150	-0.431
	0.020	0.739	0.310	**0.028**
BA at diagnosis (year)	-0.276	-0.094	-0.442	-0.409
	0.055	0.515	**0.002**	**0.038**
BA-CA (year)	-0.392	-0.221	-0.135	-0.064
	0.005	0.122	0.360	0.757
Duration of treatment	-0.065	0.012	0.450	0.509
	0.659	0.931	**0.001**	**0.008**
MPH (cm)	0.038	0.357	0.306	0.094
	0.802	**0.014**	**0.041**	0.663
FH (cm)	0.048	0.124	0.209	0.306
	0.797	0.507	0.259	0.288
MPH-FH (cm)	0.088	-0.094	0.005	0.123
	0.639	0.617	0.979	0.675

GV: growth velocity, SDS: standard deviation score, CA: chronological age, FSH: follicle-stimulating hormone, LH: luteinizing hormone, BA: bone age, MPH: methylphenidate, FH: final height, BMI: body mass index

SDS was within the normal limits during the first year of treatment, GV began to decline below -1 SDS, starting from the second year and during the treatment interval and in 58% of the patients, GV dropped below -1 SDS at least once. The cause of linear growth impairment during GnRHa treatment is unknown. Several investigators have examined the effect of gonadal suppression with GnRHa on the growth hormone axis and height velocity. Although some studies have suggested a subnormal GH secretion during treatment with GnRHa (11,15), others have not (10,12,16). Evaluation of subpopulations of children with poor growth during GnRHa therapy has also not clearly demonstrated GH deficiency (8,11,15,17,18,19). Furthermore, studies have reported no significant change in IGF-1 and IGFBP-3 concentrations, despite a decrease in the height velocity (20,12). Lack of change in IGF-1 and IGFBP-3 with decrease in sex hormones level and height velocity suggests a direct effect of sex hormones on growth. *In vitro* and animal studies have shown that sex steroids may act via locally produced IGF-1 in the target tissues without significantly raising circulating IGF-1 concentrations (4). According to Weise et al (13), height velocity SDS is correlated inversely with markers of the severity of prior estrogen exposure, including duration of precocious puberty before treatment start and Tanner breast stage. However, no correlation between estradiol and GV was found in this study (13). It is known that activation of the hypothalamus-pituitary gonad (HHG) axis in puberty results in LH dominant secretion rather than FSH in the LHRH test, thus stimulating estrogen production in the ovaries. When we examined the relationship between GV and the HHG axis there was a positive correlation between basal FSH at diagnosis and 3th year GV SDS, a negative correlation between basal LH at diagnosis and 1st and 2nd year GV SDS and a negative correlation between estradiol and first year and 4th year GV SDS values. Patients whose GV was subnormal at least once were found to have higher basal LH levels at diagnosis than LH levels of patients with normal GV. Peak LH levels of patients who showed subnormal GV twice were higher than the peak LH levels of patients who had one subnormal GV episode. These findings support the hypothesis that, during GHRHa treatment, there was an increased risk of subnormal GV as the degree of activation of the HHG axis increases. However, there was no correlation between the breast stage at diagnosis and GV SDS. In normal pubertal development, pubic hair growth also starts not long after the onset of breast development. In our study, an increased risk of subnormal GV in patients with pubic hair at diagnosis also suggests that the initiation of treatment in the later stages of puberty may increase the risk of GV decrease. Weise et al (13) have shown that height velocity SDS in the second year of treatment is correlated inversely with BA advancement and that BA was the best independent predictor of growth during GnRHa therapy. These authors hypothesized that during GnRHa therapy, when hormonal concentrations are normalized, this excessive senescence would be expected to result in decreased linear growth. Similar to these results, in our study we also found that BA at diagnosis showed a significantly negative correlation with the third and the fourth year GV SDS. Advanced BA (BA-CA) at diagnosis was found to be negatively correlated only with the 1 year GV SDS. In the study of Weise et al, (13) it was suggested that subnormal GV was related to the fact that 40% of their patients were postmenarcheal, to late onset treatment (maximum 9.4 years) and to an advanced BA (maximum BA 14 years, median BA advance 3.8 years). Of note in our study a correlation was found between BA and GV, despite the fact that all of our patients were premenarcheal, the treatment was initiated before the age of eight years and BA was not greatly advanced (maximum BA 12 years, mean BA advance 1.36 years). One of the best indicators of estrogenic effect in precocious puberty is advanced BA. In addition, the finding that advanced CA at diagnosis is correlated with GV and GV SDS only in the 1st year of treatment suggests that the effect of the removal of estrogenic activity by GnRHa treatment on GV SDS is only present at the beginning of the treatment. In our study, while advanced BA at diagnosis had no effect on GV SDS after the first year of treatment, BA at diagnosis appeared to be related to GV SDS at the 3rd and 4th years. These results suggest that subnormal GV SDS after the first year of treatment was independent of advance in BA and associated with only with the level of bone maturation. In our study, the negative correlation between age of diagnosis and 3rd and 4th year GV SDS also supports this conclusion.

It has also been hypothesized that GnRHa treatment might inhibit growth by suppression of estrogen concentrations to levels below those of prepubertal children (21). However, estrogen measurements using an ultrasensitive recombinant cell bioassay are not consistent with this hypothesis (22). Possible excessive suppression of estradiol could not be demonstrated due to the fact that the estradiol kit used in our study was not ultrasensitive. However, the significant increase in LH suppression with GnRHa treatment over the years and having more suppressed LH levels at the second year of patients with subnormal GV at 3 years of treatment were considered to be a significant effect of LH suppression on GV. However, considering the absence of a relationship between treatment dose and LH suppression, it was concluded that the degree of LH suppression varied

independently from the dose and that excessive suppression of LH should be avoided in the management of these patients.

The effect of subnormal GV seen in patients receiving GnRHa therapy with ICPP on FH is not known exactly. There are studies which report that for some patients treated with GnRHa, GV decreases so considerably that patients fail to reach their target height (23,17). In our study, positive correlations between MPH and 2nd and 3rd year GV SDS showed that LH suppression with GnRHa in precocious puberty provided appropriate growth in accordance with the patient's genetic potential. Subnormal GV was frequently observed in patients treated with GnRHa treatment, but the FH of the patients was compatible with MPH. FH and MPH-FH were not different in patients with subnormal GV and normal GV.

In our study, we found that age of diagnosis was negatively correlated with 3rd and 4th year GV SDS. A subnormal GV was observed in the 3rd year of treatment (median) and the median age of detection of subnormal GV was 9.9 (4.9-10.9) years. It was seen that patients who started treatment at 7-8 years had a lower GV and GV SDS than patients who started treatment at a younger age. Thus, it was thought that patients, who started treatment at age 7-8 years might be at risk of subnormal GV when they are 10-10.5 years old.

Study Limitations

The two limitations in our study were, firstly, a possible excessive suppression of estradiol could not be demonstrated due to the fact that the estradiol kit used in our study was not ultrasensitive. Secondly, FH and MPH-FH were not different in patients with subnormal GV and normal GV in our study. The effect of subnormal GV on FH may have been small due to occurrence of subnormal GV that is often observed at the end of treatment. New studies are needed to investigate the effect of the subnormal GV seen in the first years of treatment on FH, since the subnormal GV in our study was observed in the last years of treatment in many subjects.

Conclusion

This study shows that that during GnRHa treatment in ICPP, the risk of subnormal GV is high in the 3rd year of treatment and/or at ages 10-10.5 years, in patients with pubic hair at diagnosis, in those in whom the treatment is started at ages 7-8 years, in those who have a high baseline level and peak LH at diagnosis and in those with advanced BA and excessive LH suppression on follow-up. Subnormal GV during GnRHa treatment did not affect FH.

Ethics

Ethics Committee Approval: Retrospective study.

Informed Consent: Retrospective study.

Peer-review: Internal and external peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Nursel Muratoğlu Şahin, Semra Çetinkaya, Zehra Aycan, Concept: Nursel Muratoğlu Şahin, Design: Nursel Muratoğlu Sahin, Data Collection or Processing: Nursel Muratoğlu Şahin, Analysis or Interpretation: Nursel Muratoğlu Şahin, Asiye Uğraş Dikmen, Literature Search: Nursel Muratoğlu Şahin, Writing: Nursel Muratoğlu Şahin, Zehra Aycan.

Financial Disclosure: There is no financial funding.

References

1. Brauner R. Central precocious puberty in girls: prediction of the aetiology. J Pediatr Endocrinol Metab 2005;18:845-847.
2. Weise M, De-Levi S, Barnes KM, Gafni RI, Abad V, Baron J. Effects of estrogen on growth plate senescence and epiphyseal fusion. Proc Natl Acad Sci U S A 2001;98:6871-6876. Epub 2001 May 29
3. Byers S, Moore AJ, Byard RW, Fazzalari NL. Quantitative histomorphometric analysis of the human growth plate from birth to adolescence. Bone 2000;27:495-501.
4. Attie KM, Ramirez NR, Conte FA, Kaplan SL, Grumbach MM. The pubertal growth spurt in eight patients with true precocious puberty and growth hormone deficiency: evidence for a direct role of sex steroids. J Clin Endocrinol Metab 1990;71:975-983.
5. Pucarelli I, Segni M, Ortore M, Acardi E, Pasquino AM. Effects of combined gonadotropin-releasing hormone agonist and growth hormone therapy on adult height in precocious puberty: a further contribution. J Pediatr Endocrinol Metab 2003;16:1005-1010.
6. Klein KO, Barnes KM, Jones JV, Feuillan PP, Cutler GB Jr. Increased final height in precocious puberty after long-term treatment with LHRH agonists: the National Institutes of Health experience. J Clin Endocrinol Metab 2001;86:4711-4716.
7. Walvoord EC, Pescovitz OH. Combined use of growth hormone and gonadotropin-releasing hormone analogues in precocious puberty: theoretic and practical considerations. Pediatrics 1999;104:1010-1014.
8. Tato L, Saggese G, Cavallo L, Antoniazzi F, Corrias A, Pasquino AM, Cisternino M. Use of combined Gn-RH agonist and hGH therapy for better attaining the goals in precocious puberty treatment. Horm Res 1995;44:49-54.
9. Saggese G, Bertelloni S, Baroncelli GI, Di Nero G, Battini R. Growth velocity and serum aminoterminal propeptide of type III procollagen in precocious puberty during gonadotropin-releasing hormone analogue treatment. Acta Paediatr 1993;82:261-266.
10. Galluzi F, Salti R, Bindi G, Pasquini E, La Cauza C. Adult height comparison between boys and girls with precocious puberty after long-term gonadotrophin-releasing hormone analogue therapy. Acta Paediatr 1998;87:521-527.
11. Kamp GA, Manasco PK, Barnes KM, Jones J, Rose SR, Hill SC, Cutler GB Jr. Low growth hormone levels are related to increased body mass index and do not reflect impaired growth in luteinizing hormone-releasing hormone agonist-treated children with precocious puberty. J Clin Endocrinol Metab 1991;72:301-307.

12. Sklar CA, Rothenberg S, Blumberg D, Oberfield SE, Levine LS, David R. Suppression of the pituitary-gonadal axis in children with central precocious puberty: effects on growth, growth hormone, insulin-like growth factor-I, and prolactin secretion. J Clin Endocrinol Metab 1991;73:734-738.
13. Weise M, Flor A, Barnes KM, Cutler GB Jr, Baron J. Determinants of growth during gonadotropin-releasing hormone analog therapy for precocious puberty. J Clin Endocrinol Metab 2004;89:103-107.
14. Neyzi O, Furman A, Bundak R, Gunoz H, Darendeliler F, Bas F. Growth references for Turkish children aged 6 to 18 years. Acta Paediatr 2006;95:1635-1641.
15. Oostdijk W, Drop SL, Odink RJ, Hummelink R, Parsch CJ, Sippell WG. Long-term results with a slow-release gonadotrophin-releasing hormone agonist in central precocious puberty. Dutch-German Precocious Puberty Study Group. Acta Paediatr 1991;372:39-45.
16. Verrotti A, Ferrari M, Sabatino G, Morgese G, Chiarelli F. Serum insulin-like growth factor-I (IGF-I) and IGF binding protein-3 levels in children with precocious puberty treated with gonadotropin-releasing hormone analog without or in combination with cyproterone acetate. Gynecol Endocrinol 1997;11:243-250.
17. Saggese G, Pasquino AM, Bertelloni S, Baroncelli GI, Battini R, Pucarelli I, Segni M, Franchi G. Effect of combined treatment with gonadotropin releasing hormone analogue and growth hormone in patients with central precocious puberty who had subnormal growth velocity and impaired height prognosis. Acta Paediatr 1995;84:299-304.
18. Pasquino AM, Municchi G, Pucarelli I, Segni M, Mancini MA, Troiani S. Combined treatment with gonadotropin-releasing hormone analog and growth hormone in central precocious puberty. J Clin Endocrinol Metab 1996;81:948-951.
19. Pucarelli I, Segni M, Ortore M, Moretti A, Iannaccone R, Pasquino AM. Combined therapy with GnRH analog plus growth hormone in central precocious puberty. J Pediatr Endocrinol Metab 2000;13(Suppl 1):811-820.
20. Kanety H, Karasik A, Pariente C, Kauschansky A. Insulin-like growth factor-I and IGF binding protein-3 remain high after GnRH analogue therapy in girls with central precocious puberty. Clin Endocrinol (Oxf) 1996;45:7-12.
21. Lampit M, Golander A, Hochberg Z. Estrogen mini-dose replacement during GnRH agonist therapy in central precocious puberty: a pilot study. J Clin Endocrinol Metab 2002;87:687-690.
22. Klein KO, Baron J, Barnes KM, Pescovitz OH, Cutler GB Jr. Use of an ultrasensitive recombinant cell bioassay to determine estrogen levels in girls with precocious puberty treated with a luteinizing hormone-releasing hormone agonist. J Clin Endocrinol Metab 1998;83:2387-2389.
23. Saggese G, Bertelloni S, Baroncelli GI, Di Nero G, Battini R. Growth velocity and serum aminoterminal propeptide of type III procollagen in precocious puberty during gonadotropin-releasing hormone analogue treatment. Acta Pediatr 1993;82:261-266.

ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.0010

J Clin Res Pediatr Endocrinol 2018;10(3):247-255

Prospective Follow-up of Children with Idiopathic Growth Hormone Deficiency After Termination of Growth Hormone Treatment: Is There Really Need for Treatment at Transition to Adulthood?

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What is already known on this topic?

Continuation of growth hormone (GH) treatment in adolescents with severe, childhood-onset idiopathic GH deficiency during the period of transition to adult care is still debatable.

What this study adds?

We did not observe a clinical condition requiring growth hormone (GH) treatment in any of the study subjects, half of whom had idiopathic GH deficiency and half with transient growth hormone deficiency, during the six months subsequent to cessation of treatment.

Abstract

Objective: Continuation of growth hormone (GH) treatment in adolescents with severe childhood onset idiopathic GH deficiency (IGHD) during the transition period, irrespective of achievement of final height, is still debatable. We aimed to prospectively investigate the metabolic profile, bone mineral density (BMD) and body composition of patients with IGHD in whom GH treatments were terminated after they had reached their final height, six months after the cessation of therapy.

Methods: Twelve patients, six of whom had peak GH levels < 5 ng/mL [permanent GH deficiency (GHD), group 1], and six who had peak GH levels > 5 ng/mL (transient GHD, group 2) after insulin stimulation test were evaluated for anthropometric and laboratory parameters including fasting blood glucose (FBG), fasting insulin, lipid profile, BMD, body composition measurements and 24-hour ambulatory blood pressure monitoring before (baseline) and at six months after discontinuation of GH.

Results: No differences were found in clinical, laboratory, BMD and body composition measures between groups 1 and 2 at baseline. All IGHD patients had significant increments of body weight (BW), body mass index (BMI), BMD, total body fat (TBF), TBF%, truncal fat (TF) and TF% after GH cessation. Six months later BW, BMI, BMD and TF% was increased significantly while FBG and lipids showed no change in group 1. In group 2, TBF% and TF% were increased, FBG, total cholesterol and high-density lipoprotein decreased after six months. Changes in these parameters in group 2 were not statistically different from group1.

Conclusion: TF% increase in both groups after cessation of therapy. We did not observe a clinical condition requiring GH treatment in any of the study subjects during the follow-up period.

Keywords: Growth hormone deficiency, transition, childhood

Introduction

Growth hormone (GH) treatment is generally applied to stimulate longitudinal skeletal growth in children with idiopathic GH deficiency (IGHD) and is terminated when final height is attained and epiphyseal closure has occurred. However, it has been reported that body mass (i.e. muscle and bone mass) of adult patients with severe childhood onset GHD who had been treated with GH until they achieved their final height, was significantly less than the body mass of young adults with adult onset GHD (1). Somatic development, including body composition, muscle mass maturation and skeletal mineralization is completed during the transition from late adolescence to

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Conflict of interest: None declared
Received: 09.01.2018
Accepted: 06.03.2018

early adulthood and GH is believed to play a role in this process (2).

GHD adults receiving hormone replacement therapy were reported to have increased body fat, insulin resistance and dyslipidemia with low-density lipoprotein (LDL), high serum triglycerides (TG), and high-density lipoprotein (HDL) levels. Increased prevalence of hypertension, premature atherosclerosis, mortality from cardiovascular diseases and decreased quality of life are also observed in these patients (3). These observations suggest that GH may also play a role in the prevention of metabolic and cardiovascular diseases.

Initial small-scale trials reported between 1991-1994 have demonstrated a reduction in muscle mass and an increase in fat mass during the 3-12 months transition period after termination of GH therapy (3). Further studies evaluated the effects of discontinuation of GH on metabolic profile in the transition period. Some of these studies evaluated the effects of GH therapy on bone mineralization (4) and some on body composition (5) using different study protocols. These studies concluded that GH was necessary for better adult metabolic profile, peak bone mass and body composition in GH deficient adolescents after they had attained their final height. In contrast, a more recent study concluded that GH deficient patients properly treated during childhood could have normal bone mineral density (BMD), body composition, cardiac function, muscle strength, carbohydrate metabolism, lipid metabolism and a good quality of life when they reached their adult height (6). These authors also reported that continuation of GH therapy for an additional two years did not change any of these parameters when compared to placebo-treated or control subjects. Continuation of GH treatment in adolescents with severe GHD during the transition period, irrespective of achievement of final height, is therefore still debatable.

In this study, we aimed to investigate prospectively, six months after the cessation of therapy, the metabolic profile, BMD and body composition of patients with isolated childhood onset GHD in whom GH treatment was terminated after they reached their final height.

Methods

This was a single center, prospective clinical study carried out in accordance with good clinical practice guidelines, with appropriate ethical approval and signed informed consent. Ethical approval was given by Ankara University Ethical Committee (approval number: 06-240-13).

We evaluated insulin tolerance tests (ITT) in a group of patients with childhood onset IGHD who were followed at Ankara University, Department of Pediatric Endocrinology, who had received GH treatment during their childhood and had been off GH when they reached their final height. Twelve patients who gave consent were included in the study and followed-up prospectively.

All of these twelve patients conformed to the clinical and diagnostic criteria for isolated IGHD and had GH peaks less than 10 ng/mL following two different provocative pharmacological stimuli (levodopa and ITT) prior to beginning treatment. None of these patients had any other pituitary hormone deficiencies. All patients had received subcutaneous recombinant human GH treatment at a dose of 0.2 mg/kg/week, over six days out of seven each week until they reached their final height. Final height achievement was defined as attainment of bone age (BA) ≥14 years for girls, ≥16 years for boys, closure of epiphyseal plaques and a decreased height velocity <2 cm/year (7). Diagnosis of organic GHD, GH neurosecretory dysfunction, multiple anterior pituitary hormone deficiencies and requirement for any additional treatment for a chronic disease or a complex syndrome constituted the criteria for exclusion.

The study design is given in Figure 1. Twelve patients who achieved their final height were prospectively followed-up. They underwent basal anthropometric and laboratory evaluation including measurement of body weight (BW), calculation of body mass index (BMI), calculation of BMI for height percentage (BMI %), measurement of serum insulin like growth factor 1 (IGF-1) level, estimation of IGF-1 standard deviation score (SDS), measurement of IGF binding protein 3 (IGFBP-3) and estimation of IGFBP-3 concentration SDS, measurement of fasting blood glucose (FBG), fasting insulin (FI), fasting total cholesterol (TC), LDL-cholesterol (C), HDL-C, TG levels just before the cessation of GH therapy while they were still being administered GH. Besides, a 24-hour ambulatory blood pressure monitoring and a dual energy X-ray absorptiometry scan to asses BMD were performed in each patient. Z-scores for BMD, both for chronological age (CA) and BA were calculated according to BMD reference data for healthy Turkish children (8). Blood pressure monitoring was interpreted as systolic/diastolic overload, which was defined as the percentage of high blood pressure measurements among all systolic/diastolic measurements for night and day separately. Body composition measurements [total body fat (TBF) mass, TBF%, truncal fat (TF) mass, TF%, total body muscle (TBM) mass and TBM%] were performed via TANITA Body Composition Analyzer, BC-418 MA III.

GH therapy was discontinued after the baseline evaluations in all subjects and six weeks later each patient was re-evaluated for GHD by ITT and IGF-1, IGFBP-3 levels. A peak

serum GH level below 5 ng/mL was defined as GHD (9). Peak GH response < 5 ng/mL was defined as permanent and peak GH response > 5 ng/mL was defined as transient GHD. Patients were grouped as permanent (group 1, n = 6) and transient (group 2, n = 6) GHD, according to their peak GH response.

Six months after discontinuation of GH treatment anthropometric and laboratory evaluations similar to baseline evaluations were repeated in each patient. These parameters and baseline parameters in all patients were compared both within each group and between group 1 (permanent GHD) and group 2 (transient GHD) (9).

Serum IGF-1, IGFBP-3, fasting insulin and growth hormone levels were measured using the radioimmunoassay method by gamma counter analyser using DSL kit. SDS were calculated for IGF-1, IGFBP-3 according to the reference data of the analyser. TC, HDL-C, TG and fasting glucose were measured by the enzymatic method using the "Beckman Coulter Unicel DxC 800" analyser. LDL-C was derived from other lipid measurements. Atherogenesis index (AI) was calculated as TC/HDL-C (10). The homeostasis model assessment (HOMA) was used to evaluate insulin resistance and HOMA was calculated using the following formula: HOMA = [fasting insulin (μU/mL) X glucose (mg/dL)] /22,5x18 (11). Serum cortisol (for insulin tolerance test) was assessed by immunoenzymatic method with the Roche E170 analyser.

Statistical Analysis

The data were analyzed using the SPSS software program for Windows, version 16.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables were compared between groups using Mann-Whitney U test. For the repeated measures, we also used Mc Nemar or Wilcoxon matched-pair signed-rank test. The values for all parameters except for gender and blood pressure non-dipping ratio were expressed as mean ± SDS (median). P < 0.05 was considered as statistically significant.

Results

Patients who had received GH treatment during their childhood for IGHD and had been off GH treatment when they reached their final height were retested with ITT in our pediatric endocrinology department and among them 12 patients who agreed to participate in the study were followed-up prospectively.

All 12 patients demonstrated a significant increase in BW (p = 0.033) but not in BMI and BMI% six months after GH cessation when compared to baseline. IGF-1 and IGF-1 SDS decreased significantly (p = 0.006 and p = 0.007, respectively). FBG and HDL-C also decreased, while FI and other parameters of their lipid profiles did not change. Although BMD increased (p = 0.01), BMD z-score for CA and BA were not different after 6 months. TBF mass, TBF%, TF mass and TF% increased significantly (p values respectively 0.008; 0.028; 0.012; and 0.005). TBM mass decreased but

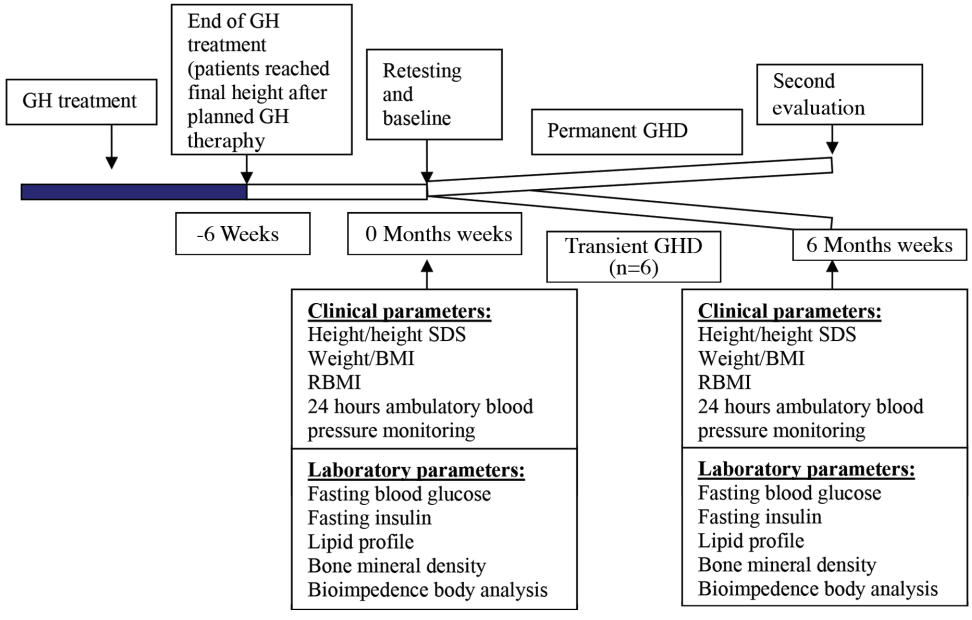

Figure 1. Study design

GH: growth hormone, GHD: growth hormone deficiency, SD: standard deviation, SDS: standard deviation score, BMI: body mass index, RBMI: relatif body mass index

Table 1A. Clinical findings at baseline and after six months in the permanent growth hormone deficiency patients

Mean ± SD (median)		Permanent GHD (group 1) n = 6		p value
		Baseline	Six months later	
Age (years)		16.10 ± 2.05 (15.47)	-	-
Bone age (years)		14.83 ± 1.75 (15.00)	-	-
Sex (female/male)		3/3	-	-
Height (cm)		163.21 ± 6.74 (163.50)	-	-
Height SDS		-0.48 ± 0.49 (-0.50)	-	-
Body weight (kg)		56.9 ± 16.95 (53.85)	60.44 ± 20.26 (56.30)	< 0.05
Body mass index (BMI)		21.6 ± 7.80 (19.23)	22.76 ± 9.48 (19.99)	< 0.05
BMI %		101.69 ± 39.6 (90.53)	105.24 ± 44.76 (92.08)	NS
Blood pressure	Day systolic overload (%)	6.2 ± 8.45 (3.45)	6.02 ± 7.92 (3.70)	NS
	Night systolic overload (%)	9.6 ± 12.90 (5.55)	4.55 ± 9.10 (0.00)	NS
	Day diastolic overload (%)	11.45 ± 14.01 (8.6)	8.22 ± 14.09 (1.85)	NS
	Night diastolic overload (%)	12.5 ± 9.48 (13.90)	9.10 ± 18.20 (0.00)	NS

SD: standard deviation, GHD: growth hormone deficiency, SDS: standard deviation score, NS: not significant

Table 1B. Laboratory findings in the permanent growth hormone deficiency (group 1) at baseline and after six months

Mean ± SD (median)	Permanent GHD (group 1) n = 6		p value
	Baseline	Six months	
IGF-1 (ng/mL)	896.36 ± 285.60 (888.00)	431.76 ± 148.87 (430.30)	**< 0.05**
IGF-1 SDS	1.83 ± 1.78 (2.21)	-1.44 ± 1.40 (-1.28)	**< 0.05**
IGFBP3 (ng/mL)	4761.66 ± 1387.87(4782. 50)	4116.00 ± 1530.96 (3595.00)	NS
IGFBP3, SDS	-1.33 ± 1.91 (-0.93)	-1.85 ± 1.78 (-2.7)	NS
Fasting blood glucose (mg/dL)	82.50 ± 6.05(84.50)	80.83 ± 5.74 (80.50)	NS
Fasting insulin (µU/mL)	12.61 ± 5.39 (11.25)	11.90 ± 4.43 (10.70)	NS
Glucose/insulin ratio	7.46 ± 2.93(7.35)	7.36 ± 1.99 (7.71)	NS
HOMA-IR	2.56 ± 1.15 (2.30)	2.36 ± 0.88 (2.19)	NS
Total cholesterol (TC) (mg/dL)	148.40 ± 34.06 (142.00)	138.50 ± 25.12(146.00)	NS
LDL cholesterol (mg/dL)	86.02 ± 28.10 (83.00)	82.50 ± 26.03 (80.50)	NS
HDL cholesterol (mg/dL)	48.38 ± 6.97 (50.60)	44.10 ± 6.29 (44.00)	NS
VLDL-cholesterol (mg/dL)	14.00 ± 9.13 (10.00)	12.16 ± 4.30 (11.50)	NS
Triglyceride (mg/dL)	70.60 ± 44.96 (52.00)	61.00 ± 21.08 (58.00)	NS
Atherogenic index (TC/HDL)	3.06 ± 0.57 (3.25)	3.18 ± 0.65 (3.33)	NS
BMD (gr/cm^2)	0.764 ± 0.116 (0.797)	0.843 ± 0.146 (0.825)	**< 0.05**
BMD z-score (Chronological age)	-2.05 ± 1.56 (-1.91)	-1.04 ± 1.48 (-1.20)	NS
BMD z-score (bone age)	-0.83 ± 0.93 (-0.90)	-0.58 ± 1.37 (-1.02)	NS
Body composition			
Total body fat mass (kg)	7.98 ± 2.45 (8.00)	9.12 ± 3.17 (10.00)	**< 0.05**
Total body fat %	15.94 ± 4.44 (18.40)	17.54 ± 5.18 (18.60)	NS
Truncal fat mass (kg)	3.46 ± 1.53 (3.10)	4.48 ± 1.61 (4.00)	NS
Truncal fat %	12.04 ± 3.76 (12.10)	13.82 ± 4.52 (14.10)	**< 0.05**
Total muscle mass (kg)	40.7 ± 7.96 (43.90)	40.72 ± 7.30 (43.00)	NS
Muscle mass %	80.32 ± 4.53 (78.30)	78.30 ± 4.18 (77.50)	NS

IGF-1: insulin like growth factor 1, IGFBP3: insulin like growth factor binding protein 3, HOMA-IR: homeostasis model assessment-Insulin resistance, BMD: bone mineral density, NS: not significant, GHD: growth hormone deficiency, LDL: low-density lipoprotein, HDL: high-density lipoprotein, VLDL: very low-density lipoprotein SDS: standard deviation score, SD: standard deviation

TBM% did not change. There were no significant changes with respect to blood pressure measurements.

Clinical and Laboratory Characteristics of Group 1 Patients at Baseline and Six Months Later

In group1 BW and BMI increased significantly after six months from discontinuation of GH (p = 0.043 and p = 0.043, respectively) however BMI% did not change (Table 1A). IGF-1 and IGF-1 SDS significantly decreased (p = 0.028 and p = 0.028, respectively) and changes in IGFBP-3 and IGFBP-3 SDS were not statistically significant. FBG, FI, lipid profile and AI showed no change. BMD increased significantly (p = 0.043), but BMD z-scores for CA and BA did not. TBF mass and TF% increased, while TBF%, TF mass, TBM mass and TBM% did not change significantly (p = 0.043) (Table 1B).

Clinical and Laboratory Characteristics of Group 2 Patients, Baseline and Six Months Later

BW, BMI and BMI% did not change significantly from baseline in group 2 (Table 2A). IGF-1, IGF-1 SD, IGFBP-3 and IGFBP-3 SD were not statistically different six months after discontinuation of GH therapy. FBG, TC and HDL-C decreased by six months after cessation of treatment (p values respectively 0.043; 0.046 and 0.046). FI, LDL-C, TG and AI, as well as BMD, BMD z-scores for CA and BA were statistically similar at baseline and at the six-month follow-up (Table 2B). TF% increased significantly in this group, but other parameters of body composition did not change (Table 2B).

Differences Between Groups 1 and 2

At baseline and after six months, group 1 and group 2 were similar with respect to all study parameters.

Discussion

It has become clear that GH and GH therapy affect more than just linear growth and have additional effect on metabolism, bone maturation and body composition. Therefore, the consequences of GH discontinuation in childhood onset GHD, after achieving final height, have become a concern among endocrinologists. There are contradictory results concerning the effect of GH on various biochemical and anthropometric parameters after discontinuation of GH in the literature. Several studies have demonstrated metabolic changes, while some others have reported no change. Thus, the clinical implication of these changes, if there are any, is debatable.

We performed this prospective study in order to evaluate the need for continuation of GH during the transition period in this group of patients. There are only a small number of studies on this topic. Some of them have evaluated effects of GH discontinuation on a specific field such as metabolic profile, or bone mass, or body composition. Most of these studies are multicenter and are made up of a heterogeneous group of subjects which include GHD patients with different etiologies (3,4,5,12).

In our study, permanent GHD subjects (group 1) had significantly increased BW and BMI at six months after discontinuation of GH however their BMI% did not change at all. In addition, the increase in BW and BMI in group 1 was not different from the increase observed in the transient GHD group, which is consistent with the literature (3,6). Johannsson et al (3) have evaluated 40 GHD patients (including organic GHD and multiple anterior pituitary hormone deficiencies) in 21 GH deficient, 19 GH sufficient

Table 2A. Clinical comparison of transient growth hormone deficiency (group 2) at baseline and after six months

Mean ± SD (median)		Transient GHD (group 2) n = 6		p value
		Baseline	Six months later	
Age (years)		16.51 ± 1.87 (16.55)	-	-
Bone age (years)		15.16 ± 0.75 (15.00)	-	-
Sex (female/male)		2/4	-	-
Height (cm)		159.93 ± 9.80 (165.10)	-	-
Height SDS		-1.23 ± 0.82 (-1.33)	-	-
Body weight (kg)		58.21 ± 20.14 (53.35)	59.92 ± 18.36 (55.62)	NS
Body mass index (BMI) (kg/m^2)		22.3 ± 5.77 (20.27)	22.61 ± 5.04 (21.15)	NS
%BMI		108.87 ± 36.23 (94.60)	104.22 ± 21.71 (96.65)	NS
Blood pressure	Day systolic overload (%)	11.4 ± 8.6 (9.50)	27.90 ± 29.24 (25.80)	NS
	Night systolic overload (%)	22.33 ± 17.62 (21.45)	32.41 ± 22.65 (34.50)	NS
	Day diastolic overload (%)	7.73 ± 8.10 (3.65)	16.33 ± 15.98 (9.80)	NS
	Night diastolic overload (%)	18.83 ± 17.44 (19.85)	38.96 ± 29.68 (43.90)	NS

GHD: growth hormone deficiency, SD: standard deviation, SDS: standard deviation score, NS: not significant

and 16 healthy controls annually, after discontinuing GH treatment for a follow-up period of two years. They have reported increased BMI in both the transient GHD and control groups, however BMI did not differ between the groups. Also, the BMI did not change in their GH deficient group over the follow-up period. Mauras et al (6) studied 58 childhood onset GHD with heterogeneous etiology after GH discontinuation. According to retest, 40 of their patients showed transient GHD while18 of their patients were GH sufficient. Twenty-five patients with transient GHD were treated with GH, 15 treated with placebo. No significant differences were found with respect to weight and BMI over two years among their study population. It is well known that GH itself has a lipolytic effect. Increase in BW in the permanent GHD group, and TF% both in the permanent and the transient group after cessation of GH therapy may be related to reduction of the GH dependent lipolytic effect. IGF-1 and IGF-1 SDS significantly decreased after discontinuation of therapy taking all twelve patients as a whole while these values did not differ in the GH sufficient group. IGFBP-3 and IGFBP-3 SD did not change statistically in either group. Significant decrease in IGF-1 level in the permanent GHD group after discontinuation of GH is consistent with the literature (3,6,13). Decrease in IGF-1 level in group 1 can be a representative factor for persisting GH deficiency. Probably due to the small sample size, no difference with respect to IGF-1 was observed between baseline and after six months of GH cessation in group 2.

We also evaluated the metabolic effects in our study population. FBG, FI and lipid profile parameters were all similar compared to baseline values after six months in the permanent GHD group. In group 2 decreases in FBG, TC, HDL-C after six months were found but their FI, LDL-C, TG and AI values did not change. All parameters were within

Table 2B. Comparison of laboratory findings at baseline and after six months in the transient growth hormone deficiency (group 2)

Mean ± SD (median)	Transient GHD (group 2) n = 6		p value
	Baseline	Six months	
IGF-1 (ng/dL)	688.00 ± 213.76 (699.00)	523.6 ± 158.61 (514.20)	NS
IGF-1 SDS	0.61 ± 1.61 (0.95)	-0.70 ± 1.35 (-0.91)	NS
IGFBP-3 (ng/dL)	4881.00 ± 1182.78 (4550.50)	5107.50 ± 1487.15 (4790.00)	NS
IGFBP-3 SDS	-0.64 ± 1.43 (-0.84)	-0.5 ± 1.79 (0.89)	NS
Fasting blood glucose (mg/dL)	91.33 ± 10.91 (91.00)	80.66 ± 4.17 (82.50)	**< 0.05**
Fasting insulin (mIU/mL)	14.00 ± 6.92 (13.25)	10.46 ± 3.39 (10.80)	NS
Glucose/insulin ratio	7.59 ± 2.86 (7.14)	8.33 ± 2.72 (7.59)	**< 0.05**
HOMA-IR	3.15 ± 1.67 (2.93)	2.07 ± 0.70 (2.18)	NS
Total cholesterol (TC) (mg/dL)	158.33 ± 31.57 (163.50)	143.50 ± 24.76 (145.00)	**< 0.05**
LDL-cholesterol (mg/dL)	93.03 ± 20.92 (99.00)	83.83 ± 22.99 (85.50)	NS
HDL-cholesterol (mg/dL)	47.73 ± 10.14 (45.45)	42.83 ± 10.28 (37.50)	**< 0.05**
VLDL-cholesterol (mg/dL)	17.50 ± 13.04 (12.00)	16.83 ± 9.19 (19.50)	NS
Triglyceride (mg/dL)	86.83 ± 64.92 (59.50)	85.5 ± 46.21 (99.00)	NS
Atherogenic index (TC/HDL)	3.41 ± 0.95 (3.10)	3.47 ± 0.95 (3.30)	NS
BMD (gr/cm^2)	0.804 ± 0.047 (0.822)	0.839 ± 0.044 (0.832)	NS
BMD z-score (chronological age)	-1.39 ± 0.64 (-1.34)	-0.92 ± 0.70 (-0.70)	NS
BMD z-score (bone age)	-0.58 ± 0.74 (-0.49)	-0.25 ± 1.14 (-0.34)	NS
Body composition			
Total body fat mass (kg)	10.00 ± 6.60 (6.3)	12.16 ± 6.44 (9.70)	NS
Total body fat %	16.43 ± 5.02 (15.80)	19.38 ± 4.35 (20.00)	NS
Truncal fat mass (kg)	4.13 ± 3.34 (2.15)	6.06 ± 3.54 (5.3)	NS
Truncal fat %	11.33 ± 6.67 (8.55)	15.54 ± 6.37 (11.20)	**< 0.05**
Total muscle mass (kg)	45.21 ± 13.80 (44.85)	46.32 ± 14.26 (46.90)	NS
Muscle mass %	79.80 ± 4.84 (80.05)	76.94 ± 4.31 (76.40)	NS

GHD: growth hormone deficiency, SD: standard deviation, SDS: standard deviation score, IGF-1: insulin like growth factor 1, IGFBP-3: insulin like growth factor binding protein 3, HOMA-IR: homeostasis model assessment-Insulin resistance, BMD: bone mineral density, HDL: high-density lipoprotein, LDL: low-density lipoprotein, VLDL: very low-density lipoprotein, NS: not significant

the normal ranges. Decrease in FBG in the transient group after six months, although not observed in group 1, may be due to the weaning effect of GH on glucose metabolism. In our opinion, no significant negative effects on glucose metabolism were observed in either group at six months after discontinuation of GH. There are contradictory results related to these parameters in the literature. Johannsson et al (3) in their study of 21 GHD, 19 GHS patients and 16 healthy controls reported that TC, LDL-C and apolipoprotein B were all higher in the permanent GHD group than the other two groups before GH discontinuation. TC and LDL-C increased in both GH deficient and GH sufficient groups but not in healthy controls at two years after discontinuation of GH. After two years the serum levels of these lipids were therefore still higher in the GH deficient group than the other groups (3). HDL-C increased in healthy controls after two years. The permanent GHD group had statistically lower HDL-C compared to the GH sufficient group after 2 years (3). In the study of Mauras et al (6) there was no significant difference with respect to either FG or homeostatic model assessment insulin resistance in GH deficient (n = 40; 25 received further GH and 15 placebo) and sufficient (n = 18) groups at baseline or throughout the 24-month study period. Their study also demonstrated no significant difference across the groups with respect to TC, LDL-C, HDL-C, TC/HDL ratio and TG at baseline, at baseline vs. month 12 and at baseline vs. month 24 (6). Carroll et al (13) reported similar lipoprotein profiles in GH continuation and discontinuation groups at 12 months.

We observed that BMD increased significantly in GH deficient subjects at six months after discontinuation of GH therapy whereas BMD Z-scores for CA and BA did not change. Group 2 demonstrated no change in BMD and BMD z-scores for CA and BA after six months. Limited previous studies have reported an increase in BMD of GH deficient patients after discontinuation of GH treatment at final height (4,12). Fors et al (12) reported similar BMD values at baseline and after two years among three groups (40 childhood onset GHD retested of whom 21 were GH deficient and 19 GH sufficient in comparison with 16 healthy controls), after discontinuation of GH therapy at or near final height. They concluded that bone mass measured by DXA continued to rise for two years, but bone markers showed that bone formation decreased during the same period. This study does not rule out the possibility of slow long-term loss of bone in adults with GHD (12). Some studies showed bone loss in longitudinal measurements in the same population (4,13,14,15). Kaufman et al (14) showed that in childhood-onset GHD in a group of full-grown men treated with GH, they had low adult bone mass, despite prior GH substitution. In a large multi-center prospective study of 149 young adults with childhood-onset GHD who had completed linear growth and who were randomized to receive 12.5 μg/kg/d; 25 μg/kg/d or no treatment for two years the following was reported; over two years, total bone mineral content (BMC) and BMD were significantly increased in the control group, but increases in both treatment groups were greater than that of the controls and that there was no significant dose effect (4). The authors suggested that GH treatment after attainment of final height should not be discontinued for optimal progress to peak bone mass. However, in this study BMD z-scores were not evaluated and it was not shown whether the increase of BMD in the control group was enough for a normal BMD z-score. In our study, BMD z-scores did not decrease after six months in either group. Moreover, a statistically significant increase was detected in the permanent GHD group, this may be due to either small number of cases in this group, or the lasting effect of GH on bone metabolism. Baroncelli et al (15) showed in16 GHD adolescents that lumbar BMD area was < -2 SD in 70% and lumbar BMD volume was between 0 and -2 SD of normal at final height. They showed that after discontinuation of GH the timing of lumbar peak BMD area and volume were delayed in GHD patients and that lumbar BMD area and volume were increased until peak, then significantly decreased two years after peak compared with controls (15).

We have demonstrated that TF% increased significantly but that TBF mass, TBF%, TF mass, TBM mass and TBM% did not change in the permanent GHD group six months after GH discontinuation. We have also shown that, TF% and TBF mass increased significantly but TBF%, TF mass, TBM mass and TBM% did not change in the transient GHD group. The amount of increase of TF% was not different in the transient GHD and permanent GHD groups. In an earlier study, Ogle et al (16) compared eight adolescent GHD patients (whose GH treatment had ceased) with seven age matched healthy subjects for 12 months. They observed that lean body mass (LBM) decreased significantly in patients with GHD at 12 months whereas there was a non-significant increase in LBM in the control group. The percentage of body fat increased in all patients with GHD at six and 12 months with no significant increase in the control group. The mean android (trunk)/gynoid (legs) fat ratio increased, though non-significantly, in patients with GHD at 12 months while no change was observed in the control group. Vahl et al (17) showed that TBF increased and resumption of GH increased LBM after discontinuation of GH for 1 year.

Johannsson et al (3) evaluated 40 GHD patients annually, after discontinuing GH treatment for two years. They showed that the amount of total body and abdominal fat

mass throughout the study and increase in these masses were more marked in permanent GHD patients than in the transient GHD and control subjects (3). Attanasio et al (5) randomized 149 GHD patients who received GH until final height as GH treated (58 pediatric dose, 59 adult dose) and not treated groups. GH treated patients gained a significant amount of LBM and lost significant fat mass compared to the no treatment group during two years. There was no dose effect (5). A multi-centered study reported similar results for one year, with respect to continuation or discontinuation GH treatment (18).

The strengths of our study are its prospective design and the simultaneous evaluation of many metabolic parameters. In addition, our study group consisted of only idiopathic isolated GHD patients. This enables us to rule out the effects of other hormone deficiencies, probable inappropriate/non-homogenous replacement therapies and the effects of other organic pathologies and their treatments such as chemotherapy or radiotherapy.

Study Limitations

The limitations of our study are the short follow-up period and small sample size. Thus, in our opinion this study can be accepted as a well-designed pilot study which should be extended in terms of numbers of subjects and duration of follow-up for further and more robust information.

Conclusion

BW, BMI and truncal adiposity increased after cessation of GH if GHD was permanent. However, these increases were not significant compared to the transient GHD group. Despite discontinuation of GH, the pubertal increase in BMD continued in GHD patients. Although FBG was not high during GH therapy, it decreased to a safer range after cessation of therapy while FI showed no change. Although we did not observe a clinical condition requiring GH treatment in any of the study subjects during the follow-up period, which coincided with the transition period, longer follow-up is needed to assess the need for treatment during the transition period. In our opinion, until sufficient evidence accumulates, patient follow-up in terms of the above-mentioned parameters and treatment only of patients with a clinical need, instead of routine continuation of GH during the transition period, might be a safe and feasible option.

Ethics

Ethics Committee Approval: Ethical approval was given by Ankara University Ethical Committee (approval number: 06-240-13).

Informed Consent: Informed consent was taken.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Emine Çamtosun, Zeynep Şıklar, Merih Berberoğlu, Concept: Merih Berberoğlu, Design: Merih Berberoğlu, Data Collection or Processing: Emine Çamtosun, Zeynep Şıklar, Merih Berberoğlu, Analysis or Interpretation: Emine Çamtosun, Zeynep Şıklar, Merih Berberoğlu, Literature Search: Emine Çamtosun, Writing: Emine Çamtosun, Zeynep Şıklar, Merih Berberoğlu

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Attanasio AF, Howell S, Bates PC, Frewer P, Chipman J, Blum WF, Shalet SM. Body composition, IGF-I and IGFBP-3 concentrations as outcome measures in severely GH-deficient (GHD) patients after childhood GH treatment: a comparison with adult onset GHD patients. J Clin Endocrinol Metab 2002;87:3368-3372.
2. Chamberlain SH, Levy RA. Transition care of patients with growth hormone deficiency from pediatric endocrinologists to adult endocrinologists. Endocr Pract 2012;18:256-268.
3. Johannsson G, Albertsson-Wikland K, Bengtsson BA. Discontinuation of growth hormone (GH) treatment: metabolic effects in GH-deficient and GH-sufficient adolescent patients compared with control subjects. Swedish Study Group for Growth Hormone Treatment in Children. J Clin Endocrinol Metab 1999;84:4516-4524.
4. Shalet SM, Shavrikova E, Cromer M, Child CJ, Keller E, Zapletalová J, Moshang T, Blum WF, Chipman JJ, Quigley CA, Attanasio AF. Effect of growth hormone (GH) treatment on bone in postpubertal GH-deficient patients: a 2-year randomized, controlled, dose-ranging study. J Clin Endocrinol Metab 2003;88:4124-4129.
5. Attanasio AF, Shavrikova E, Blum WF, Cromer M, Child CJ, Paskova M, Lebl J, Chipman JJ, Shalet SM; Hypopituitary Developmental Outcome Study Group. Continued growth hormone (GH) treatment after final height is necessary to complete somatic development in childhood-onset GH-deficient patients. J Clin Endocrinol Metab 2004;89:4857-4862.
6. Mauras N, Pescovitz OH, Allada V, Messig M, Wajnrajch MP, Lippe B; Transition Study Group. Limited efficacy of growth hormone (GH) during transition of GH-deficient patients from adolescence to adulthood: a phase III multicenter, double-blind, randomized two-year trial. J Clin Endocrinol Metab 2005;90:3946-3955. Epub 2005 Apr 26
7. Cohen P, Rogol AD, Deal CL, Saenger P, Reiter EO, Ross JL, Chernausek SD, Savage MO, Wit JM; 2007 ISS Consensus Workshop participants. Consensus statement on the diagnosis and treatment of children with idiopathic short stature: a summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. J Clin Endocrinol Metab 2008;93:4210-4217. Epub 2008 Sep 9
8. Goksen D, Darcan S, Coker M, Kose T. Bone mineral density of healthy Turkish children and adolescents. J Clin Densitom 2006;9:84-90. Epub 2006 Mar 27
9. Thomas M, Massa G, Maes M, Beckers D, Craen M, François I, Heinrichs C, Bourguignon JP; Belgian Study Group for Paediatric Endocrinology (BSGPE). Growth hormone (GH) secretion in patients with childhood-

onset GH deficiency: retesting after one year of therapy and at final height. Horm Res 2003;59:7-15.

10. Malaspina JP, Bussiere H, Le G. The total cholesterol/HDL cholesterol ratio: a suitable atherogenesis index. Atherosclerosis 1981;40:373-375.
11. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-419.
12. Fors H, Bjarnason R, Wirent L, Albertsson-Wikland K, Bosaeust L, Bengtsson BA, Johannsson G. Currently used growth-promoting treatment of children results in normal bone mass and density. A prospective trial of discontinuing growth hormone treatment in adolescents. Clin Endocrinol (Oxf) 2001;55:617-624.
13. Carroll PV, Drake WM, Maher KT, Metcalfe K, Shaw NJ, Dunger DB, Cheetham TD, Camacho-Hübner C, Savage MO, Monson JP. Comparison of continuation or cessation of growth hormone (GH) therapy on body composition and metabolic status in adolescents with severe GH deficiency at completion of linear growth. J Clin Endocrinol Metab 2004;89:3890-3895.
14. Kaufman JM, Taelman P, Vermeulen A, Vandeweghe M. Bone mineral status in growth hormone-deficient males with isolated and multiple pituitary deficiencies of childhood onset. J Clin Endocrinol Metab 1992;74:118-123.
15. Baroncelli GI, Bertelloni S, Sodini F, Saggese G. Longitudinal changes of lumbar bone mineral density (BMD) in patients with GH deficiency after discontinuation of treatment at final height; timing and peak values for lumbar BMD. Clin Endocrinol (Oxf) 2004;60:175-184.
16. Ogle GD, Moore B, Lu PW, Craighead A, Briody JN, Cowell CT. Changes in body composition and bone density after discontinuation of growth hormone therapy in adolescence: an interim report. Acta Paediatr Suppl 1994;399:3-7.
17. Vahl N, Juul A, Jorgensen JO, Orskov H, Skakkebaek NE, Christiansen JS. Continuation of growth hormone (GH) replacement in GH-deficient patients during transition from childhood to adulthood: a two-year placebo-controlled study. J Clin Endocrinol Metab 2000;85:1874-1881.
18. Monson JP. Indications for GH replacement in adolescents and young adults. J Endocrinol Invest 2005;28(Suppl 5):52-55.

ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.5119

J Clin Res Pediatr Endocrinol 2018;10(3):256-263

Nationwide Study of Turner Syndrome in Ukrainian Children: Prevalence, Genetic Variants and Phenotypic Features

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What is already known on this topic?

Turner syndrome (TS) is one of the most common genetic disorders associated with abnormalities of chromosome X. A significant delay at initial diagnosis of TS has been reported in all populations.

What this study adds?

The most common karyotype in Ukrainian Turner syndrome (TS) patients was 45,X. In this population, TS was accompanied by a lower frequency of cardiac and renal malformations compared to other countries.

Abstract

Objective: We aimed to investigate the prevalence of Turner syndrome (TS) in the Ukrainian population, the frequency of karyotype variants, the age of children at diagnosis, the degree of short stature and phenotypic features in TS girls.

Methods: A retrospective analysis was made in 538 TS girls aged 0.11-18.2 years within the time period of 2005-2015 with detailed examination of 150 patients.

Results: The prevalence of TS in Ukraine is 77.5 in 100.000 live female births. The average age at diagnosis is 9.33 ± 4.93 years. The relative proportions of karyotypic abnormalities found were: 45,X (59.3%); mosaicism 45,X/46,XX (22.9%); and structural abnormalities in chromosome X (17.8%). The most frequently encountered findings were growth delay (98.8%), shortening of the 4th and 5th metacarpal bones (74.6%), abnormal nails (73.3%), broad chest (60.7%), short neck (58.6%), hypertelorism of nipples (51.4%), malformations of the cardiovascular (19.6%) and urinary systems (13.8%) and pathology related to vision (20.1%) and hearing (22.0%).

Conclusion: In the Ukrainian population, the highest proportion of patients with TS had a karyotype 45,X. TS was accompanied by a lower frequency of malformations of internal organs compared to other countries.

Keywords: Turner syndrome, prevalence, growth retardation, karyotype, phenotype, malformations

Introduction

Turner syndrome (TS) is one of the most common genetic disorders associated with abnormalities of chromosome X (1). The incidence of this syndrome has been reported to vary from 25 to 210 per 100.000 live female births in different populations. This variability has been attributed to the prevalence of mosaic forms and the lack of classical features of the disease (1,2,3,4,5). A significant delay at initial diagnosis of TS has been well documented (3,6,7,8). The average age of TS diagnosis varies widely from 4.1 ± 5.1 years in the United State of America (USA) up to 13.74 years old in Albania (6,9,10,11). Diagnosis in patients with a 45,X karyotype is usually made at an earlier age than other variants (9). Karyotype 45,X has been reported in between 32-74% patients, while 9.2-31% patients are carriers of different variants of chromosome X structural abnormalities and a mosaic karyotype is present in between 9-56.3% of patients (12,13,14,15,16,17,18,19). Delay in growth is commonly associated with TS and is present in most affected girls. Final height in 45,X patients who did not receive treatment with recombinant growth hormone (rGH) was typically

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Conflict of interest: None declared
Received: 16.08.2017
Accepted: 29.12.2017

less than 142-145 cm, which is about 20 cm lower than the average height of a healthy female population (20,21). The most serious malformations of TS include congenital and acquired heart diseases, such as aneurysm and aortic dissection, valvular disease, hypertension, thromboembolic disease and myocardial infarction (22,23). Sybert and McCauley (23) reported cardiac pathology in 56% of TS patients. Defects of the urinary tract, including pyelocaliceal system defects, horseshoe kidney and other anomalies of kidney location are observed in 30-40% of TS patients (24,25). Epicanthus, palpebral ptosis and strabismus are the major stigmata of disembryogenesis of eyes and eye appendages encountered in patients with TS (26,27). Hearing defects in patients with TS are characterized by a high frequency of otitis media, assumed to be caused by abnormalities in the eustachian tube and middle ear (20,28,29). Patients with TS are reported to have a higher frequency of autoimmune diseases, including autoimmune thyroid disease and celiac disease (30,31,32,33). Several researchers in Europe have reported increased levels of antithyroid antibodies in patients with TS, the frequency ranging from 36 to 64.8% (6,30,31,32,33,34,35). Of these, 21.2-70.4% were reported to have subclinical or clinical hypothyroidism (30,31,33,34,35). The aims of our study were to investigate the prevalence of TS among children in Ukraine. In addition we also aimed to determine age at initial diagnosis, frequency of different karyotype variants, degree of growth delay and prevalence of different phenotypic features of TS.

Methods

In this study the Ukrainian Pediatric TS Registry, created in 2004, was used. The registry included children diagnosed with TS between ages 0.11 and 18.2 years old, identified by regional Ukrainian pediatric endocrinologists. TS registration cards contained information on date of birth, age at diagnosis, karyotype, height (cm), height standard deviation (SD) [World Health Organization (WHO), 2007] (36), weight (kg), body mass index [BMI (kg/m²)] derived from WHO percentile tables for girls of appropriate age (36) and Tanner stage of sexual development (37). Data on phenotypic features of the girls was also recorded. Biochemical and hormonal parameters available from the records included: thyroid stimulating hormone (TSH), free thyroxine (fT4), thyroid peroxidase antibodies (TPOAb), luteinising hormone, follicle stimulating hormone, estradiol, insulin-like growth factor-1, results of clonidine stimulation test for GH, bone age according to the Greulich and Pyle method. A retrospective analysis of 538 registration cards of TS girls who were registered between the years 2005 and 2015 was conducted. Depending on the karyotype's variant, the patients were divided into three groups. The first group included patients with 45,X (n = 319). The second group consisted of patients with the mosaic karyotype 45,X/46,XX (n = 123) and the third group of girls had structural abnormalities of chromosome X, such as 46,Xi(Xq), 45,X/46,Xi(Xq), 45,X/46,X + mar, 46,X, del(X)(Xq) and 45,X/46,Xdel (n = 96). The physical development of girls with TS were compared with healthy girls in the control group aged from 10 months to 18 years old. The control group included 525 healthy girls. This group had been under observation during the health-care examinations in 2005-2008. The exams took place in our Clinic and in preschools and schools of the Ukraine. All girls in the study and control groups were divided into 5 age groups as follows: younger than 1 year of age; 1 to 3 years; 4 to 7 years; 8 to 11 years and older than 12 years of age. In addition 150 girls with TS were examined in the Ukrainian Research Center of Endocrine Surgery of the Ministry of Health of Ukraine and in the National Children's Specialized Hospital "OHMATDYT". Diagnosis of TS was confirmed by determination of karyotype in blood leukocytes. In these 150 all parameters from TS registration cards were re-evaluated. To study anomalies of internal organs, these patients underwent ultrasound of internal organs and echocardiography. An audiogram was performed in all patients with hearing loss. Also, all children were examined by an ophthalmologist and otolaryngologist to confirm the presence or absence of any abnormal features. To assess thyroid function in patients with different TS karyotype, thyroid function tests (TSH and fT4) and TPOAb concentrations were determined. In the scope of our study, we did not assess the prevalence of celiac disease.

Statistical Analysis

Statistical analysis of the results was performed by using Statistica 10 (StatSoft, USA). Standard non-parametric statistical tests and Kruskal-Wallis test or Student's test in the case of normal distribution were used. For the analysis of qualitative data (%) for two or more independent groups χ^2 Pearson was used. One-Way analysis of variance test was used for the quantitative data analyses in groups. The data are presented as mean values ± SD or as median and 25th and 75th percentiles (first and third quartiles) [median (25; 75)] for parametric and nonparametric distributions respectively. A p value of < 0.05 was taken as an indicator of significant difference. This study was approved by the Ethics Committee of Ukrainian Research Center of Endocrine Surgery MoH of Ukraine (approval number: 12 from 14.10.2013). All procedures performed in the studies involving patients were in accordance with the ethical

standards of the Institution on clinical practice and with the 1964 Helsinki Declaration, as amended. The parents or legal guardians of patients signed informed consent forms in which they agreed to the treatment and all the diagnostic procedures required.

Results

Data from the Ukrainian Pediatric TS Registry shows that the prevalence of TS among children aged 0-18 years was 77.5 per 100.000 female live births during the study period. Over the last five years there were 17-25 new TS cases with registered annually (38). Among girls with TS (n = 538), different karyotype variants were found. However, monosomy 45,X was identified most often, in 59.32 % of the patients ($p < 0.001$), than mosaicism 45,X/46,XX (22.90 %) and structural abnormalities of chromosome X in 17.78 % patients. The structural abnormalities were further divided as follows: 46,Xi(Xq) in 5.11 %; 45,X/46,XX(Xq) in 6.9 %; 45,X/46,X + mar in 3.16 %; 46,X,del(X)(Xq) in 1.87 %; and 45,X/46,X,del in 0.74 % of patients. In Ukraine the mean age of diagnosis of TS in children was 9.33 ± 4.93 years. However, age of diagnosis depended on the karyotype and was lowest in children with 45,X as compared to children with structural abnormalities of chromosome X ($p = 0.013$), Age of diagnosis was 8.96 ± 5.28 years in 45,X patients (n = 303), 10.49 ± 3.95 years in patients with structural abnormalities of chromosome X (n = 87) and 9.50 ± 4.41 years in 45,X/46,XX (n = 111) patients. TS was diagnosed in the first year of life in 1.62 %, at ages 1-4 years in 3.60 %, at ages 5-7 years in 9.46 %, at 8-11 years (the age when normal puberty is expected to start in girls) in 18.92 % and between 12-17 years, when puberty would be expected to have begun in 66.40 % of the patients. Phenotypic manifestations of TS in children had significant variability. Growth delay was a constant feature (98.82 % of patients). Shortening of 4th and 5th metacarpal bones (74.62 % patients) followed by abnormal nails (73.31 %), broad chest (60.67 %), short neck (58.63 %), sexual developmental delay (57.32 %) and hypertelorism of nipples (51.37 %) were the most frequently observed findings. These manifestations of TS were most frequent in patients with karyotype 45,X and significantly less frequent in patients with 45,X/46,XX ($p = 0.023$) and structural abnormalities of chromosome X ($p = 0.035$).

Malformations of the cardiovascular system was the most common pathology of internal organs in TS patients (19.62 %). These consisted of aortic stenosis (in 5.32 % of the patients), coarctation of the aorta and bicuspid aortic valve (in 2.63 % and 2.02 % of the cases respectively). Malformations of the cardiovascular system were found more often in children with mosaicism (26.18 %) and in cases of structural abnormalities of chromosome X (21.62 %) compared to the karyotype 45,X (15.85 %) ($p = 0.02$). Urinary tract malformations were observed less frequently in the patients (13.82 %) but were significantly more common ($p = 0.017$) in patients with karyotype 45,X (14.76 %) and less so in cases with karyotype 45,X/46,XX (8.28 %) and structural abnormalities of chromosome X (2.75 %). The main malformations of urinary tract were doubling pyelocaliceal renal system (3.38 %), renal hypoplasia (3.36 %) and ureter malformations (3.31 %). Frequency of vision and hearing defects were 20.08 % and 22.01 % respectively. Otitis was the most common pathology pertaining to the ears and was observed most frequently in children with monosomy X ($p < 0.01$). Optic and otic pathologies found are shown in Table 1. Frequency of autoimmune thyroid disease in TS girls was 48.45 %. It was proved by elevated levels of TPOAb and appropriate ultrasound changes, and it did not differ significantly between karyotypes ($p > 0.05$). Among TS girls with elevated TPOAb levels subclinical (48.76 %) and clinical hypothyroidism (29.14 %, $p < 0.05$) were found more frequently. 11.87 % of patients were euthyroid, and 10.23 % of girls had subclinical hyperthyroidism. Until 2013, in Ukraine there was no state programme of free treatment with rGH for girls with TS, thus most patients were untreated. The analysis of growth in girls with TS who did not receive treatment with rGH compared to the control group of appropriate age revealed a significant difference in the growth of children in all age groups (Table 2). The difference was noticeable in the first year of life, increased

Table 1. The spectrum of pathology of vision and hearing in Turner syndrome girls with different karyotypes

Pathology	All TS girls n = 346	45,X n = 200	45,X/46,XX n = 84	Structural abnormalities of chromosome X n = 62	p
Amblyopia, %	5.43	4.91	5.26	4.62	p > 0.05
Strabismus, %	6.00	5.32	5.20	8.11	p > 0.05
Myopia, %	8.65	8.04	10.57	8.14	p > 0.05
Frequent otitis, %	12.62	17.37	10.54	5.46*	p < 0.01
Hearing loss, %	9.39	10.62	5.28	8.17	p > 0.05

TS: Turner syndrome, *: the difference between groups of patients with karyotype 45X and structural abnormalities of chromosome X

with age and was highest in those girls aged 14 years old, probably due to the lack of pubertal growth spurt in TS patients. The difference in final height between TS girls and controls was 24.4 ± 1.7 cm ($p < 0.001$). Analysis of final height in girls who did not receive rGH revealed no significant difference among patients with different karyotypes (Table 3). In our TS group of pubertal age, spontaneous (without hormonal stimulation) sexual development, assessed by the appearance of thelarche, occurred in 14.62% (n = 18). There was no significant difference in the age of onset of puberty among groups with different karyotypes (Table 4).

Spontaneous menstruations was reported in 38.82% of TS girls having signs of sexual development. It is of note that among girls with spontaneous puberty and karyotype 45,X, there were three girls at Tanner stage 2, three at Tanner stage 3 after the age of 15 years and three girls who had spontaneous menarche at age of 15.1 (14.30; 16.20) years. The frequency of body weight disorders in TS girls was assessed by comparing their BMI values with that of girls of similar age in the control group. Most of the TS girls (68.1%) had a normal BMI ($p = 0.021$), although 13.82% were overweight, 6.88% were obese and 11.20% were underweight (BMI <15th centile). The average BMI in patients with TS was 53.23 ± 27.06th percentile that was significantly higher ($p < 0.05$) compared to the control group (50.62 ± 27.63th percentile) (Table 5). According to our data, BMI in patients with TS increased with age and reached a maximum in children over the age of 12 years, but did not exceed the normal range. The highest BMI values were found in girls with structural abnormalities of chromosome X ($p < 0.05$) (Table 6).

Discussion

The current study aimed to assess the prevalence, age at initial diagnosis, incidence of different variants of the karyotype, phenotypic characteristics, presence of associated components and physical and sexual development in Ukrainian TS patients. It was found that in 2015 the prevalence of TS among children 0-18 years in Ukraine was 77.5 per 100.000 live female births. The

Table 2. Height in Turner syndrome girls (not receiving recombinant growth hormone treatment) and in the control group, in different age groups

Age group	Height (cm) in the TS group, mean ± SD, n = 502	Height (cm) in the control group, mean ± SD, n = 525	p
Younger than 1 year	61.45 ± 8.12	66.69 ± 7.02	< 0.001
1-3 years	82.06 ± 7.02	89.38 ± 8.39	< 0.001
4-7 years	100.93 ± 8.42	114.21 ± 8.32	< 0.001
8-11 years	120.72 ± 8.33	137.99 ± 8.64	< 0.001
12-17 years	136.87 ± 7.83	160.58 ± 7.14	< 0.001

SD: standard deviation, TS: Turner syndrome

Table 3. Final height in Turner syndrome girls with different karyotype

Karyotype	Final height (cm) median (25; 75)	SD median (25; 75)
45,X (n = 80)	140.90 (137.00; 145.00)	-3.38 (-4.00; -2.70)
45,X/46,XX (n = 23)	142.02 (136.50; 148.00)	-3.12 (-4.10; -2.20)
Structural abnormalities of chromosome X (n = 26)	140.75 (137.00; 144.00)	-3.27 (-4.00; -2.60)
All TS patients with closed growth plates (n = 129)	141.09 (137.00; 145.00)	-3.31 (-4.00; -2.60)

TS: Turner syndrome, SD: standard deviation

Table 4. Frequency of spontaneous sexual development and age of puberty in Turner syndrome girls with different karyotypes

Karyotype	Girls with spontaneous sexual development, %	Age of puberty, years median (25,75)
45,X (n = 61)	11.56	15.76 (13.28; 16.65)
45,X/46,XX (n = 31)	25.82	14.20 (13.10; 16.20)
Structural abnormalities of chromosome X (n = 31)	9.62	16.11 (14.80; 16.60)

Table 5. Assessment of body weight using percentile tables in children of all ages with Turner syndrome

Investigated groups	Age groups, mean ± SD percentile				
	< 1 year	1-3 years	4-7 years	8-11 years	12-17 years
TS girls body weight percentile (n = 538)	65.33 ± 29.54	56.48 ± 26.58	49.13 ± 28.25	57.38 ± 27.14	55.17 ± 27.28
Control group body weight percentile (n = 525)	67.60 ± 27.03	52.06 ± 29.69	52.14 ± 28.40	50.16 ± 29.97	44.73 ± 22.62
p*	0.84	0.58	0.64	0.06	< 0.001

SD: standard deviation, TS: Turner syndrome, *: p value between body mass index in Turner syndrome girls and the control group

incidence rate is consistent with other reports, although is a higher figure when compared to some other countries such as Denmark (3,5), Germany (9), Albania (6) and Japan (2). Age of initial diagnosis of TS was 9.33 ± 4.93 years with a maximum frequency of initial registration of the disease in puberty, most likely because of the referrals of patients with growth delay or delay/absence of sexual development or menstruation at normal female pubertal ages. The highest proportion of early primary diagnosis of TS in Ukraine was found in patients with karyotype 45,X, who were diagnosed at a mean age of 8.96 ± 5.28 years, a finding which can be explained by the presence of typical features of the disease in these girls. Though the age of the diagnosis is older than in a Belgian population (where the average age of diagnosis in 2003 was 6.6 years (9)), it was lower than in a Denmark (where the average age at diagnosis for the entire TS group was reported as 15.1 years, or 13.3 years for 45, X patients) (11). In Ukraine, the largest proportion of patients diagnosed with TS (59.32 %) were 45,X, similar to patients from Poland, United Kingdom (UK) and USA (17,18,19). Diagnosis of different karyotype may vary depending on the different methods of analysis and the type of biological material that has been used. In our study, only cytogenetic analysis of peripheral blood lymphocytes was used to determine the karyotype. However, Hook and Warburton (39) suggested that all live birth girls with karyotype 45,X, actually have a mosaic karyotype because some of their organs and tissues will contain more cell lines with normal or aberrant sex chromosomes, which cannot be determined by peripheral blood sample analysis alone. This may be a rationale for further research of karyotype in other tissues in 45,X girls, especially in those who have signs of spontaneous puberty or mild growth delay. We found a lower frequency of cardiovascular and urinary tract malformations in TS girls compared to the USA (40,41,42), UK (43), Egypt (44), Denmark (45) and France (46). The lower frequency of malformations of the cardiovascular system may be explained by the lack of routine cardiac magnetic resonance imaging (MRI) in girls with TS, in the absence of clinical symptoms in our country. Thus we believe that these results are in need of further confirmation by focused examination to detect the above mentioned pathology. Even in the absence of clinical manifestations, presence of aortic dilatation and associated abnormalities need to be evaluated in pediatric patients with TS. The frequency of pathology of vision and hearing was also lower in Ukraine compared to other countries (20,27,28,29). The lower frequency of hearing defects can be due to the fact that only patients with complaints on hearing loss were assesed by audiogram. TS girls in the Ukraine appear to have a lower frequency of malformations of internal organs. However, we believe that the insufficient diagnostics of both the cardiovascular system and that of auricular pathology need to be taken into account in this conclusion.

Table 6. Body mass index percentile in children with Turner syndrome of different karyotypes

Karyotype	BMI percentile, mean ± SD
Control group	50.63 ± 27.66
45,X	53.09 ± 26.73
45,X/46,XX	54.11 ± 27.23
Structural abnormalities of chromosome X	62.16 ± 28.40
[1]p	0.004
[2]p	0.03
[3]p	< 0.001

[1]: difference among patients with TS karyotype 45,X and X chromosome structural abnormalities, [2]: difference among patients with TS karyotype 45,X/46,XX and X chromosome structural abnormalities, [3]: difference among control group (karyotype 46,XX) and X chromosome structural abnormalities, BMI: body mass index, TS: Turner syndrome, SD: standard deviation

Studies on the frequency of autoimmune thyroid disease have shown increased TPOAb levels in 48.45 % of our patients, a finding that is similar to figures reported from Italy (32) and Denmark (33), but which is less than in Albania (6) and Poland (35). Also, among TS girls with elevated TPOAb levels the number of patients with subclinical and clinical hypothyroidism was greater (77.9 %) as compared to other European countries. Thus, researchers reported 31.4 % of patients with subclinical hypothyroidism in Poland, Silesia (30), 21.2 % with clinical and subclinical hypothyroidism in Italy (31), 33 % with clinical hypothyroidism in Denmark (33) and 24 % with hypothyroidism among all patients with TS and 65 % patients with positive thyroid antibodies in Greece (34); other authors from Poland (Warsaw) reported 20 % of patients with subclinical hypothyroidism (35).

Height of girls with TS who did not receive treatment with rGH shows that they were significantly shorter in all age groups, compared with the control group, a finding consistent with other studies (21,22). The progression of the degree of growth delay increased with age and was more pronounced in puberty. Final height in TS patients who did not receive treatment with rGH was significantly lower, compared to healthy Ukrainian women.

Most girls with TS (68.1 %) had normal body weight. However, overweight was detected in 13.82 % of patients, obesity in 6.88 % and 11.20 % of the patients were underweight. The frequency of overweight in children with TS was higher than in the general population in all age groups with a significant difference in puberty, which coincides with the findings of other authors (47,48,49). The highest BMI and the highest rate of overweight were observed in patients with structural

abnormalities of chromosome X. To our knowledge, this data had not been described previously. It is well known that TS is a syndrome of disproportionate anthropometry and body composition. Dual-energy X-ray absorptiometry (DEXA) can be helpful to estimate the visceral fat and skeletal muscle mass. We can hypothesize that TS girls with structural abnormalities of chromosome X (especially those with isochromosome Xq) might have skewed body composition with increased skeletal muscle mass versus total fat mass and hence, increased BMI. In Ukrainian population among structural abnormalities of chromosome X majority patients were girls with isochromosome Xq abnormalities. In our study we used BMI only, that may not reflect the real body composition, however only few authors investigated the effect of karyotype on body composition in children (12,50). Further studies (including those with using of DEXA) are needed to estimate the total and regional distribution of fat and muscle mass in girls with different karyotypes.

Study Limitations

There are several limitations to this study. There was no national screening for celiac disease. Also we did not perform the evaluation of the hearing loss and MRI of the heart for all TS patients. DEXA with evaluation of the total and regional distribution of fat and muscle mass in girls with different karyotype is needed for the appropriate BMI evaluation.

Conclusion

Tthe highest proportion of patients with TS in Ukraine had a karyotype 45,X. TS was accompanied by a lower frequency of malformations of internal organs as compared to figures from other countries. This leads us to conclude that more widespread use of cardiac MRI, audiogram screening and celiac disease in all patients with TS in Ukraine is urgently needed. Additionally, the implementation of genetic testing to identify genes associated with malformations (*ZFYVE9, TIMP1, PRKX, KDM6A*) can lead to a higher detection rate of aortic aneurysm formation, congenital urinary malformations and other anomalies (51). Earlier diagnosis of TS would allow more timely medical, psychological and social assistance for girls with TS and their families. It is assumed that pediatricians and family physicians will provide an active and targeted search for TS among girls, especially in those with a delay of growth and sexual development. This targeted approach would contribute to the prevention of short stature by earlier rGH therapy. Expanded screening for malformations, especially cardiac and renal malformation which TS girls are particularly at risk of would also serve to ameliorate some of the associated morbidity seen in TS patients.

Acknowledgment

We would like to thank all Ukrainian regional endocrinologists for their input in Ukrainian Pediatric TS Registry. We are grateful to N. Pogadayeva and Y. Shcherbak from National Children's Specialized Hospital "OHMATDYT" for referring patients.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of Ukrainian Scientific Center of Endocrine Surgery MoH of Ukraine (approval number: 12 from 14.10.2013). All procedures performed in the studies involving patients were in accordance with the ethical standards of the Institution on clinical practice and with the 1964 Helsinki Declaration, as amended.

Informed Consent: The parents or legal guardians of patients signed informed-consent forms in which they agreed to the treatment and all the diagnostic procedures required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Nataliya Zelinska, Iryna Shevchenko, Evgenia Globa, Concept: Nataliya Zelinska, Design: Nataliya Zelinska, Iryna Shevchenko, Evgenia Globa, Data Collection or Processing: Iryna Shevchenko, Analysis or Interpretation: Iryna Shevchenko, Nataliya Zelinska, Evgenia Globa, Literature Search: Iryna Shevchenko, Writing: Iryna Shevchenko, Nataliya Zelinska, Evgenia Globa.

Financial Disclosure: This work was supported by the Ministry of Health of Ukraine.

References

1. Gravholt CH. Epidemiological, endocrine and metabolic features in Turner syndrome. Eur J Endocrinol 2004;151:657-687.
2. Hibi I, Takano K. Basic and Clinical Approach to Turner Syndrome. Excerpta Medica International Congress Series 1014: Amsterdam, 1993;169-176.
3. Gravholt CH, Stochholm K. The epidemiology of Turner syndrome. International Congress Series, 2006;1298:139-145.
4. Hook EB, Warburton D. The distribution of chromosomal genotypes associated with Turner's syndrome: livebirth prevalence rates and evidence for diminished fetal mortality and severity in genotypes associated with structural X abnormalities or mosaicism. Hum Genet 1983;64:24-27.
5. Gravholt CH, Juul S, Naeraa RW, Hansen J. Prenatal and postnatal prevalence of Turner's syndrome: a registry study. BMJ 1996;312:16-21.

6. Hoxha P, Babameto-Laku A, Vyshka G, Gjoka K, Minxuri D, Myrtaj E, Çakërri L. Turner syndrome in Albania and the efficacy of its treatment with growth hormone. J Pediatr Endocrinol Metab 2015;28:1227-1234.
7. Wikiera B, Barg E, Konieczna A. Glab E, Basiak A. Factors influencing the frequency and the time of Turner's syndrome diagnosis:7th ESPE/LWPES Joint Meetings Paediatric Endocrinology. Lyon 2005;241.
8. Schweizer R, Blumenstock G, Mangelsdorf K, Ehehalt S, Rössner L, Dorn T, Binder G, Ranke MB. Prevalence and incidence of endocrine disorders in children: results of a survey in Baden-Wuerttemberg and Bavaria 2000-2001. Klin Padiatr 2010;222:67-72. Epub 2010 Feb 10
9. Massa G, Verlinde F, De Schepper J, Thomas M, Bourguignon JP, Craen M, de Zegher F, François I, Du Caju M, Maes M, Heinrichs C; Belgian Study Group for Paediatric Endocrinology. Trends in age at diagnosis of Turner syndrome. Arch Dis Child 2005;90:267-268.
10. Nabhan ZM, Eugster EA. Medical care of girls with Turner Syndrome: where are we lacking. Endocr Pract 2011;17:747-752.
11. Stochholm K, Juel S, Juel K, Naeraa RW, Gravholt CH. Prevalence, Incidence, Diagnostic Delay, and Mortality in Turner Syndrome. J Clin Endocrinol Metab 2006;91:3897-3902. Epub 2006 Jul 18
12. Yeşilkaya E, Bereket A, Darendeliler F, Baş F, Poyrazoğlu Ş, Küçükemre Aydın B, Darcan Ş, Dündar B, Büyükinan M, Kara C, Sarı E, Adal E, Akıncı A, Atabek ME, Demirel F, Çelik N, Özkan B, Özhan B, Orbak Z, Ersoy B, Doğan M, Ataş A, Turan S, Gökşen D, Tarım Ö, Yüksel B, Ercan O, Hatun Ş, Şimşek E, Ökten A, Abacı A, Döneray H, Özbek MN, Keskin M, Önal H, Akyürek N, Bulan K, Tepe D, Emeksiz HC, Demir K, Kızılay D, Topaloğlu AK, Eren E, Özen S, Abalı S, Akın L, Selver Eklioğlu B, Kaba S, Anık A, Baş S, Ünüvar T, Sağlam H, Bolu S, Özgen T, Doğan D, Deniz Çakır E, Şen Y, Andıran N, Çizmecioğlu F, Evliyaoğlu O, Karagüzel G, Pirgon Ö, Çatlı G, Can HD, Gürbüz F, Binay Ç, Baş VN, Fidancı K, Polat A, Gül D, Açıkel C, Demirbilek H, Cinaz P, Bondy C. Turner Syndrome and Associated Problems in Turkish Children: A Multicenter Study. J Clin Res Pediatr Endocrinol 2015;7:27-36.
13. Freriks K, Timmermans J, Beerendonk CC, Verhaak CM, Netea-Maier RT, Otten BJ, Braat DD, Smeets DF, Kunst DH, Hermus AR, Timmers HJ. Standardized multidisciplinary evaluation yields significant previously undiagnosed morbidity in adult women with Turner syndrome. J Clin Endocrinol Metab 2011;96:1517-1526. Epub 2011 Jul 13
14. de Araújo C, Galera BB, Galera MF, de Medeiros SF. Clinical and cytogenetic aspects of the Turner syndrome in the Brazilian Western region. Rev Bras Ginecol Obstet 2010;32:381-385.
15. Kammoun I, Chaabouni M, Trabelsi M, Ouertani I, Kraoua L, Chelly I, M'rad R, Ben Jemaa L, Maâzoul F, Chaabouni H. Genetic analysis of Turner syndrome: 89 cases in Tunisia. Ann Endocrinol (Paris) 2008;69:440-445. Epub 2008 Jun 9
16. Daggag H, Srour W, El-Khateeb M, Ajlouni K. Analysis of Turner syndrome patients within the Jordanian population, with a focus on four patients with Y chromosome abnormalities. Sex Dev 2013;7:295-302. Epub 2013 Aug 29
17. Prakash S, Guo D, Maslen CL, Silberbach M, Milewicz D, Bondy CA; GenTAC Investigators. Single-nucleotide polymorphism array genotyping is equivalent to metaphase cytogenetics for diagnosis of Turner syndrome. Genet Med 2014;16:53-59. Epub 2013 Jun 6
18. Iyer NP, Tucker DF, Roberts SH, Moselhi M, Morgan M, Matthes JW. Outcome of fetuses with Turner syndrome: a 10-year congenital anomaly register based study. J Matern Fetal Neonatal Med 2012;25:68-73. Epub 2011 Apr 4
19. Wikiera B, Bieniasz J, Barg E, Głab E, Noczyńska A. Lipoprotein a (Lp(a)) in patients with Turner's syndrome. Pediatr Endocrinol Diabetes Metab 2010;16:29-32.
20. Rappold GA, Durand C, Decker E, Marchini A, Schneider KU. New roles of SHOX as regulator of target genes. Pediatr Endocrinol Rev 2012;9(Suppl 2):733-738.
21. De Lemos-Marini SH, Morcillo AM, Baptista MT, Guerra G Jr, Macial-Guerra AT. Spontaneous final height in Turner's syndrome in Brazil. J Pediatr Endocrinol Metab 2007;20:1207-1214.
22. Gonzalez L, Witchel SF. The Patient with Turner Syndrome: Puberty and Medical Management Concerns. Fertil Steril 2012;98:780-786. Epub 2012 Aug 9
23. Sybert VP, McCauley E. Turner's Syndrome. N Engl J Med 2004;351:1227-1238.
24. Chang P, Tsau YK, Tsai WY, Tsai WS, Hou JW, Hsiao PH, Lee JS. Renal malformations in children with Turner's syndrome. J Formos Med Assoc 2000;99:796-798.
25. Di Pinto D, Balestracci A, Dujovne N, de Palma I, Adragna M, Delgado N. Nephrourologic pathology in girls with Turner syndrome. Arch Argent Pediatr 2010;108:353-357.
26. Bondy CA; Turner Syndrome Study Group. Care of girls and women with Turner syndrome: a guideline of the Turner Syndrome Consensus Study Group. J Clin Endocrinol Metab 2007;92:10-25. Epub 2006 Oct 17
27. Denniston AK, Butler L. Ophthalmic features of Turner's syndrome. Eye (Lond) 2004;18:680-684.
28. Hultcrantz M. Ear and hearing problems in Turner's syndrome. Acta Otolaryngol 2003;123:253-257.
29. Dhooge I J, De Vel E, Verhoye C, Lemmerling M, Vinck B. Otologic disease in Turner syndrome. Otol Neurotol 2005;26:145-150.
30. Gawlik A, Gawlik T, Januszek-Trzciakowska A, Patel H, Malecka-Tendera E. Incidence and dynamics of thyroid dysfunction and thyroid autoimmunity in girls with Turner's syndrome: a long-term follow-up study. Horm Res Paediatr 2011;76:314-320. Epub 2011 Oct 13
31. Grossi A, Crinò A, Luciano R, Lombardo A, Cappa M, Fierabracci A. Endocrine autoimmunity in Turner syndrome. Ital J Pediatr 2013;39:79.
32. Calcaterra V, Klersy C, Muratori T, Caramagna C, Brizzi V, Albertini R, Larizza D. Thyroid ultrasound in patients with Turner syndrome: influence of clinical and auxological parameters. J Endocrinol Invest 2011;34:260-264. Epub 2010 May 28
33. Mortensen KH, Cleemann L, Hjerrild BE, Nexo E, Locht H, Jeppesen EM, Gravholt CH. Increased prevalence of autoimmunity in Turner syndrome-influence of age. Clin Exp Immunol 2009;156:205-210. Epub 2009 Feb 26
34. Livadas S, Xekouki P, Fouka F, Kanaka-Gantenbein C, Kaloumenou I, Mavrou A, Constantinidou N, Dacou-Voutetakis C. Prevalence of thyroid dysfunction in Turner's syndrome: a long-term follow-up study and brief literature review. Thyroid 2005;15:1061-1066.
35. Kucharska AM, Czarnocka B, Demkow U. Anti-natrium/iodide symporter antibodies and other anti-thyroid antibodies in children with Turner's syndrome. Adv Exp Med Biol 2013;756:131-138.
36. Child growth standards: http://www.who.int/childgrowth/standards/height_for_age/en/.
37. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child 1969;44:291-303.
38. Zelinska N, Shevchenko I, Globa Y, Pogadaeva N. Nationwide study of Turner syndrome in Ukraine. The 54th Annual ESPE Meeting (Barselona 1-3 October). - ESPE Abstracts 2015;84:3-1239.
39. Hook EB, Warburton D. Turner syndrome revisited: review of new data supports the hypothesis that all viable 45,X cases are cryptic mosaics with a rescue cell line, implying an origin by mitotic loss. Hum Genet 2014;133:417-424. Epub 2014 Jan 30

40. Völkl TM, Degenhardt K, Koch A, Simm D, Dörr HG, Singer H. Cardiovascular anomalies in children and young adults with Ullrich-Turner syndrome: the Erlangen experience. Clin Cardiol 2005;28:88-92.

41. Kim HK, Gottliebson W, Hor K, Backeljauw P, Gutmark-Little I, Salisbury SR, Racadio JM, Helton-Skally K, Fleck R. Cardiovascular anomalies in Turner syndrome: spectrum, prevalence, and cardiac MRI findings in a pediatric and young adult population. AJR Am J Roentgenol 2011;196:454-460.

42. Thomas J, Yetman AT. Management of cardiovascular disease in Turner syndrome. Expert Rev Cardiovasc Ther 2009;7:1631-1641.

43. Elsheikh M, Casadei B, Conway GS, Wass JA. Hypertension is a major risk factor for aortic root dilatation in women with Turner's syndrome. Clin Endocrinol (Oxf) 2001;54:69-73.

44. Hamza RT, Shalaby MH, Hamed LS, Abdulla DBA, Elfekky SM, Sultan OM. Renal anomalies in patients with Turner syndrome: is scintigraphy superior to ultrasound? Am J Med Genet A 2016;170:355-362. Epub 2015 Nov 30

45. Mortensen KH, Andersen NH, Gravholt CH. Cardiovascular phenotype in Turner syndrome-integrating cardiology, genetics, and endocrinology. Endocr Rev 2012;33:677-714. Epub 2012 Jun 15

46. Donadille B, Rousseau A, Zenaty D, Cabrol S, Courtillot C, Samara-Boustani D, Salenave S, Monnier-Cholley L, Meuleman C, Jondeau G, Iserin L, Duranteau L, Cabanes L, Bourcigaux N, Bonnet D, Bouchard P, Chanson P, Polak M, Touraine P, Lebouc Y, Carel JC, Léger J, Christin-Maitre S. Cardiovascular findings and management in Turner syndrome: insights from a French cohort. Eur J Endocrinol 2012;167:517-522. Epub 2012 Jul 16

47. O'Gorman CS, Syme C, Lang J, Bradley TJ, Wells GD, Hamilton JK. An evaluation of early cardiometabolic risk factors in children and adolescents with Turner syndrome. Clin Endocrinol (Oxf) 2013;78:907-913. Epub 2013 Apr 6

48. Blackett PR, Rundle AC, Frane J, Blethen SL. Body mass index (BMI) in Turner syndrome before and during growth hormone (GH) therapy. Int J Obes Relat Metab Disord 2000;24:232-235.

49. Zaki ME, Afifi HH. Body composition in Egyptian Turner syndrome girls. Indian J Hum Genet 2013;19:150-153.

50. Sari E, Bereket A, Yeşilkaya E, Baş F, Bundak R, Aydın BK, Darcan Ş, Dündar B, Büyükinan M, Kara C, Adal E, Akıncı A, Atabek ME, Demirel F, Çelik N, Özkan B, Özhan B, Orbak Z, Ersoy B, Doğan M, Ataş A, Turan S, Gökşen D, Tarım Ö, Yüksel B, Ercan O, Hatun Ş, Şimşek E, Ökten A, Abacı A, Döneray H, Özbek MN, Keskin M, Önal H, Akyürek N, Bulan K, Tepe D, Emeksiz HC, Demir K, Kızılay D, Topaloğlu AK, Eren E, Özen S, Demirbilek H, Abalı S, Akın L, Eklioğlu BS, Kaba S, Anık A, Baş S, Unuvar T, Sağlam H, Bolu S, Özgen T, Doğan D, Çakır ED, Şen Y, Andıran N, Çizmecioğlu F, Evliyaoğlu O, Karagüzel G, Pirgon Ö, Çatlı G, Can HD, Gürbüz F, Binay Ç, Baş VN, Fidancı K, Gül D, Polat A, Acıkel C, Cinaz P, Darendeliler F. Anthropometric findings from birth to adulthood and their relation with karyotpye distribution in Turkish girls with Turner syndrome. Am J Med Genet A 2016;170:942-948. Epub 2016 Jan 20

51. Trolle C, Nielsen MM, Skakkebæk A, Lamy P, Vang S, Hedegaard J, Nordentoft I, Ørntoft TF, Pedersen JS, Gravholt CH. Widespread DNA hypomethylation and differential gene expression in Turner syndrome. Sci Rep 2016;6:34220.

ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.0029

J Clin Res Pediatr Endocrinol 2018;10(3):264-273

Neonatal Features of the Prader-Willi Syndrome; The Case for Making the Diagnosis During the First Week of Life

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What is already known on this topic?

Early diagnosis in Prader-Willi syndrome allows appropriate management of feeding difficulties to be implemented and is important for counselling as well as minimising parental uncertainty and anxiety. The prevalence of preterm birth and low birthweight is increased in Prader-Willi syndrome and is a potential source of diagnostic confusion.

What this study adds?

Although the prevalence of preterm birth (26%) and low birthweight (24%) is increased in Prader-Willi syndrome, diagnosis in preterm infants is not significantly delayed. However, clinical diagnosis in our cohort was made later than 28 days in 34% of the patients despite presence of classic features of hypotonia and cryptorchidism in males and need for nasogastric feeding in over 80%. Comparison with non-affected siblings shows that mothers detect reduced fetal movement in 80% of patients with Prader-Willi syndrome, a feature which can facilitate early diagnosis.

Abstract

Objective: Early diagnosis is of proven benefit in Prader-Willi syndrome (PWS). We therefore examined key perinatal features to aid early recognition.

Methods: Data were collected from case records of subjects attending a multi-disciplinary clinic and from a retrospective birth questionnaire.

Results: Ninety patients (54 male-36 female) were seen between 1991-2015, most with paternal deletion (n = 56) or maternal isodisomy (n = 26). Features included cryptorchidism in 94% males, preterm birth (26%), birthweight < 2500 g (24%), polyhydramnios (23%), breech presentation (23%) and need for nasogastric feeding (83%). Reduced fetal movements (FM) were reported in 82.5% patients compared with 4% healthy siblings. Of 35 children born since 1999, 23 were diagnosed clinically within 28 days while diagnosis in 12 was > 28 days: 1-12 months in seven; and 3.75-10.5 years in five. Typical PWS features in these 12 infants included hypotonia (100%), feeding difficulties (75%), cryptorchidism (83% males) and reduced FM (66%). Causes other than PWS including neuromuscular disease were considered in nine patients.

Conclusion: Neonatal hypotonia, reduced FM, feeding difficulties and cryptorchidism should immediately suggest PWS, yet late diagnosis continues in some cases. Awareness of the typical features of PWS in newborn units is required to allow prompt detection even in the presence of confounding factors such as prematurity.

Keywords: Prader-Willi syndrome, hypotonia, fetal movement, nasogastric feeding

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Conflict of interest: None declared
Received: 25.01.2018
Accepted: 18.03.2018

Introduction

Prader-Willi syndrome (PWS) is a neurodevelopmental disorder resulting from absence of paternally imprinted genes in the 15q11-13 region, caused by deletion in the paternal chromosome (del15q) in about 70% of cases or disomy of the maternal chromosome [maternal uniparental disomy of chromosome 15 (mUPD15)] in about 25% (1). The natural history of PWS is complex, with different phenotypic features developing at different ages (2,3). Before the advent of confirmatory molecular genetic testing, diagnosis was made solely according to age-related clinical criteria (4). Prompt diagnosis of PWS is not only beneficial for educating families about the condition but also for facilitating early therapeutic interventions such as speech therapy, physiotherapy and dietetics to help with feeding difficulties and prevent early onset obesity (5,6,7). Moreover, timely diagnosis will enable growth hormone (GH) therapy to be implemented early so as to improve linear growth and motor development (8). So typical are the features of PWS that, especially with publications highlighting the phenotype (9,10,11,12,13,14) and widely available molecular genetic analysis, it might be expected that diagnosis would be made at, or shortly after, birth.

Hypotonia is a relatively common problem in the newborn period, affecting not only otherwise healthy preterm infants but also those with sepsis, neurological disease and metabolic disorders. The challenge to the clinician, therefore, is to identify the minority of infants with PWS from the majority of babies in whom hypotonia is a transient feature and from those who are suffering from a different condition such as spinal muscular atrophy (SMA) (15). Our clinical experience indicates that the diagnosis of PWS continues to be made beyond the newborn period in some cases.

An increased incidence of preterm birth and low birth weight (LBW) has been reported in PWS, together with obstetric symptoms such as reduced fetal movement (FM), polyhydramnios and malpresentation (16,17,18). Some typical features of PWS infants, such as hypotonia and inability to suck necessitating tube feeding, are also common in unaffected preterm infants. It might be conjectured, therefore, that the diagnosis of PWS is more difficult when the infant is preterm.

A multidisciplinary PWS clinic has been held at our centre in the Royal Hospital for Children in Glasgow since 1991, providing data on a heterogeneous Scottish cohort. The aim of the study was to identify key diagnostic features of PWS to facilitate early diagnosis. These include obstetric and newborn data, feeding difficulties requiring assisted feeding and the prevalence of reduced FMs. In addition, we looked for an association between prenatal and perinatal characteristics and genotype, to further develop previous work (16,17,18).

Methods

All patients with proven PWS attending the multidisciplinary clinic in Glasgow, Scotland, since its inception in 1991 until December 2015 were included. Data on genotype, BW, gestation, delivery method, obstetric symptoms, postnatal complications and timing of diagnosis were collected and analysed from three sources: a birth questionnaire completed by the parent(s); the subject's case records; and maternal obstetric records, when available. It was decided, however, that the systematic recording of phenotypic features such as hair and eye colour, bifrontal narrowing, characteristic eyes, small hands and feet and weak cry should not be included within the scope of the study. This is because these data were recorded inconsistently, if at all, during the newborn period and by multiple observers and it was felt appropriate to confine this retrospective study to the collection of hard data.

Endocrine problems were documented by detailing the number of patients undergoing GH stimulation testing either with insulin or arginine and the outcome, and defining GH deficiency as peak values <15 mU/L. The numbers of patients treated with GH injections as well as the numbers of patients diagnosed with either hypothyroidism or cortisol deficiency were also documented.

The questionnaire invited parents with more than one child to submit information on the BW, gestation and delivery method of their other children. Mothers were also asked to retrospectively estimate the degree of *in utero* FM in their affected and unaffected children using a simple 5 point scoring system ranging from 1: much less than expected to 5: much more than expected.

BW was assessed according to United Kingdom reference standards (Least Mean Square Growth program; http://www.healthforallchildren.com) and expressed in kilograms and standard deviations (SD). The incidences of preterm birth (gestation <37 completed weeks), LBW, defined as BW <2500 g, small for gestational age (SGA), defined as BW $<10^{th}$ percentile and operative delivery were compared with healthy siblings and contemporary Scottish population data (19).

Statistical Analysis

All analyses were done using Minitab (version 13.1) at a significance level of 5%. Data distribution was assessed for normality using Anderson-Darling test. Parametric data

are presented as mean (± SD) and non-parametric data as median (range) or median (range) (interquartile range) Quantitative variables were compared using t-tests and analysis of variance or Kruskal-Wallis and Mann-Whitney U tests. Qualitative variables were compared using chi-squared or Fisher's exact tests.

Ethical Aspects

Approval was initially granted by the Ethical Committee of the Royal Hospital for Sick Children in Glasgow in 2004 and data collection completed in 2011. Following the tragic loss of the lead investigator, Wendy Paterson in 2012, the study was relaunched in 2015 to update the data and to include more recent patients. This second phase of the study was approved by the National Health Service of the United Kingdom Research Ethics Authority (reference 15/NW/0900). In both study periods written informed consent was obtained from parents/guardians and subjects aged ≥16 years while children < 16 years were invited to give assent.

Results

Ninety (54 male) subjects with PWS born between 1950 and 2015 (median 1994) were included in the study (Table 1). Excluding eight deaths (male/female = 5/3) at the ages of 15, 18, 23, 25, 27, 27, 29 and 45 years, the median (range) age of 82 patients on 01/01/2016 was 20.8 (0.9-65.7) years. Twenty-nine patients had undergone GH stimulation testing of whom 17 were GH insufficient-median (range) levels 7.2 (< 0.1-14.8) mU/L; and 12 were GH sufficient-levels 31.6 (17.4-78.1 mU/L). A total of 20 patients were treated with GH injections during the study period. Hypothyroidism was not documented, and no patient was referred with elevation of thyroid stimulating hormone on newborn screening programme, which was introduced in Scotland in 1979. No patient was diagnosed with adrenal insufficiency during the study period, or received hydrocortisone therapy.

Questionnaires were sent to 72 families and returned by 60 (83%). Reasons for questionnaires not being sent to 18 families included adoption/fostering and parents no longer being contactable. Comparative birth data were collected for 97 siblings (56 male).

Genetics

Genetic diagnosis of PWS was confirmed in 89 of the 90 subjects, as follows: paternal deletion (del15q) in 56 (62%); maternal disomy (mUPD15) in 26 (29%); translocation (chromosome 15-16) in one (1%); imprinting defect in two (2%) and four tested elsewhere (4%). One subject was initially reported to have no defect on standard PWS genetic screening but later Comparative Genomic Hybridization Array analysis revealed a deletion in paternal 15q11.2 incorporating the *NDN* (NECDIN) gene (OMIM 602117). *NDN* is important for normal hypothalamic development and it has been suggested that loss of *NDN* contributes to both the hypothalamic hypogonadism and neurological features of PWS (19). No defect was found in one other, older subject who nevertheless satisfied the Holm consensus criteria for PWS (4).

Prenatal, Perinatal and Postnatal Data

Data for all PWS subjects and a comparison between term and preterm infants are shown in Table 1. Mean (± SD) BW was significantly lower in the study group versus healthy siblings: 2.68 (0.6) (n = 89) vs 3.34 (0.6) kg (n = 91); and -1.03 (1.03) vs -0.182 (1.02) SD ($p < 0.001$); prevalence of SGA significantly higher -32/89 (36%) versus 10/91 (11%). Median (range) PWS gestation was lower at 39 (30-43) versus 40 (33-42) weeks while preterm birth occurred significantly more often; in 23 (25.8%) patients compared with three (3.2%) siblings ($p = 0.03$).

Table 1. Prenatal, perinatal and postnatal characteristics of 90 patients with Prader-Willi syndrome seen in a single centre between 1991 and 2015 according to term and preterm status

	PWS all (n = 90)	PWS term (n = 66)	PWS preterm (n = 23)	Term vs preterm
Gender (M/F)	54/36	40/26	13/10	
Median (range) BW (kg)	2.68 (1.18-3.99) (n = 89)	2.97 (1.9-3.99) (n = 66)	1.95 (1.18-2.78) (n = 23)	p < 0.001
Median (range) BW SDS (n)	-1.10 (-3.27 to 1.92) (89)	-1.12 (-3.27 to 1.12) (66)	-0.62 (-3.21 to 1.92) (23)	p = 0.26
% SGA	36 (n = 32/89)	36.4 (n = 24/66)	34.8 (n = 8/23)	p = 0.9
Median (range) gestation (wks)	39 (30-43) (n = 89)	40 (37-43) (n = 66)	34 (30-36) (n = 23)	p < 0.001
Median (range) duration of hospital stay (days)	27 (0-730) (n = 71)	21 (1-730) (n = 51)	36.5 (0-134) (n = 20)	p = 0.01
Median (range) duration of NGF (days)	30 (2-480) (n = 71)	25 (3-480) (n = 50)	42 (2-231) (n = 21)	p = 0.04
Median (range) fetal movement score	1 (1-4) (n = 80)	2 (1-4) (n = 60)	1 (1-3) (n = 20)	p = 0.05
Time to clinical diagnosis (days)	70 (1-16801) (n = 87)	152 (1-16801) (n = 65)	24 (2-2995) (n = 22)	p = 0.16

PWS: Prader-Willi syndrome, BW: birthweight, SDS: standard deviation scores, SGA: small for gestational age, wks: weeks, NGF: nasogastric feeding,

Polyhydramnios was reported in 10/44 (22.7%) mothers. Breech presentation was recorded in 15/65 (23.1%) with one transverse lie. Mode of delivery was spontaneous vaginal in 38/86 subjects (44%), Caesarean section in 38/86 (44%), forceps-assisted delivery in six and ventouse extraction in three. FMs were reduced with a median score in PWS of 1 in the preterm and 2 in the term infants respectively (Table 1).

Figure 1 shows that whereas sibling gestation was normally distributed, the PWS group suggested a bimodal, flattened distribution with peaks at both 34 and 40 weeks. This was more marked in the del15q than the mUPD15 groups.

Fetal Movement Scores

Decreased FM were present in 66/80 (82.5%) with normal movement in 14 (17.5%). Figure 2 shows comparison between 55 patients (including six with normal movement) and their unaffected siblings, with significantly lower scores for PWS ($p < 0.001$). Mothers who had had non-affected babies (n = 55) scored their affected babies significantly lower ($p = 0.02$) than those who had had only one (affected) baby (n = 25)-median scores 1 versus 2. Thus 8/25 (32%) of the mothers with experience of only one pregnancy, carrying a PWS-affected infant, reported normal FMs compared with 6/55 (11%) of those with more than one child although the FM scores for the former group were still significantly lower than for unaffected children ($p < 0.001$).

Feeding Difficulties

Nasogastric feeding (NGF) was given to 75 (86%) infants with no assisted feeding in 12 and no data in three. Six of the infants who did not receive assisted feeding were diagnosed after four months of age. Median (range) duration of NGF in days was 30 (2-480) (n = 71) and was longer in preterm (n = 21) at 42 (2-231) than in term infants (n = 49) at 25 (3-480).

Hypogonadism in Male Prader-Willi Syndrome Infants

Data on testicular position was available in all but five of the 54 males. Forty-six (94%) of the 49 boys had cryptorchidism, which was bilateral in 40, unilateral in five, unspecified in one. Only three (5.5%) male patients had normally descended testes. Cryptorchidism was present in all but one of the 12 preterm males.

Hypoplasia of Labia Minora in Female Prader-Willi Syndrome Infants

This was documented as present in six girls at birth, not present (i.e. normal) in a further six, and not documented otherwise.

Timing of Clinical and Molecular Diagnosis

Table 1 shows that age at clinical diagnosis was extremely variable during the study period and also that preterm infants were diagnosed sooner than term infants although this difference was not significant.

Table 2 shows data on the timing of clinical (n = 87) and genetic diagnosis (n = 83) with subjects stratified by

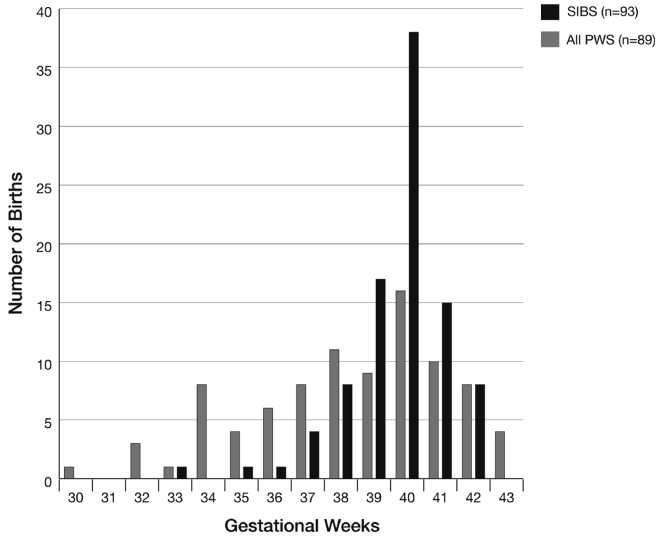

Figure 1. Comparison of gestational age between 89 Prader-Willi syndrome patients and 93 unaffected siblings

Fetal movements were retrospectively scored by the mothers as 1: much less than expected, 2: a bit less than expected, 3: about as much as expected, 4: a bit more than expected and 5: a lot more than expected

Sibs: siblings, PWS: Prader-Willi syndrome

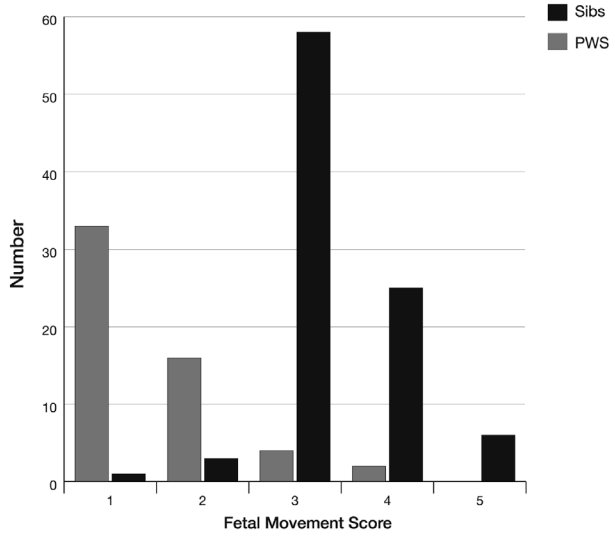

Figure 2. Comparison of fetal movement in 55 Prader-Willi syndrome subjects and 93 unaffected siblings

Sibs: siblings, PWS: Prader-Willi syndrome

decade of birth. Median (range) age at diagnosis during the study period was 2.3 months (1 day-46 years) for clinical and 10 months (4 days-46.5 years) for genetic diagnosis. Since 1991, time to genetic diagnosis has been 28 days (4 days-10.6 years). Age at diagnosis showed an apparent improvement during the study period although the number of as yet undiagnosed patients, particularly during the 2010-2015 period, remains uncertain.

Age at Clinical Diagnosis in Patients Born from the Year 2000 Onwards

Analysis of patients born since 1 January 2000 showed that of 35 patients (16 males) the clinical diagnosis was made within the first week of life in 12 infants, between days 8 and 27 in 11, aged 4 weeks to 12 months in seven, and after 5 years in five. Among the 12 infants who were diagnosed clinically within a week of birth, sepsis was considered in two and neuromuscular disease in another. Table 3 gives details on the 12 patients diagnosed ≥4 weeks from year 2000 onwards. Feeding difficulties and hypotonia at birth were present in all these patients, with NGFs given to nine. Other features included reduced FMs (9), prematurity (4), LBW (4), and bilateral cryptorchidism (5/6 males). Hypotonia was attributed in three cases to birth asphyxia, birth injury and benign congenital hypotonia. Magnetic resonance imaging (MRI) was carried out in three. Five children had developed hyperphagic obesity by the time that PWS was confirmed.

Genotype vs Prenatal and Perinatal Features

Table 4 shows that both maternal and paternal age were significantly greater in the mUPD15 group compared with the del15q group. Also, median year of birth was later in the mUPD15 group than in the del15q group-2004 vs 1994-although this was not significant (p = 0.11). The prevalence of diminished FMs, birthweight and gestation and operative delivery was similar between the two groups.

Discussion

This study of 90 patients with PWS, drawn from a geographically defined area over a 25-year period, confirms and extends existing knowledge of the prenatal, perinatal and postnatal features of PWS, the dominant trait being presence of hypotonia. It should be noted that our study is confined to describing only those infants whose hypotonia was caused by PWS so that the prevalence of neonatal hypotonia in Scotland during the study period and hence the proportion of those hypotonic infants who actually had PWS is unknown.

Intrauterine hypotonia causes polyhydramnios, malpresentation and reduced FMs leading to an increased prevalence of operative delivery. All these features were seen in this study, consistent with previous reports (9,12,17,18). Of note, breech presentation was found in 23% of our cohort compared with 0.2% in the Scottish population while 44% were delivered by caesarean section in contrast to Scottish population data showing a rate of 8.6% in 1975-76, rising to 29.9% in 2014-15 (20).

We found increased incidence of LBW (23.6%) and SGA (36%), contrasting with 12.7% for SGA in the Scottish population (21) although SGA prevalence in PWS has been reported to be higher, ranging from 53-65% (9,22). It has been suggested that SGA in PWS may result from a failure to express paternal genes which improve placental function (22).

Table 2. Mean ± standard deviation and median (range) times (days) to clinical (n = 87) and molecular (n = 83) diagnosis by decade of birth

Decade of birth	Mean ± SD	Median (range) (IQR)	p compared to prior decade	Comparison between all decades p
Clinical diagnosis				
< 1980 (n = 19)	3558 ± 4548	1468 (194-16801) (840-4200)	-	-
1980-1989 (n = 17)	415 ± 681	97 (1-2557) (9-612)	p < 0.001	-
1990-1999 (n = 16)	248 ± 422	74 (1-1534) (3-225)	p = 0.61	p < 0.001
2000-2009 (n = 26)	404 ± 887	19 (2-3835) (7-128)	p = 0.91	-
> = 2010 (n = 9)	6.1 ± 3.4	5.0 (2-12) (3.5-9)	p = 0.01	-
Molecular diagnosis				
< 1980 (n = 16)	8797 ± 3458	8401 (4556-16984) (6452-10319)	-	-
1980-1989 (n = 18)	3890 ± 2556	3658 (183-8949) (1544-5470)	p < 0.001	-
1990-1999 (n = 15)	276 ± 494	73 (4-1790) (14-293)	p < 0.001	p < 0.001
2000-2009 (n = 25)	476 ± 958	56 (7-3857) (13-228)	p = 0.82	-
> = 2010 (n = 9)	11.8 ± 4.52	12.0 (5-19) (7.5-15)	p = 0.006	-

SD: standard deviation, IQR: interquartile range

The prevalence of preterm birth (25.8%) in our cohort markedly exceeds the current Scottish figures of 7.3% (19). This increase is similar to Lionti's study (27.4%) (22) and higher than two earlier French studies which reported rates of 13/86 (15%) and 2/19 (10.5%) versus 6% in the general population (12,17). Preterm birth in PWS may be partly due to polyhydramnios from reduced fetal swallowing causing uterine distension and inducing early labour (14,17). The distribution of gestational age seen in our cohort appeared to be bimodal. This finding has been previously reported by Butler et al (16). However these authors report bimodal distribution only in mUPD15 whereas we found bimodality only in the deletion group. More data on larger populations are needed to confirm this possible bimodal pattern.

In contrast to our expectations that features in common with prematurity might cause delay in the diagnosis of PWS, we found that affected premature infants tended to be diagnosed earlier than their term counterparts. This might be attributable to preterm infants receiving more clinical attention as well as having a longer stay in hospital.

While most prenatal features of PWS, such as polyhydramnios and abnormal presentation are too non-specific to be of value, reduced FM is of importance in indicating the congenital nature of the hypotonia, prompting consideration of disorders such as PWS or neurological conditions such as SMA. Careful comparison of maternal retrospective evaluation of FMs in affected

Table 3. Clinical data on 12 of 35 children with Prader-Willi born from the year 2000 onwards in whom clinical diagnosis was made at or after 28 days

Patient no	Sex	Age at clinical diagnosis	FM score	BW (kg)	Gestation (weeks)	Floppy at birth?	UDT	NGF (duration)	Comment
1	M	4 w	1	1.96	36	y	b	y (4 w)	Had MRI of brain for possible neurological diagnosis
2	M	4 w	2	3.38	39	y	b	y (12 m)	Diagnosed at end of newborn period
3	M	4 w	3	2.5	42	y	b	n	Poor suck and floppy with dislocated hips
4	F	6 w	1	1.94	34	y		y (17 w)	Mother noticed very reduced FM compared with co-twin
5	F	7 w	2	2.29	35	y		y (2 d)	Admitted day 5 with hypothermia, tube fed for 2 days, discharged day 10. Diagnosis made because of persistent hypotonia
6	M	3 m	4	2.61	38	y	b	y (10 m)	MRI and EEG carried out in view of neonatal hypotonia and poor feeding
7	F	7 m	1	2.68	38	y		y (2 w)	Hypotonia attributed to birth asphyxia (Apgar 4 at 5 minutes). Persistent hypotonia led to diagnosis
8	F	10.5 y	1	2.76	39	y		y (7 d)	Very floppy and difficult to feed at birth, hyperphagic from 3 years
9	M	3.9 y	1	2.7	37		b	y (4 w)	Bruised at birth. MRI scan performed at 3 w in view of hypotonia and poor feeding. Developmental delay and weight gain led to diagnosis
10	F	4.6 y	3	3.24	40	y		n	"Like a rag doll" with no cry and poor feeding. Diagnosed as benign congenital hypotonia. Weight gain from 3 years and mother suspected PWS from internet
11	F	3.7 y	3	3.1	41	y		y (3 d)	Difficult to feed and tube fed briefly. Developmental delay followed by increase in weight at 3 years prompted diagnosis
12	M	4.2 y	2	1.8	36	y	No	n	Reduced fetal movement compared to siblings reported. Went home day 3. Increase in weight noted at 1 year. Initial genetic screening reported normal but deletion in PWS region detected by CGH Array

d: days, m: months, y: years, b: bilateral, F: female, M: male, FM: fetal movement, BW: birthweight, UDT: undescended testis, NGF: nasogastric feeding, MRI: magnetic resonance imaging, EEG: electroencephalogram, PWS: Prader-Willi syndrome, CGH: comparative genomic hybridization

and unaffected infants shows a striking difference, with 82.5% reporting reduced movements in PWS infants. This is consistent with the figures of 85% and 88% reported in the studies of Miller et al (23) and Gross et al (9), both of which assessed FMs using antenatal ultrasound.

In our study mothers who had only experienced one pregnancy with a PWS infant were less likely to score FM as low. However, scores given by uniparous mothers were still significantly lower than those for the unaffected babies of other mothers. Furthermore, preterm infants had a lower median score for FMs than term babies, but despite this term babies' scores were still significantly lower compared with unaffected babies. Table 3 shows that eight of twelve patients diagnosed ≥4 weeks of age had reduced FMs, awareness of which could have secured earlier diagnosis. Subjective maternal reporting, even for uniparous women, appears reliable and increased awareness of this among obstetricians and neonatologists is important (18).

The most constant feature of PWS at birth is hypotonia, Dudley and Muscatelli citing a 97% incidence (17). Gunay-Aygun et al (24) cite "hypotonia with poor suck" as the sole factor from birth to two years, sufficient to prompt genetic testing for PWS. Feeding difficulty is also common in PWS, NGF being instituted in 86% of our cohort. Late-diagnosed infants had shorter durations of NGF reflecting failure to recognise the condition and its severity. In the few cases where NGF was not commenced, some mothers described enormous difficulties in feeding their infants. The value of prompt diagnosis in reducing the period of hospitalisation and duration of NGF has been shown by Bacheré et al (25). The very high rate of male hypogonadism in PWS is confirmed by this study with a prevalence of 90% amongst preterm boys compared with 30% prevalence reported in unaffected preterm boys (26).

During the study period the median age at diagnosis of PWS appeared to improve with a fall from 9 to 5 days between 2000-2009 and 2010-2015. However it is important to note that the number of as yet undiagnosed children with PWS is currently unknown, and that the true median age at diagnosis may prove to be higher.

Consistent with previous studies we have shown an increase in parental age with mUPD15 compared with del15q (16). We also showed that the ratio of mUPD15 to del15q rose during the study, consistent with the rise

Table 4. Parental, fetal and perinatal characteristics of 90 patients with Prader-Willi syndrome seen in a single centre 1991-2015 according to molecular genetic defect

	mUPD15	del15q	p value
Mean (SD) maternal age (yrs)	34.6 (6.2)	26.4 (6.1)	**<0.001**
[n]	[23]	[51]	
Mean (SD) paternal age (yrs)	34.6 (7.2)	29.6 (6.4)	**0.004**
[n]	[23]	[49]	
% decreased fetal movement scores	79.2	81.6	0.91
[n]	[19]	[40]	
% Caesarean section	40	49.1	0.54
[n]	[10]	[27]	
% Vaginal delivery	56	50	0.54
[n]	[14][a]	[28][b]	
Median (range) gestation (weeks)	38 (32-42)	39 (30-43)	0.52
[n]	[25]	[56]	
Preterm <37 weeks	6/25	16/56	0.66
(%)	(24)	(28.6)	
Mean (SD) birth weight	2.73 (0.57)	2.60 (0.57)	0.36
[n]	[25]	[56]	
Mean (SD) birth weight SDS	-0.82 (0.83)	-1.18 (1.01)	0.13
[n]	[n=25]	[56]	
Number (%) SGA	5 (20)	25 (44.6)	**0.02**
[n]	[25]	[56]	

mUPD15: maternal uniparental disomy of chromosome 15, del15q: deletion in 15q 11-13 region of paternal chromosome, [a]: includes two forceps and one ventouse deliveries; [b]: includes three forceps and two ventouse deliveries, SGA: small for gestational age, SD: standard deviation

in parental ages in Scotland between 1977 and 2015-from 26.1 to 29.4 and 28.6 to 32.3 years for mothers and fathers respectively (20). The relationship between maternal age and disomy has been hypothesized to result from a phenomenon known as trisomy rescue (16,27). No other significant difference was found between the two genotypes.

Early diagnosis in PWS is highly desirable. Therapies to counter muscular hypotonia, including GH therapy and physiotherapy training programmes, may be especially useful in the early years. During this period PWS infants and young children may have impaired motor development and skill acquisition which has been reported to affect further development of social abilities and cognitive function (5). GH production is reduced at all ages in PWS but is more marked at younger ages and reduced muscle mass is present from infancy so that early GH therapy may alleviate deficits associated with PWS hypotonia, including improved cognitive development (7,8). Moreover, feeding difficulties are a major problem during infancy and early detection of PWS enables speech and language therapy help with this problem as well as with language acquisition and cognitive development. Indeed, so prevalent are feeding difficulties and the need for NGFs in PWS that this diagnosis can be virtually excluded in older patients with obesity and developmental delay if there is no history of neonatal feeding problems. Beyond infancy parents and carers need to restrict calorie intake in order to prevent the onset of obesity (5,6). Thus an additional benefit of early diagnosis is the opportunity to educate parents and others involved in the welfare of the patient as to the natural history of this complex condition and to not only care for the child, but also to seek help themselves, when necessary (5,7,8).

Given the typical features of PWS, particularly the findings of hypotonia and feeding difficulty at birth, it might be expected that diagnosis would be consistently early. The additional feature of cryptorchidism in almost all males is most helpful, especially when combined with hypotonia. By contrast, hypoplasia of the labia minora was documented in only a minority of female patients. This partly reflects the lack of systematic documentation during the study period, but also the limited diagnostic value of such a subjective feature. The finding of unexplained hypotonia at birth, especially if accompanied by undescended testes in boys, should immediately prompt a search for the phenotypic features of PWS. These include characteristic up-slanting eyes with narrow palpebral fissures (so-called "almond eyes"), narrow nose, bi-frontal narrowing and sticky saliva resulting in the "string sign" (28), small hands and feet, and weak cry. Whilst we decided that it was not possible in this retrospective study to accurately quantify and evaluate these traits individually, a constellation of these features in a hypotonic infant is an important adjunct to diagnosis. However, we would argue that it is actually making the essential connection between neonatal hypotonia and the possibility of PWS in the first place-which only then prompts a search for phenotypic features-which is essential and yet not being made consistently in clinical practice in our experience. Thus our study shows that in Scotland diagnosis of PWS was first suspected well after the first week of life in 34% of patients during the past 15 years. This is despite the presence of hypotonia in all 12 of the late-diagnosed cases with recourse to NGF in nine, reduced FMs in eight, and cryptorchidism in 5/6 boys.

Also, the distress caused to parents when diagnosis is delayed, even for a matter of days, should not be underestimated, especially when investigations for neurological disease, such as MRI and muscle biopsy are contemplated. The mother of patient 7 in Table 3, eventually diagnosed at 9 months, wrote "As the hospital didn't know what was wrong we were not allowed to go home until our daughter could take the bottle. We secretly cup fed her so as to be able to get home as they wouldn't let us tube feed. On finding out about her condition we realised that she ticked all the boxes and we feel that the hospital should have recognised the condition. Instead we were told she had cerebral palsy."

Study Limitations

The main limitation of our study was its retrospective nature. However, in including all genetically proven PWS patients who have attended our clinic up to 2015, our cohort size was maximised. In addition, a temporal analysis of the speed of clinical and molecular diagnosis at a dedicated, specialised PWS clinic was made possible. A further limitation of our study was the subjective nature of data obtained from the mothers, especially concerning the degree of FM in affected and unaffected children. It is reassuring to compare the subjective recall of our mothers which proved to be consistent with the radiologically measured degree of FM reported by Miller et al (23) and Gross et al (9).

In our opinion the diagnosis of PWS can and should be made within days of birth provided that the hypotonia and the feeding difficulties, which will invariably be evident

to the mother and midwife, are appreciated. Subsequent rapid genetic confirmation of PWS is essential since previous work in our centre showed clinical misdiagnosis in 11/31 patients at the time our multi-disciplinary clinic was established (29). In the present study only one patient did not show molecular genetic evidence of PWS despite fulfilling the Holm criteria.

Conclusion

We believe that, although features such as preterm birth, LBW, SGA, operative delivery and malpresentation are not specific to PWS, their combined presence in the context of hypotonia and feeding difficulties should evoke PWS. Indeed it has been suggested that when a combination of polyhydramnios, SGA and asymmetric intrauterine growth co-exist in the absence of morphological abnormalities, prenatal methylation studies for PWS should be performed (9).

Acknowledgment

The authors gratefully acknowledge Dr. David Young, Research and Development Office, Royal Hospital for Sick Children, Glasgow, for his assistance with the statistical analyses and all the families who took part in the study. We also thank the many clinicians who referred their patients to our clinic and shared their patients' care with us.

Ethics

Ethics Committee Approval: Approval was initially granted by the Ethical Committee of the Royal Hospital for Sick Children in Glasgow in 2004 and data collection completed in 2011. Following the tragic loss of the lead investigator, Wendy Paterson in 2012, the study was relaunched in 2015 to update the data and to include more recent patients. This second phase of the study was approved by the National Health Service of the United Kingdom Research Ethics Authority (reference 15/NW/0900).

Informed Consent: In both study periods written informed consent was obtained from parents/guardians and subjects aged ≥16 years while children <16 years were invited to give assent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Wendy Forsyth Paterson, Ruth McGowan, M. Guftar Shaikh, Malcolm Donaldson, Concept: Filiz Mine Çizmecioğlu, Jeremy Huw Jones, Wendy Forsyth Paterson, Malcolm Donaldson, Design: Filiz Mine Çizmecioğlu, Jeremy Huw Jones, Wendy Forsyth Paterson, Malcolm Donaldson, Data Collection or Processing: Filiz Mine Çizmecioğlu, Jeremy Huw Jones, Wendy Forsyth Paterson, Sakina Kherra, Mariam Kourime, Analysis or Interpretation: Filiz Mine Çizmecioğlu, Jeremy Huw Jones, Wendy Forsyth Paterson, Sakina Kherra, Mariam Kourime, Malcolm Donaldson, Literature Search: Filiz Mine Çizmecioğlu, Jeremy Huw Jones, Wendy Forsyth Paterson, Malcolm Donaldson, Writing: Filiz Mine Çizmecioğlu, Jeremy Huw Jones, Wendy Forsyth Paterson, Ruth McGowan, M. Guftar Shaikh, Malcolm Donaldson.

Financial Disclosure: No funding or grants were used in support of this work.

References

1. Cassidy SB, Schwartz S, Miller JL, Driscoll DJ. Prader-Willi syndrome. Genet Med 2012;14:10-26. Epub 2011 Sep 26
2. Donaldson MD, Chu CE, Cooke A, Wilson A, Greene SA, Stephenson JB. The Prader-Willi syndrome. Arch Dis Child 1994;70:58-63.
3. Donaldson MDC. The natural history of Prader-Willi syndrome. KIGS Biannual Report 2002;1:11-21.
4. Holm VA, Cassidy SB, Butler MG, Hanchett JM, Greenswag LR, Whitman BY, Greenberg F. Prader-Willi syndrome: consensus diagnostic criteria. Pediatrics 1993;91:398-402.
5. Goldstone AP, Holland AJ, Hauffa BP, Hokken-Koelega AC, Tauber M; speakers contributors at the Second Expert Meeting of the Comprehensive Care of Patients with PWS. Recommendations for the diagnosis and management of Prader-Willi syndrome. J Clin Endocrinol Metab 2008;93:4183-4197. Epub 2008 Aug 12
6. Irizarry KA, Miller M, Freemark, Haqq AM. Prader Willi syndrome: Genetics, Metabolomics, hormonal function, and new approaches to therapy. Adv Pediatr 2016;63:47-77.
7. Angulo MA, Butler MG, Cataletto ME. Prader-Willi syndrome: a review of clinical, genetic, and endocrine findings. J Endocrinol Invest 2015;38:1249-1263. Epub 2015 Jun 11
8. Festen DA, Wevers M, Lindgren AC, Böhm B, Otten BJ, Wit JM, Duivenvoorden HJ, Hokken-Koelega AC. Mental and motor development before and during growth hormone treatment in infants and toddlers with Prader-Willi syndrome. Clin Endocrinol (Oxf) 2008;68:919-925. Epub 2007 Nov 19
9. Gross N, Rabinowitz R, Gross-Tsur V, Hirsch HJ, Eldar-Geva T. Prader-Willi syndrome can be diagnosed prenatally. Am J Med Genet A 2014;167:80-85. Epub 2014 Oct 22
10. Miller SP, Riley P, Shevell MI. The neonatal presentation of Prader-Willi syndrome revisited. J Pediatr 1999;134:226-228.
11. Õiglane-Shlik E, Žordania R, Varendi H, Antson A, Mägi ML, Tasa G, Bartsch O, Talvik T, Ounap K. The neonatal phenotype of Prader-Willi syndrome. Am J Med Genet A 2006;140: 1241-1244.
12. Bigi N, Faure JM, Coubes C, Puechberty J, Lefort G, Sarda P, Blanchet P. Prader-Willi syndrome: is there a recognizable fetal phenotype? Prenat Diagn 2008;28:796-799.
13. Haugen G, Rønnestad A, Kroken M. Variations in fetal phenotype in Prader-Willi syndrome. Prenat Diagn 2009;29:294.
14. Butler MG. Benefits and limitations of prenatal screening for Prader-Willi syndrome. Prenat Diagn 2016;37:81-94. Epub 2016 Oct 12
15. Parano E, Pavone L, Falsperla R, Trifiletti R, Wang C. Molecular basis of phenotypic heterogeneity in siblings with spinal muscular atrophy Ann Neurol 1996;40:247-251.

16. Butler MG, Sturich J, Myers SE, Gold JA, Kimonis V, Driscoll DJ. Is gestation in Prader-Willi syndrome affected by the genetic subtype? J Assist Reprod Genet 2009;26:461-466. Epub 2009 Sep 17

17. Dudley O, Muscatelli F. Clinical evidence of intrauterine disturbance in Prader-Willi syndrome, a genetically imprinted neurodevelopmental disorder. Early Hum Dev 2007;83:471-478. Epub 2006 Oct 18

18. Whittington JE, Butler JV, Holland AJ. Pre-, peri- and postnatal complications in Prader-Willi syndrome in a UK sample. Early Hum Dev 2008;84:331-336. Epub 2007 Oct 4

19. Information Services Division (ISD) Scotland website: www.isdscotland.org/Health-Topics/Maternity-and-Births/Publications/2015-11-24/2015-11-24-Births-Report

20. Miller NL, Wevrick R, Mellon PL. Necdin, a Prader-Willi syndrome candidate gene, regulates gonadotropin-releasing hormone neurons during development. Hum Mol Genet 2009;18:248-260. Epub 2008 Oct 17

21. Montgomery Sardar C, Kinmond S, Siddique J, Cooper A, McGowan S, Paterson W, Donnelly S, Gault EJ, Donaldson M. Short Stature Screening by Accurate Length Measurement in Infants with a Birth Weight < 9th Centile. Horm Res Paediatr 2015;83:400-407. [Epub ahead of print

22. Lionti T, Reid SM, White SM, Rowell MM. A population-based profile of 160 Australians with Prader-Willi syndrome: Trends in diagnosis, birth prevalence and birth characteristics. Am J Med Genet A 2015;167:371-378. Epub 2014 Nov 25

23. Miller JL, Lynn CH, Driscoll DC, Goldstone AP, Gold JA, Kimonis V, Dykens E, Butler MG, Shuster JJ, Driscoll DJ. Nutritional phases in Prader-Willi syndrome. Am J Med Genet A 2011;155:1040-1049. Epub 2011 Apr 4

24. Gunay-Aygun M, Schwartz S, Heeger S, O'Riordan MA, Cassidy SB. The changing purpose of Prader-Willi syndrome clinical diagnostic criteria and proposed revised criteria. Pediatrics 2001;108:92.

25. Bacheré N, Diene G, Delagnes V, Molinas C, Moulin P, Tauber M. Early diagnosis and multidisciplinary care reduce the hospitalisation time and duration of tube feeding and prevent early obesity in PWS infants. Horm Res 2008;69:45-52. Epub 2007 Dec 4

26. Nah SA, Yeo CS, How GY, Allen JC Jr, Lakshmi NK, Yap TL, Jacobsen AS, Low Y, Ong CC. Undescended testis: 513 patients' characteristics, age at orchidopexy and patterns of referral. Arch Dis Child 2014;99:401-406. Epub 2013 Nov 13

27. Cho SY, Ki CS, Sohn YB, Maeng SH, Jung YG, Kim SJ, Jin DK. The proportion of uniparental disomy is increased in Prader-Willi syndrome due to an advanced maternal childbearing age in Korea. J Hum Genet 2013;58:150-154. Epub 2013 Jan 10

28. Stephenson JB. Neonatal Presentation of Prader-Willi Syndrome. Am J Dis Child 1992;146:151-152.

29. Chu CE, Cooke A, Stephenson JB, Tolmie JL, Clarke B, Parry-Jones WL, Connor JM, Donaldson MD. Diagnosis in Prader-Willi syndrome. Arch Dis Child 1994;71:441-442.

CASE REPORT

DOI: 10.4274/jcrpe.5501

J Clin Res Pediatr Endocrinol 2018;10(3):274-278

Severe Early Onset Obesity due to a Novel Missense Mutation in Exon 3 of the Leptin Gene in an Infant from Northwest India

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What is already known on this topic?

Congenital leptin deficiency due to mutations of the leptin gene is a rare cause of early-onset obesity with less than 50 cases reported to date.

What this study adds?

This article presents an Indian infant with severe early-onset obesity caused by a novel mutation in the leptin gene.

Abstract

Monogenic obesity, caused by mutations in one of the genes involved in the control of hunger and satiety, is a rare cause of early onset obesity (EOO). The most common of the single gene alterations affect the leptin gene (*LEP*), resulting in congenital leptin deficiency that manifests as intense hyperphagia, EOO and severe obesity associated with hormonal and metabolic alterations. Only eight mutations of *LEP* associated with congenital leptin deficiency have been described in humans to date. In this study, we report a novel, homozygous, missense mutation in exon 3 of the *LEP* gene (chr7:127894610;c.298G > A) resulting in the amino acid substitution of asparagine for aspartic acid at codon 100 (p.Asp100Asn) in a 10-month-old infant who presented to us with severe hyperphagia and EOO. She was subsequently found to have low serum leptin concentrations. Additionally, a homozygous missense variation of unknown significance in exon 11 of Bardet-Biedl syndrome-1 gene (chr11:66291279; G > A; Depth 168x) was detected. Significant abnormalities of lipid parameters were also present in our patient. Both parents were thin but there was a family history suggestive of EOO in a paternal uncle and a cousin. In conclusion, we report the second patient from India with a novel mutation of the *LEP* gene associated with severe obesity.

Keywords: Congenital leptin deficiency, monogenic obesity, leptin gene, novel mutation, early onset obesity, India

Introduction

Severe early onset obesity (EOO) may be caused by alterations in genes that regulate appetite, body weight and energy homeostasis (1). The most common single-gene alterations that cause severe EOO include mutations in the leptin (*LEP*), leptin receptor (*LEPR*), preopiomelanocortin, prohormone convertase 1 or melanocortin 4 receptor genes, which together account for 3-5% of non-syndromic cases (1). These genes are involved in the control of hunger and satiety through the leptin-melanocortin signaling pathway in the hypothalamus. Of these genes, the most commonly affected is the *LEP* gene. Homozygous mutations in *LEP* cause the recessively inherited congenital leptin deficiency which manifests as severe EOO (1). Other characteristic manifestations include impaired satiety, intense hyperphagia, a normal birth weight and rapid weight gain during early infancy (1). These children also develop several hormonal and metabolic abnormalities associated with obesity in older children and adults (2). In addition, they may have reduced T-cell number and function, resulting in increased predisposition to infections and high rates of mortality during childhood (3). After the first report of a frameshift mutation in *LEP* in two severely obese cousins

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This manuscript was presented as a poster in UPEK 2016 (Turkish National Pediatric Endocrinology Congress).

Conflict of interest: None declared
Received: 13.10.2017
Accepted: 29.11.2017

from a consanguineous United Kingdom family of Pakistani origin (4), several other patients with frameshift, missense or deletion mutations in *LEP* have been reported (5,6,7,8, 9,10,11,12,13,14,15,16,17). These reports have emanated from several countries and especially from regions with high rates of consanguinity. Approximately 80 % of about 50 patients described in the literature so far come from Central Pakistan (4,5,6,7,8). In this communication, we report a novel homozygous missense mutation in *LEP* associated with low serum leptin concentrations, hyperphagia and severe EOO in an infant from Northwest India.

Case Report

A 10-month-old girl was referred to our endocrine unit for evaluation of excessive and rapid weight gain. She was born at full term by normal vaginal delivery and weighed 3.0 kg [-0.52 standard deviation score (SDS)] at birth. She is the second child of healthy, non-obese parents with third degree consanguinity. There was no history to suggest gestational diabetes, hypertension, hypothyroidism or excess weight gain by mother during pregnancy. Parents noticed increased appetite at about two months of age. She started demanding feeds at half to one hourly intervals. Subsequently, there was a rapid gain in her weight to 9.5 kg (+3.14 SDS) at four months and 15 kg (+8.17 SDS) at six months of age. There was no history of lethargy, dryness of skin, constipation, excessive hair growth, seizures, visual or sleep disturbances. There was a history of EOO in a paternal uncle and a male cousin.

Physical examination revealed generalized body fat distribution, a rounded face and deep skinfolds (Figure 1A, 1B). There were no stigmata of a syndrome or underlying endocrinopathy, except acanthosis nigricans in axillae and neck folds (Figure 1C). The vital parameters were normal. Her weight was 19 kg (+7.38 SDS), length 71.0 cm (-0.24 SDS) and body mass index 37.7 kg/m^2 (+10.94 SDS). Anthropometric calculations were done using the World Health Organizetion (WHO) Anthroplus software (version 1.0.4 WHO, Geneva, Switzerland). Ophthalmological evaluation did not show retinitis pigmentosa. Systemic examination was unremarkable.

Laboratory investigations revealed normal routine hematological and biochemical parameters, except for serum liver aminotransferases. The results of other laboratory evaluations are shown in Table 1. Abdominal ultrasound showed normal morphology of kidneys, a liver span of 12 cm (normal 6.3-9.6 cm) and features of hepatic steatosis. Magnetic resonance imaging of the brain, with fine cuts through the pituitary and hypothalamus, showed no abnormality. In view of intense hyperphagia followed by

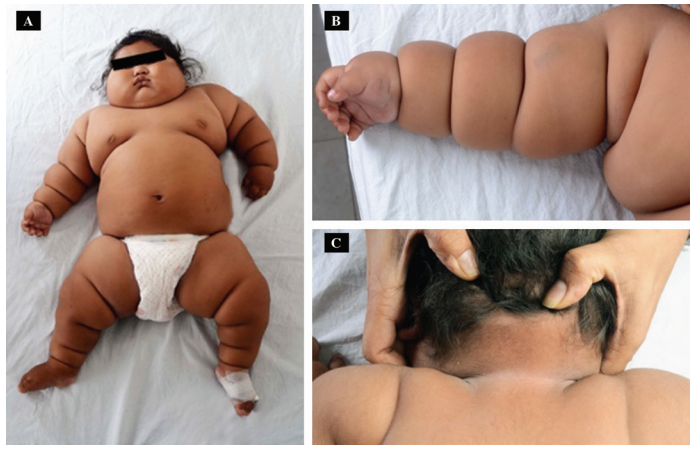

Figure 1. Clinical photographs of the patient showing A) generalized body fat distribution, B) deep skin folds and C) acanthosis nigricans

Table 1. Hormonal, metabolic and other laboratory results of the patient at presentation

Variable	Patient's value	Reference range
Fasting blood glucose (mg/dL)	92	70-100
Fasting C-peptide (ng/mL)	4.08	1.1-4.4
Fasting plasma insulin (mIU/L)	18.56	2.6-24.9
HbA1c (%)	5.4	4.0-5.8
Plasma cortisol (nmol/L)	473	171-536
Plasma ACTH (pg/mL)	8.0	5.0-60
Triiodothyronine (ng/mL)	**2.56**	0.8-2.0
Thyroxine (µg/dL)	11.20	4.8-12.7
TSH (µIU/mL)	5.23	0.27-4.2
Random growth hormone (ng/mL)	0.44	0-2.5
Luteinizing hormone (mIU/mL)	0.100	< 1-3.3
Follicle-stimulating hormone (mIU/mL)	3.38	< 1-7.1
Estradiol (pmol/L)	5.0	0- < 73.5
Testosterone (nmol/L)	0.087	< 1.0
25-hydroxy vitamin D (ng/mL)	**7.87**	20.0-40.0
Parathyroid hormone (pg/mL)	27.71	15-65
Total cholesterol (mg/dL)	**129.2**	150-200
Low-density lipoprotein cholesterol (mg/dL)	51.7	0-130
High-density lipoprotein cholesterol (mg/dL)	**30.0**	35-55
Serum leptin (ng/mL)	**1.25**	2.0-5.6
Serum adiponectin (mg/mL)	6.2	5.0-7.5
Aspartate aminotransferase (U/L)	**53**	3-30
Alanine aminotransferase (U/L)	**89**	7-45

HbA1c: haemoglobin A1C, ACTH: adrenocorticotropic hormone, TSH: thyroid-stimulating hormone

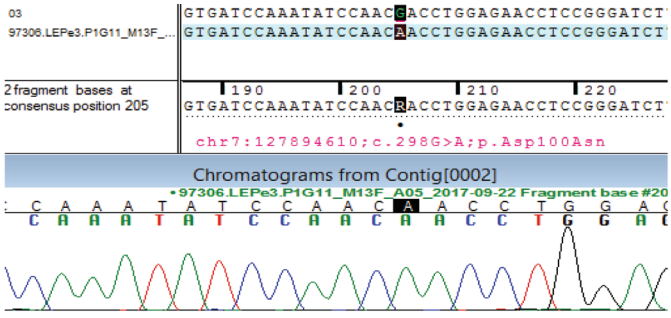

Figure 2. Sequence chromatogram showing homozygous missense mutation in exon 3 of the leptin gene (chr7:127894610;c.298G > A) resulting in the amino acid substitution of asparagine for aspartic acid at codon 100 (p.Asp100Asn)

rapid weight gain, early age of onset, family history of EOO and low level of circulating leptin, a diagnosis of monogenic obesity due to *LEP* gene mutation was considered. Written informed consent was obtained from the parents for conducting all laboratory studies and for publishing clinical information and photographs.

For genetic studies, genomic DNA extracted from blood was used to perform targeted gene capture using a custom capture kit on Illumina HiSeq 2000 sequencing platform (Illumina, California, USA). Sequencing identified a homozygous missense mutation in exon 3 of the *LEP* gene (chr7:127894610;c.298G > A) resulting in the amino acid substitution of asparagine for aspartic acid at codon 100 (p.Asp100Asn). Validation of the identified mutation was done by Sanger sequencing to exclude false positivity (Figure 2). The Asp100Asn variant lies in the functional domain of the leptin protein and has not been reported in the 1000 Genomes database. It has a minor allele frequency of 0.0008 % in the Exome Aggregation Consortium (ExAC) database. The *in silico* predictions of the effect of the mutation are "probably pathogenic" by Polyphen-2 (HumDiv) and "pathogenic" by Sorting Intolerant From Tolerant, Log ratio test and MutationTaster2. The reference codon is conserved across species.

Sequencing also revealed a homozygous missense variation in exon 11 of Bardet-Biedl syndrome-1 (*BBS1*) gene (chr11:66291279; G > A; Depth 168x) resulting in amino acid substitution of isoleucine for valine at codon 346 (p.Val346Ile). This variant has a minor allele frequency of 0.16 % and 0.1 % in the 1000 Genomes and ExAC databases respectively. The *in silico* prediction of the effect of the mutation is "pathogenic" by only MutationTaster2. The reference codon is conserved across mammals. This *BBS1* mutation is classified as a variant of uncertain significance based on the above evidence. Sanger sequencing of exon 3 of the *LEP* gene and exon 11 of *BBS1* gene of the unaffected parents identified the same variations as in the index patient but with heterozygous inheritance.

Discussion

The majority of children with EOO have simple obesity (18). However, about 5 % of all children with EOO may have monogenic obesity caused by mutations in one of the several genes involved in the regulation of appetite and body weight (1). Even rarer are the syndromic forms of EOO such as BBS, Prader-Willi syndrome and Beckwith-Wiedemann syndrome caused by genetic, epigenetic and genomic alterations (1). The most common and treatable form of monogenic obesity is due to mutations in the *LEP* gene manifesting as hyperphagia and rapid weight gain starting from early infancy (4). The clinical manifestations in the index patient were similar to the previously reported patients (4,5,6,7,8). Additionally, our patient exhibited severe abnormalities of lipid parameters, usually found in patients with congenital leptin deficiency during late childhood or even adulthood, at 10 months of age (8). However, other common obesity-associated complications such as abnormalities of glucose homeostasis and blood pressure were not detected in our patient (2,8). The mild elevation of serum aminotransferases was possibly related to hepatic steatosis commonly seen in patients with obesity and dyslipidemia (19).

The mutation (chr7:127894610;c.298G > A) in our patient that led to amino acid substitution of asparagine for aspartic acid at codon 100, has not been described previously. However, a different missense variation (Asp100Tyr) affecting the same codon has been reported (13). Interestingly, the affected patient had high circulating levels of mutant leptin (functional studies showed that leptin was biologically inactive) (13), unlike the characteristically absent or nearly absent circulating leptin in *LEP* gene mutations (5,6,7,8).We presume that the low serum leptin concentrations secondary to the mutated *LEP* gene resulted in severe hyperphagia and severe EOO in our patient. The serum concentrations of leptin were even lower than the recent local normative data for children (mean serum levels 1.4 ± 0.5, range 1.04-3.71 ng/mL) (20).

Leptin is an important afferent, peripheral, humoral signal to the appetite-regulating network in the hypothalamus and affects food intake and energy expenditure. It is an important predictor of weight gain even during early infancy (21). Therefore, low levels of leptin or its biological inactivity resulting from mutations in *LEP* may disturb metabolic balance, leading to severe obesity and related metabolic disorders. Leptin replacement normalises these hormonal

and metabolic alterations, suggesting that leptin deficiency or inactivity is the predominant determinant of obesity associated disorders in these patients (3,9,13).

The finding of *BBS1* gene mutation in our patient is intriguing. BBS is a known cause of a syndromic form of EOO and BBS proteins are required for *LEPR* signalling (22). However, leptin resistance rather than leptin deficiency is the characteristic finding in obese patients with BBS (23). The obesity usually manifests by 2-3 years of age, unlike that found in patients with *LEP* gene mutation which manifests in early infancy (22,23). Furthermore, our patient did not show the usual BBS stigmata, such as retinitis pigmentosa, kidney dysfunction, polydactyly, behavioural problems and hypogonadism (22). Low levels of circulating leptin and compromised leptin signalling may account for the extreme obesity seen in this case.

A significant majority of the previously reported patients belong to consanguineous families of the Arain tribe, who live in the Central Punjab, Pakistan (5,6,7). Incidentally, our patient hails from a geographical area in Indian Punjab approximately 30 miles away from this location, where most patients so far described live (5,6,7). Although families of the Arain community have a scattered presence across Northern India, including Punjab, their consanguinity rates are lower. Our patient does not belong to the Arain community, although third degree consanguinity was present. The first reported patient from India also came from North India (16). The geographic location points to either the operation of natural selection (carrier advantage) or random genetic drift (chance founder effects) for *LEP* gene in this population.

The limitations of our study include the lack of functional studies to understand the mechanism of disease manifestations in the patient. Also, we could not screen other affected family members for the mutation detected in our patient.

In summary, we report an infant with congenital leptin deficiency due to a novel mutation of the *LEP* gene, manifesting as severe EOO and dyslipidemia. This is only the second case from India with *LEP* gene mutation in the published literature. In patients with EOO, identifying those with *LEP* gene mutations is important, as recombinant human leptin therapy offers substantial clinical benefits in these patients.

Ethics

Informed Consent: Written informed consent was obtained from the parents.

Peer-review: Externally peer-reviewed.

Authorship Contribution

Concept: Devi Dayal, Design: Devi Dayal, Data Collection or Processing: Keerthivasan Seetharaman, Balasubramaniyan Muthuvel, Ashish Agarwal, Analysis or Interpretation: Devi Dayal, Inusha Panigrahi, Literature Search: Devi Dayal, Keerthivasan Seetharaman, Writing: Devi Dayal.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Serra-Juhé C, Martos-Moreno GÁ, Bou de Pieri F, Flores R, González JR, Rodríguez-Santiago B, Argente J, Pérez-Jurado LA. Novel genes involved in severe early-onset obesity revealed by rare copy number and sequence variants. PLoS Genet 2017;13:e1006657.
2. Dayal D, Jain H, Attri SV, Bharti B, Bhalla AK. Relationship of high sensitivity C-reactive protein levels to anthropometric and other metabolic parameters in Indian children with simple overweight and obesity. J Clin Diag Res 2014;8:5-8. Epub 2014 Aug 20
3. Farooqi IS, Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C, Sanna V, Jebb SA, Perna F, Fontana S, Lechler RI, DePaoli AM, O'Rahilly S. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J Clin Invest 2002;110:1093-1103.
4. Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA, Cheetham CH, Earley AR, Barnett AH, Prins JB, O'Rahilly S. Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 1997;387:903-908.
5. Fatima W, Shahid A, Imran M, Manzoor J, Hasnain S, Rana S, Mahmood S. Leptin deficiency and leptin gene mutations in obese children from Pakistan. Int J Pediatr Obes 2011;6:419-427. Epub 2011 Aug 19
6. Saeed S, Butt TA, Anwer M, Arslan M, Froguel P. High prevalence of leptin and melanocortin-4 receptor gene mutations in children with severe obesity from Pakistani consanguineous families. Mol Genet Metab 2012;106:121-126. Epub 2012 Mar 10
7. Saeed S, Bonnefond A, Manzoor J, Shabir F, Ayesha H, Philippe J, Durand E, Crouch H, Sand O, Ali M, Butt T, Rathore AW, Falchi M, Arslan M, Froguel P. Genetic variants in LEP, LEPR, and MC4R explain 30% of severe obesity in children from a consanguineous population. Obesity (Silver Spring) 2015;23:1687-1695. Epub 2015 Jul 14
8. Shabana, Hasnain S. The p. N103K mutation of leptin (LEP) gene and severeearlyonsetobesity in Pakistan. Biol Res 2016;49:23.
9. Paz-Filho G, Mastronardi C, Delibasi T, Wong ML, Licinio J. Congenital leptin deficiency: diagnosis and effects of leptin replacement therapy. Arq Bras Endocrinol Metabol 2010;54:690-697.
10. Andiran N, Celik N, Andiran F. Homozygosity for two missense mutations in the leptin receptor gene (P316:W646C) in a Turkmenian girl with severe early-onset obesity. J Pediatr Endocrinol Metab 2011;24:1043-1045.
11. Mazen I, El-Gammal M, Abdel-Hamid M, Amr K. A novel homozygous missense mutation of the leptin gene (N103K) in an obese Egyptian patient. Mol Genet Metab 2009;97:305-308. Epub 2009 Apr 9
12. Mazen I, Amr K, Tantawy S, Farooqi IS, El Gammal M. A novel mutation in the leptin gene (W121X) in an Egyptian family. Mol Genet Metab Rep 2014;1:474-476.

13. Wabitsch M, Funcke JB, Lennerz B, Kuhnle-Krahl U, Lahr G, Debatin KM, Vatter P, Gierschik P, Moepps B, Fischer-Posovszky P. Biologically inactive leptin and early-onset extreme obesity. N Engl J Med 2015;372:48-54.

14. Altawil AS, Mawlawi HA, Alghamdi KA, Almijmaj FF. A Novel Homozygous Frameshift Mutation in Exon 2 of LEP Gene Associated with Severe Obesity: A Case Report. Clin Med Insights Pediatr 2016;10:115-118.

15. Fischer-Posovszky P, von Schnurbein J, Moepps B, Lahr G, Strauss G, Barth TF, Kassubek J, Mühleder H, Möller P, Debatin KM, Gierschik P, Wabitsch M. A new missense mutation in the leptin gene causes mild obesity and hypogonadism without affecting T cell responsiveness. J Clin Endocrinol Metab 2010;95:2836-2840. Epub 2010 Apr 9

16. Thakur S, Kumar A, Dubey S, Saxena R, Peters AN, Singhal A. A novel mutation of the leptin gene in an Indian patient. Clin Genet 2014;86:391-393. Epub 2013 Dec 5

17. Zhao Y, Hong N, Liu X, Wu B, Tang S, Yang J, Hu C, Jia W. A novel mutation in leptin gene is associated with severe obesity in Chinese individuals. Biomed Res Int 2014;2014:912052. Epub 2014 Feb 23

18. Dayal D, Soni V, Das G, Bhunwal S, Kaur H, Bhalla AK. Longitudinal observations on growth patterns of obese infants: Developing country perspectives. Preliminary study. Pediatria Polska 2017;92:397-400.

19. Kamble R, Sodhi KS, Thapa BR, Saxena AK, Bhatia A, Dayal D, Khandelwal N. Liver acoustic radiation force impulse (ARFI) in childhood obesity: comparison and correlation with biochemical markers. J Ultrasound 2016;20:33-42.

20. Tenneti N, Dayal D, Sharda S, Panigrahi I, Didi M, Attri SV, Sachdeva N, Bhalla AK. Concentrations of leptin, adiponectin and other metabolic parameters in non-obese children with Down syndrome. J Pediatr Endocrinol Metab 2017;30:831-837.

21. Treviño-Garza C, Estrada-Zúñiga CM, Mancillas-Adame L, Villarreal-Martínez L, Villarreal-Pérez JZ, Rodríguez-Balderrama I, Montes-Tapia FF, de la O Cavazos ME. Adding Multiple Adipokines into the Model do not Improve Weight Gain Prediction by Leptin Levels in Newborns. J Clin Res Pediatr Endocrinol 2016;8:321-324. Epub 2016 Apr 18

22. Priya S, Nampoothiri S, Sen P, Sripriya S. Bardet-Biedl syndrome: Genetics, molecular pathophysiology, and disease management. Indian J Ophthalmol 2016;64:620-627.

23. Feuillan PP, Ng D, Han JC, Sapp JC, Wetsch K, Spaulding E, Zheng YC, Caruso RC, Brooks BP, Johnston JJ, Yanovski JA, Biesecker LG. Patients with Bardet-Biedl Syndrome Have Hyperleptinemia Suggestive of Leptin Resistance. J Clin Endocrinol Metab 2011;96:528-535. Epub 2011 Jan 5

CASE REPORT

DOI: 10.4274/jcrpe.5335

J Clin Res Pediatr Endocrinol 2018;10(3):279-283

Sirolimus-Induced Hepatitis in Two Patients with Hyperinsulinemic Hypoglycemia

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What is already known on this topic?

Sirolimus is an alternative for the treatment of congenital hyperinsulinism unresponsive to diazoxide and octreotide.

What this study adds?

This is the first report of sirolimus-induced hepatitis in pediatric patients with hyperinsulinemic hypoglycemia.

Abstract

Sirolimus has been reported to be effective in the treatment of the diffuse form of congenital hyperinsulinism (CHI), unresponsive to diazoxide and octreotide, without causing severe side effects. Two newborns with CHI due to homozygous *ABCC8* gene mutations were started on sirolimus aged 21 and 17 days, due to lack of response to medical treatment. A good response to sirolimus was observed. At follow-up after ten and two months of treatment, liver enzymes were found to be increased [serum sirolimus level 1.4 ng/mL (normal range: 5-15), aspartate aminotransferase (AST): 298U/L, alanine aminotransferase (ALT): 302U/L and serum sirolimus level: 9.9 ng/mL, AST: 261U/L, ALT: 275U/L, respectively]. In Case 1, discontinuation of the drug resulted in normalization of liver enzymes within three days. Two days after normalization, sirolimus was restarted at a lower dose, which resulted in a repeated increase in transferases. In Case 2, a reduction of sirolimus dose caused normalization of liver enzymes within ten days. When the dose was increased, enzymes increased within three days. Sirolimus was discontinued in both cases.

The rapid normalization of liver enzyme levels after sirolimus withdrawal or dose reduction; elevation of transaminases after restart or dose increase and rapid normalization after sirolimus withdrawal were findings strongly suggestive of sirolimus-induced hepatitis.

To the best of our knowledge, this is the first report of sirolimus-induced hepatitis in CHI. Sirolimus is a promising drug for CHI patients who are unresponsive to medical treatment, but physicians should be vigilant for adverse effects on liver function.

Keywords: Hyperinsulinemic hypoglycemia, sirolimus, hepatitis, liver enzymes

Introduction

Congenital hyperinsulinism (CHI) is characterized by inappropriate insulin secretion despite hypoglycemia. It is a heterogeneous disorder with the clinical manifestations ranging from severe hypoglycemia in the newborn period to mild hypoglycemia in childhood (1,2). The incidence is approximately 1:30.000 live births but is increased in populations with a high prevalence of consanguinity (3). Most cases of CHI are caused by autosomal recessive mutations in the *ABCC8* and *KCNJ11* genes (1).

Historically the treatment of severe, diffuse CHI, unresponsive to diazoxide and octreotide was subtotal pancreatectomy. This surgery has been associated with a high incidence of insulin-dependent diabetes, persistent hypoglycemia and exocrine pancreatic insufficiency (4). As a novel agent, the mammalian target of rapamycin

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Conflict of interest: None declared
Received: 15.09.2017
Accepted: 29.11.2017

(mTOR) inhibitor, sirolimus, has been recommended for the treatment of the diffuse form of CHI, unresponsive to diazoxide and octreotide. It has been reported to be a safe agent in pediatric cases (5,6,7). Herein, we report two cases of diazoxide and octreotide unresponsive CHI, due to homozygous *ABCC8* gene mutations in which sirolimus had to be discontinued because of drug related hepatotoxicity.

Case Reports

Case 1

A female infant presented with severe hypoglycemia on the first day of life. CHI was diagnosed based on laboratory findings. She was normoglycemic with intravenous (iv) glucose, diazoxide, iv glucagon and octreotide on day 16 but the reduction in glucose requirement was not successful during the next five days (Table 1). She also had congenital hypothyroidism with normal thyroid ultasonography (TSH: >100 uIU/mL, sT4:0.7 ng/dL) and was euthyroid with L-thyroxine (12 mcg/kg/day).

18F-DOPA positron emission tomography/computed tomography (PET/CT) scanning could not be performed but sequence analysis identified a novel homozygous p.H59P (c.176A>C) missense mutation in the proband's *ABCC8* gene. *In silico* analysis predicted the variant was likely to be pathogenic and that the affected residue was highly conserved across species (Alamut, Rouen, France). The identification of a recessively inherited ABCC8 mutation in the patient was consistent with diffuse pancreatic disease.

Table 1. The clinical features of the patients before sirolimus

	Case 1	Case 2
Sex	Female	Female
Birth weight	3300 g	3100 g
Gestation week	39 wks	36 wks
Blood glucose level (mg/dL)	26	20
Blood insulin level (mIU/mL)	55	43
Genetic result	*ABCC8* gene	*ABCC8* gene
	p.H59P homozygote	p.A1185E homozygote
Treatment before sirolimus		
GPR	18 mg/kg/min	11 mg/kg/min
Diazoxide	15 mg/kg/d	20 mg/kg/d
Octreotide	40 mcg/kg/d	40 mcg/kg/d
Glucagon	0.01 mg/kg/h	
Age at sirolimus treatment	Postnatal 21st day	Postnatal 17th day

Min: minute

After consent from the parents, sirolimus was started at a dose of 0.5 mg/m^2/day on day 21. The serum level of sirolimus and laboratory tests (full blood count, kidney and liver function tests, lipid profile, electrolytes) were checked every five days, to maintain the serum sirolimus concentration between 5-15 ng/dL. The patient was discharged on day 72 with oral feeding, subcutaneous octreotide (40 mcg/kg/d) and oral sirolimus (3 mg/m^2/day). The sirolimus level and biochemical markers were checked at monthly intervals.

Since she was normoglycemic, the octreotide dose was decreased during follow-up. At the age of 10 months the patient presented with diarrhea. At this time, she was being treated with octreotide (6 mcg/kg/d) and sirolimus (3.1 mg/m^2/day) and was normoglycemic. Her laboratory tests revealed elevated liver enzymes (Table 2). The coagulation tests, bilirubin levels, alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT) and abdominal ultrasound results were all normal. Although the sirolimus level was below the therapeutic range (1.4 ng/mL), it was discontinued due to its known hepatotoxic effect. The liver enzyme

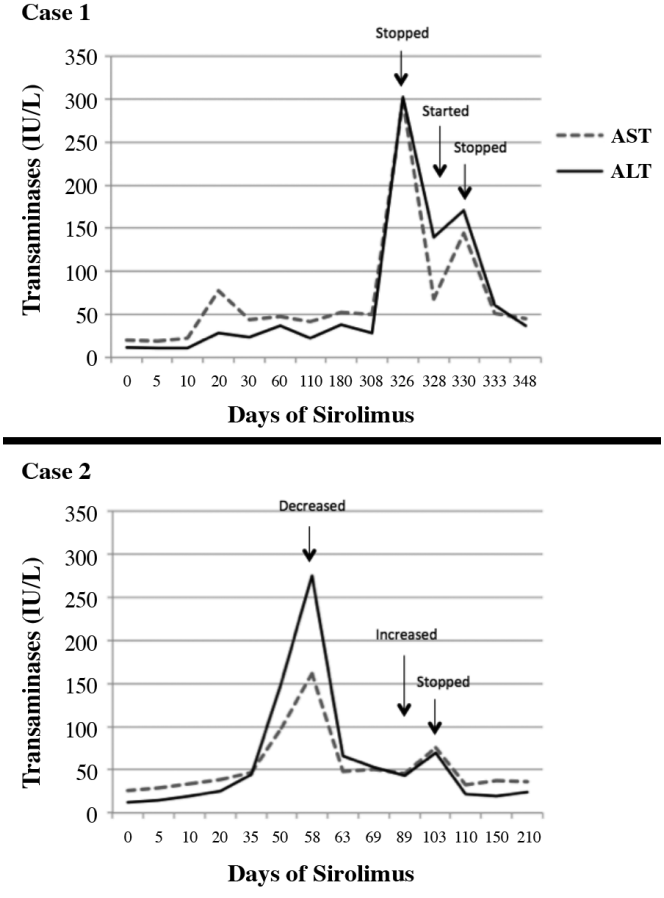

Figure 1. Liver enzyme levels (AST: aspartate aminotransferase, ALT: alanine aminotransferase) of Case 1 and Case 2 during sirolimus treatment and immediately after cessation

levels during dose adjustments are shown in the Figure 1. After sirolimus was discontinued, the octreotide dose was increased to 45 mcg/kg/d to achieve normoglycemia and four months later the patient was switched to octreotide-long-acting release (LAR). She is currently 18 months of age with normal neuromotor development and normoglycemia, treated solely with octreotide-LAR (15 mg/monthly, 41 mcg/kg/d) and oral feedings with three hourly intervals. Her most recent HbA1c level was 4.9% (30 mmol/mol) and also, she is euthyroid on L-thyroxine treatment.

Case 2

This female infant was referred to our clinic on day 14 of life with CHI resistant to medical therapy (Table 1) and the reduction in glucose requirement was not successful. She had also congenital hypothyroidism with normal thyroid ultrasound (TSH: >100 uIU/mL, sT4:0.9 ng/dL) and was euthyroid with L-thyroxine (8 mcg/kg/d).

Sequence analysis identified a previously reported homozygous missense mutation, p.A1185E (c.3554C>A), in ABCC8 (8). The presence of a homozygous mutation in the patient was in keeping with diffuse pancreatic disease. After consent from the parents was obtained, sirolimus (0.5 mg/m^2/day) was added, due to no reduction in the glucose requirement by day 17. Serum levels of sirolimus were checked every five days to maintain a therapeutic serum level as before. Neither clinical nor laboratory side effects were observed. She was discharged at age 40 days with sirolimus 0.4 mg/m^2/day and octreotide 23 mcg/kg/d.

One month later, routine blood tests for side effects revealed elevated liver enzymes (Table 2) without any clinical symptoms. Sirolimus level at this time was 9.9 ng/mL in the middle of the therapeutic range. All other laboratory tests (blood count, kidney function tests, ALP, GGT, bilirubin levels) and abdominal ultrasound revealed normal results. The liver enzyme levels during dose adjustments are shown in Figure 1. As sirolimus was discontinued, the dose of octreotide was increased from 10 to 45 mcg/kg/d. Although, the glucose levels were generally close to the lower limit of normal, with frequent oral feedings and applying a maximum dose of octreotide, we were able to protect the patient from severe hypoglycemia (a glucose level <50 mg/dL). Subcutaneous octreotide was switched to octreotide-LAR five months later. The patient is currently 13 months of age and normoglycemic with octreotide-LAR (15 mg/monthly, 45 mcg/kg/d) and oral feedings at 4 hours intervals. Last HbA1c is 4.2% (22 mmol/mol) and also, she is euthyroid on L-thyroxine treatment.

Discussion

The aim of treatment in CHI is to achieve normoglycemia and to prevent neurological damage. However, the clinical management of severe, diffuse CHI, unresponsive to medical treatment is still a vexing clinical problem (4). In a recent study, mTOR inhibitor, sirolimus, has been reported to be a novel agent for the treatment of diazoxide unresponsive CHI. Therapy with sirolimus achieved normoglycemia with no major adverse effect in four cases (5). We now report two further cases with severe CHI due to a homozygous ABCC8 mutation. Both were successfully treated with sirolimus consistent with previous reports, but sirolimus had to be discontinued because of drug-induced hepatitis.

In adult studies, various side effects of mTOR inhibitors have been reported which include bone marrow suppression, dyslipidemia, immunosuppression, elevation of liver enzymes, renal dysfunction, pneumonitis and stomatitis. These were reversible with dose reduction (9,10). In children, this drug was reported to be well tolerated in several studies with normal or high doses (1-6 mg/m^2/d) (11,12,13). The main side effect reported in these studies was oral mucositis. However, in a recent study, Szymanowski et al (14) investigated the efficacy and adverse effect profile of sirolimus in the treatment of severe CHI. These authors detected adverse events such as hypertriglyceridemia, anemia, stomatitis, sepsis, varicella zoster and gut dysmotility in 80% of their patients, but also reported a 30% therapeutic success rate.

Hepatotoxicity is another known side effect of sirolimus, resulting in transient and mild increase in liver enzymes. Its incidence was reported to be 17% in patients with

Table 2. The liver enzyme levels of the patients during hepatotoxic period of sirolimus

	Case 1				Case 2			
	Day 0	**Day 3**	**Day 5**	**Day 7**	**Day 0**	**Day 10**	**Day 32**	**Day 35**
AST (0-40 U/L)	298	68	144	51	261	32	46	76
ALT (0-40 U/L)	302	140	171	61	275	35	43	80
Sirolimus dose	3.1	2	Stopped		0.4	0.2	0.3	Stopped
(mg/m^2/d)	Stopped	Restarted			Decreased		Increased	

AST: aspartate aminotransferase, ALT: alanine aminotransferase

renal transplant (15). Senniappean et al (5) and Méder et al (6) reported mild, transient elevation of liver enzyme concentrations. These increases were less than double the normal range and resolved spontaneously or with reduction in sirolimus dose (5,6). Although sirolimus appears to be safe in terms of hepatotoxicity, cases with severe sirolimus-induced hepatitis have been reported. One report was that of a patient with renal transplantation who received sirolimus as an initial immunosuppressive in the post-transplant period (16). At the 16th month post-transplant, increased liver enzyme levels were detected [maximum aspartate aminotransferase (AST): 368 IU/L, alanine aminotransferase (ALT): 579 IU/L] with a serum sirolimus level of 6.3 ng/dL. After sirolimus withdrawal, quick normalization of aminotransferases was observed. Jacques et al (17) reported another case with renal transplantation. In the second month of sirolimus, biochemical tests showed acute hepatitis (AST: 861 IU/l, ALT: 609 IU/L) with signs of hepatic insufficiency. The serologic and autoimmune markers for hepatitis were normal. Despite a normal sirolimus level (10 ng/mL), it was withdrawn and transaminase levels normalized within five weeks. In our two cases, after sirolimus was discontinued in one case and decreased in the other, the normalization of transaminases was observed within a few days.

While octreotide is usually well tolerated in most patients with CHI, octreotide induced hepatitis has been reported in a few patients (18,19,20,21). Hepatitis was found to develop even with doses within the normal range, but the withdrawal of octreotide resulted in resolution. The rapid normalization of liver enzyme levels after sirolimus withdrawal and dose reduction, in our first and second case respectively, followed by elevation of transaminases after restart or dose increase and rapid normalization after sirolimus was again withdrawn while the patient continued with octreotide treatment provides robust evidence of sirolimus-induced hepatitis.

Fortunately, both patients are now normoglycemic with octreotide-LAR and frequent feedings. This observation suggests that this entity may tend to become milder over time. It also suggests that a good response to octreotide-LAR may be expected as the patients get older.

Octreotide may affect thyroid hormones and may cause hypothyroidism with a concomitant low TSH level. However, hypothyroidism with elevated TSH levels was also reported in two cases with octreotide treated CHI due to *ABCC8* gene mutation (19,20). Similarly, our cases had elevated TSH levels with a low free thyroxine that is a characteristic finding for primary hypothyroidism. This is most probably a coincidental finding since patients on octreotide therapy usually develop central hypothyroidism marked by low TSH. Further tests will be done for the etiology of primary hypothyroidism in later years.

In conclusion, sirolimus is a promising drug for diazoxide and octreotide unresponsive CHI patients, but physicians should be vigilant for its adverse effects which may necessitate the withdrawal of the drug.

Ethics

Informed Consent: Informed consent was taken from the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Belma Haliloğlu, Muhittin Çelik, Heybet Tüzün, Mehmet Nuri Özbek, Concept: Belma Haliloğlu, Design: Belma Haliloğlu, Data Collection or Processing: Belma Haliloğlu, Heybet Tüzün, Mehmet Nuri Özbek, Avni Kaya, Analysis or Interpretation: Belma Haliloğlu, Sian Ellard, Sarah E. Flanagan, Literature Search: Belma Haliloğlu, Writing: Belma Haliloğlu, Sarah E. Flanagan.

Financial Disclosure: The genetic studies were funded by the Medical Research Council (grant number: 98144). Sarah E. Flanagan has a Sir Henry Dale Fellowship jointly funded by the Wellcome Trust and the Royal Society (grant number: 105636/Z/14/Z).

References

1. Flanagan SE, Kapoor RR, Hussain K. Genetics of congenital hyperinsulinemic hypoglycemia. Semin Pediatr Surg 2011;20:13-17.
2. de Lonlay P, Fournet JC, Touati G, Groos MS, Martin D, Sevin C, Delagne V, Mayaud C, Chigot V, Sempoux C, Brusset MC, Laborde K, Bellane-Chantelot C, Vassault A, Rahier J, Junien C, Brunelle F, Nihoul-Fékété C, Saudubray JM, Robert JJ. Heterogeneity of persistent hyperinsulinaemic hypoglycaemia. A series of 175 cases. Eur J Pediatr 2002;161:37-48.
3. Arnoux JB, Verkarre V, Saint-Martin C, Montravers F, Brassier A, Valayannopoulos V, Brunelle F, Fournet JC, Robert JJ, Aigrain Y, Bellanné-Chantelot C, de Lonlay P. Congenital hyperinsulinism: current trends in diagnosis and therapy. Orphanet J Rare Dis 2011;6:63.
4. Güemes M, Hussain K. Hyperinsulinemic hypoglycemia. Pediatr Clin of North Am 2015;62:1017-1036. Epub 2015 May 13
5. Senniappan S, Alexandrescu S, Tatevian N, Shah P, Arya V, Flanagan S, Ellard S, Rampling D, Ashworth M, Brown RE, Hussain K. Sirolimus therapy in infants with severe hyperinsulinemic hypoglycemia. N Engl J Med 2014;370:1131-1137.
6. Méder Ü, Bokodi G, Balogh L, Körner A, Szabó M, Pruhova S, Szabo AJ. Severe Hyperinsulinemic Hypoglycemia in a Neonate: Response to Sirolimus Therapy. Pediatrics 2015;136:1369-1372.
7. Abraham MB, Shetty VB, Price G, Smith N, Bock Md, Siafarikas A, Resnick S, Whan E, Ellard S, Flanagan SE, Davis EA, Jones TW, Hussain K, Choong CS. Efficacy and safety of sirolimus in a neonate with persistent hypoglycaemia following near-total pancreatectomy for hyperinsulinaemic hypoglycaemia. J Pediatr Endocrinol Metab 2015;28:1391-1398.

8. Arya VB, Guemes M, Nessa A, Alam S, Shah P, Gilbert C, Senniappan S, Flanagan SE, Ellard S, Hussain K. Clinical and histological heterogeneity of congenital hyperinsulinism due to paternally inherited heterozygous ABCC8/KCNJ11 mutations. Eur J Endocrinol 2014;171:685-695. Epub 2014 Sep 8

9. Ventura-Aguiar P, Campistol JM, Diekmann F. Safety of mTOR inhibitors in adult solid organ transplantation. Expert Opin Drug Saf 2016;15:303-319. Epub 2016 Jan 28

10. Sankhala K, Mita A, Kelly K, Mahalingam D, Giles F, Mita M. The emerging safety profile of mTOR inhibitors, a novel class of anticancer agents. Target Oncol 2009;4:135-142. Epub 2009 Apr 21

11. Nadal M, Giraudeau B, Tavernier E, Jonville-Bera AP, Lorette G, Maruani A. Efficacy and Safety of Mammalian Target of Rapamycin Inhibitors in Vascular Anomalies: A Systematic Review. Acta Derm Venereol 2016;96:448-452.

12. Zou L, Liu Y, Pang L, Ju J, Shi Z, Zhang J, Chen X, Su X, Hu L, Shi X, Yang X. Efficacy and safety of rapamycin in treatment of children with epilepsy complicated with tuberous sclerosis. Zhonghua Er Ke Za Zhi 2014;52:812-816.

13. Schachter AD, Benfield MR, Wyatt RJ, Grimm PC, Fennell RS, Herrin JT, Lirenman DS, McDonald RA, Munoz-Arizpe R, Harmon WE. Sirolimus pharmacokinetics in pediatric renal transplant recipients receiving calcineurin inhibitor co-therapy. Pediatr Transplant 2006;10:914-919.

14. Szymanowski M, Estebanez MS, Padidela R, Han B, Mosinska K, Stevens A, Damaj L, Pihan-Le Bars F, Lascouts E, Reynaud R, Ferreira C, Bansept C, de Lonlay P, Saint-Martin C, Dunne MJ, Banerjee I, Arnoux JB. mTOR Inhibitors for the Treatment of Severe Congenital Hyperinsulinism: Perspectives on Limited Therapeutic Success. J Clin Endocrinol Metab 2016;101:4719-4729. Epub 2016 Oct 3

15. Groth CG, Backman L, Morales JM, Calne R, Kreis H, Lang P, Touraine JL, Claesson K, Campistol JM, Durand D, Wramner L, Brattström C, Charpentier B. Sirolimus (rapamycine)- based therapy in human renal transplantation: similar efficacy and different toxicity compared with cyclosporine. Sirolimus European Renal Transplant Study Group. Transplantation 1999;67:1036-1042.

16. Niemczyk M, Wyzgał J, Perkowska A, Porowski D, Paczek L. Sirolimus-associated hepatotoxicity in the kidney graft recipient. Transpl Int 2005;18:1302-1303.

17. Jacques J, Dickson Z, Carrier P, Essig M, Guillaudeau A, Lacour C, Bocquentin F, Aldigier JC, Rerolle JP. Severe sirolimus-induced acute hepatitis in a renal transplant recipient. Transpl Int 2010;23:967-970. Epub 2010 May 20

18. Avatapalle B, Padidela R, Randell T, Banerjee I. Drug-induced hepatitis following use of octreotide for long-term treatment of congenital hyperinsulinism. BMJ Case Rep 2012:2012.

19. Levy-Khademi F, Irina S, Avnon-Ziv C, Levmore-Tamir M, Leder O. Octreotide-associated cholestasis and hepatitis in an infant with congenital hyperinsulinism. J Pediatr Endocrinol Metab 2015;28:449-451.

20. Koren I, Riskin A, Barthlen W, Gillis D. Hepatitis in an infant treated with octreotide for congenital hyperinsulinism. J Pediatr Endocrinol Metab 2013;26:183-185.

21. Ben-Ari J, Greenberg M, Nemet D, Edelstein E, Eliakim A. Octreotide-induced hepatitis in a child with persistent hyperinsulinemia hypoglycemia of infancy. J Pediatr Endocrinol Metab 2013;26:179-182.

CASE REPORT

DOI: 10.4274/jcrpe.5188

J Clin Res Pediatr Endocrinol 2018;10(3):284-288

A Novel Variant c.97C>T of the Growth Hormone Releasing Hormone Receptor Gene Causes Isolated Growth Hormone Deficiency Type Ib

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What is already known on this topic?

Isolated growth hormone deficiency (IGHD) is a sporadic disease with insufficient or deficient production of growth hormone (GH). IGHD type 1b is caused by mutations in either the *GH-1* gene or the growth hormone releasing hormone receptor gene.

What this study adds?

We report a previously undescribed genetic defect, the c.97C>T variant of the growth hormone releasing hormone receptor gene, which results in severe growth retardation, approaching growth arrest, in the homozygous state. The present case provides new data on genetic causes of isolated growth hormone deficiency type 1b and describes the phenotype of this novel mutation.

Abstract

Congenital isolated growth hormone deficiency (IGHD) type 1b is an autosomal recessive genetic condition caused by mutations of growth hormone (GH)-1 or the growth hormone releasing hormone receptor (*GHRH-R*) genes. Affected subjects present with symptoms of growth hormone deficiency (GHD) with low but detectable levels of growth hormone (GH), short stature and responsiveness to GH therapy. We describe a 13-month old girl with severe growth failure who showed a low GH response to two GH provocation tests and a modest increase of insulin-like growth factor-1 (IGF-1) to an IGF-1 generation test. Whole exome sequencing revealed a novel homozygous variant of the *GHRH-R* gene (c.97C>T), leading to a premature stop codon. Administration of recombinant human GH improved linear growth. This is the first report of a c.97C>T mutation of the *GHRH-R* gene.

Keywords: Congenital isolated growth hormone deficiency, growth hormone releasing hormone receptor, failure to thrive, short stature

Introduction

Isolated growth hormone deficiency (IGHD) is a sporadic disease with a prevalence ranging from 1:3480 to 1:10 000 live births (1). It is defined as an insufficient or deficient production of growth hormone (GH) by the pituitary gland. Its complex etiology involves a spectrum of hypothalamic defects, pituitary abnormalities or combined conditions, which can be structurally detected by brain imaging in only 26.8% of affected patients (2).

Familial IGHD is classified into four distinct types with different clinical manifestation and inheritance patterns. The two most frequent types of IGHD are types 1a and 1b, characterized by an autosomal recessive trait; type 2 is transmitted as an autosomal dominant defect while type 3 appears with an X-linked inheritance pattern. Type 1a IGHD presents as an entire *GH-1* gene deletion with undetectable serum GH levels, extremely short stature and possible development of anti-GH antibodies after recombinant human GH administration (3,4). Type 1b IGHD presents with a milder phenotype, caused by mutations to either

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Conflict of interest: None declared

Received: 05.08.2017

Accepted: 14.12.2017

the *GH-1* gene or to the GH releasing hormone receptor (*GHRH-R*) gene with low but detectable levels of serum GH, short stature and a positive response to GH therapy with immunologic tolerance (5). Type 2 IGHD patients may also present with low serum GH levels without development of anti-GH antibodies. IGHD-type 3 has been associated with occasional agammaglobulinemia (6).

The *GHRH-R* gene is located on the short arm of chromosome 7. A number of mutations within the specific locus of the *GHRH-R* gene have been reported in IGHD type 1b subjects, leading to loss of the receptor function and thus to growth failure. We present a novel mutation of the GHRH-R, leading to IGHD type 1b in a 13-months old Greek girl, the youngest patient with a GHRH-R mutation reported so far.

Case Report

A 13-month old girl was admitted to our department due to failure to thrive. She was the second child of healthy, unrelated parents, whose heights were 190 cm (father) and 175 cm (mother). An ethical review board approval and informed consent from both parents of the proband presented here were obtained, in accordance with national laws.

The patient was the product of a 37 weeks gestation. During the 4th-8th gestational week, the mother experienced vaginal bleeding. Intrauterine growth retardation was diagnosed in the 8th gestational week due to placental insufficiency. Additionally, the mother admitted she was smoking during the entire pregnancy period. The newborn was asymmetrical and small for gestational age (SGA), with a birth weight of 2420 g (< 3rd percentile, z-score: -1.93), and a length of 44 cm (< 3rd percentile, z-score: -2.76) (Figure 1). Head circumference was 34.5 cm (70th percentile, z-score: 0.52). She was partially breast-fed during the first 30 days of life. Due to the infant's unwillingness to take formula milk, she was transferred to the pediatric gastroenterology department where a 24-hour nasogastric tube was placed at the age of nine months and hypercaloric oral supplements were administered, without significant effect on body weight gain (Figure 1).

On physical examination, at 13 months of age, the infant was small and skinny, not resembling obese GH deficient neonates. Her length was 60 cm (< 3rd percentile, z-score: -6.03) and her weight 5470 g (< 3rd percentile, z-score: -4.35). Head circumference was 45 cm (40th percentile, z-score: -0.27) and head shape was triangular with open

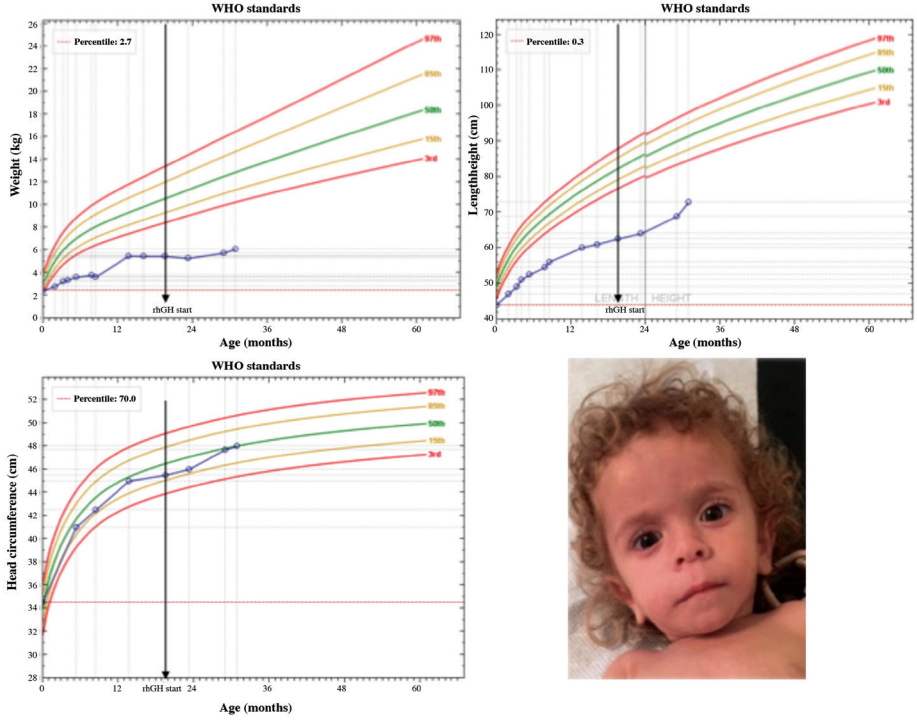

Figure 1. Growth chart for weight-for-age, height-for-age and head circumference-for-age (Anthro World Health Organization software) along with a clinical photograph of the patient at 15 months of age showing typical facial features of a patient with growth hormone deficiency

WHO: World Health Organization

fontanelles. Hair was very sparse and ears were low set. Nasal bridge was hypoplastic and dental development was significantly retarded (one tooth). Motor milestones were delayed; she was able to sit but could not stand. Systematic clinical examination of the heart, lungs and abdomen did not reveal abnormal findings.

Complete blood count, haemoglobin levels and glucose concentrations as well as renal function were within the normal range for her age. Karyotype analysis showed a normal female genotype of 46 XX. Thyroid and adrenal hormone levels were normal. Serological indices for celiac disease or food allergy were negative. Serum GH response to clonidine, glucagon and arginine stimulation tests revealed very poor response, with a peak GH value of 4.77 ng/mL, demonstrating IGHD (Table 1). An IGF-1 generation test after administering GH at a dose of 33 μg/kg for four consecutive days showed low IGF-1 levels with a modest response (Table 1). After 12 months of GH treatment, serum IGF-1 level rose to 23 ng/mL. Bone age was two months at the chronological age of 13 months. Magnetic resonance imaging of the brain revealed a normal pituitary gland and normal hypothalamus.

At the chronological age of 19 months the patient was administered GH at a starting dose of 0.28 mg/kg/week, subcutaneously. After ten months, GH dose was increased to 0.35 mg/kg/week. At the age of 22 months she started to walk. At the chronological age of 24 months she presented a 12 month phalangeal and a nine month carpal bone age. After ten months of medication she gained 7 cm in length (8.14 cm/year), 300 g in weight and her head circumference had increased by 2.2 cm. After one year of treatment (at chronological age of 31 months) the patient had achieved a length of 73.5 cm (< 3rd percentile, z-score: -5.2), a weight of 6100 g (< 3rd percentile, z-score: -5.65) and a head circumference of 48 cms (50th percentile, z-score: -0.02) (Figure 1).

Table 1. Growth hormone provocation and insulin-like growth factor 1 generation test values

Time (min)	GH (ng/mL)			IGF-1 (ng/mL)	
	Clonidine	Glucagon	Arginine	IGF-1 generation test	
0	0.441	1.65	1.35	0 day	8.9
30	1.37	0.96	2.98	1st day	11.3
60	3.45	2.3	3.63	2nd day	13.4
90	1.1	4.77	1.18	3rd day	13.6
120	0.595	2.03	1.1	4th day	11.7
150	-	0.84	1.27	-	-
180	-	0.44	-	-	-

GH: growth hormone, IGF-1: insulin-like growth factor 1, min: minute

Due to the facial features of the patient, Silver-Russell syndrome has been suspected. The absence of the clinical criteria of Price et al (7) along with a deletion/duplication analysis with array genomic hybridization, Silver-Russell syndrome was excluded. Additionally, presence of intrauterine growth retardation, along with facial characteristics and delayed eruption of teeth, could suggest a possible diagnosis of 3M syndrome. Triple whole exome sequencing (WES) of the affected girl and parents (CentoXome GOLD®) using Illumina technology was performed. No mutation on *CUL7*, *OBSL1* or *CCDC8* genes, the mutations leading to 3M syndrome were found. A novel homozygous nonsense variant in the *GHRH-R* gene, the c.97C > T (p.Gln33*) was detected. The observed variant creates a premature stop codon and is classified as likely pathogenic-class 2 variant. Parental genotyping detected the novel variant in the mother in a heterozygous form, but it was not found in the father. It is suspected that a large deletion not detectable by WES in the paternal allele is present. The detected c.97C > T variant of the *GHRH-R* gene has never been reported before and not listed, in the CentoMD.

Discussion

The present report describes an unknown GHRH-R mutation, in an infant girl of Greek origin, with a clinical appearance resembling a SGA state, rather than congenital GHD (8,9). To the best of our knowledge this is the youngest patient described in the literature to date with a mutation of the *GHRH-R* gene. The patient presented with a skinny appearance and showed a low IGF-1 response to an IGF-1 generation test. These two unexpected findings probably relate to caloric insufficiency caused by placental insufficiency (10), possibly due to the mother smoking throughout the pregnancy.

Some of the causes of congenital IGHD are *GHRH-R* gene defects. These gene defects are being described more frequently in the literature (11). Currently more than thirty-three mutations in the *GHRH-R* gene have been shown to cause impaired GHRH-GH-IGF-1 axis function, whereas no mutations in the *GHRH* gene have been reported. The large majority of these cases showed an autosomal recessive model of inheritance (12). Mutations of GHRH-R, classified into six different types, cause defective GHRH functionality (13). Null-type GHRH-R mutations lead to unmeasurable IGF-1 levels and are accompanied by mild ocular disorders (14). Missense GHRH-R variants -such as p.G369V or p.T257A- result in partial loss of receptor function due to defective ligand binding and milder phenotypes, occasionally

accompanied by hypoglycemia (15). Splice site mutations of untranslated and coding regions have been reported to lead to gross indels with loss of 5' regulatory/exon 1 region, leading to fully impaired GHRH-R expression (12). Other splice-disrupting, single nucleotide polymorphisms like intronic mutations, lead to instability of the produced mRNA, truncated GHRH-R and autosomal recessively inherited IGHD (16). Nonsense type mutations lead to loss-of-function changes (17), whereas functional variants of the GHRH-R promoter affect promoter activity and thus decrease expression of the receptor gene (18).

Herein, we present a previously undescribed homozygous *GHRH-R* gene mutation, c.97C > T (p.Gln33*) in a child with IGHD type 1b. Clinical and biochemical phenotype of the affected individual comprises severe short stature, low weight gain, low maximum GH values after a battery of provocation tests, inadequate response to IGF-1 generation testing, normal brain imaging and growth acceleration after GH therapy. The reported mutation is predicted to lead to a premature stop codon and thus it signals the termination of translation of the relevant messenger RNA. Defective translation of the gene results in a shorter encoded protein and thus an impaired form of GHRH receptor. The novel mutation probably affects the receptor in terms of both sequence and structure, leading to the inhibition of GHRH binding to its receptor and thus to disruption in GH secretion signaling. According to the recommendations of the American College of Medical Genetics and Genomics, the novel mutant is classified as likely pathogenic, class 2.

Since the variant was detected in the maternal DNA in heterozygous state, but not in the paternal genome, the precise pattern of inheritance can not be confirmed. A suggested large deletion in the exact region of the paternal *GHRH-R* locus could explain the inability to detect the mutation via father WES analysis. Nevertheless, based on the finding of a healthy, unaffected heterogyzous mother, it could be assumed that the variant presented here, p.Gln33*, represents an autosomal recessive inheritance trait.

Intrauterine growth restriction is closely associated with placental quality, functionality and therefore adequacy. Multiple layers of associations have been suggested for the causes of fetal growth restriction and SGA offspring. *In utero* exposure to tobacco constitutes a known risk factors for both conditions. From a meta-analytic approach, even exposure to tobacco smoke during pregnancy is associated with low birth weight (19). Exposure of offspring to tobacco metabolites through maternal milk during infancy has also been suggested (20). Nevertheless, cohort studies have provided evidence that maternal smoking during pregnancy or early infantile life exert a long-term negative effect on growth (21). The presented case constitutes a paradigm of mixture between nature and nurture. Apart from the detected defect in *GHRH-R* gene sequence, *in utero* environment and after birth conditions have contributed to the phenotype. Synergistic effects of genetics and epigenetic conditions are not fully understoood and remain to be elucidated.

In conclusion, we report a novel homozygous c.97C > T (p.Gln33*) GHRH-R mutation determined in a Greek infant girl with IGHD. Heterozygosity of the reported variant was not associated with pathological phenotypes in the unaffected family member c.97C > T.

Ethics

Informed Consent: Written parental consent for this case report has been given.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Assimina Galli-Tsinopoulou, Aggeliki N Kleisarchaki, Rivka Kauli, Zvi Laron, Concept: Assimina Galli-Tsinopoulou, Design: Assimina Galli-Tsinopoulou, Eleni P. Kotanidou, Zvi Laron, Data Collection or Processing: Eleni P. Kotanidou, Aggeliki N. Kleisarchaki, Rivka Kauli, Analysis or Interpretation: Rivka Kauli, Assimina Galli-Tsinopoulou, Zvi Laron, Literature Search: Eleni P. Kotanidou, Aggeliki N. Kleisarchaki, Assimina Galli-Tsinopoulou, Writing: Assimina Galli-Tsinopoulou, Eleni P. Kotanidou, Aggeliki N. Kleisarchaki, Rivka Kauli, Zvi Laron.

Financial Disclosure: Authors have no financial disclosure to declare.

References

1. Thomas M, Massa G, Craen M, de Zegher F, Bourguignon JP, Heinrichs C, De Schepper J, Du Caju M, Thiry-Counson G, Maes M. Prevalence and demographic features of childhood growth hormone deficiency in Belgium during the period 1986-2001. Eur J Endocrinol 2004;151:67-72.
2. Maghnie M, Lindberg A, Koltowska-Häggström M, Ranke MB. Magnetic resonance imaging of CNS in 15,043 children with GH deficiency in KIGS (Pfizer International Growth Database). Eur J Endocrinol 2013;168:211-217.
3. Cogan JD, Phillips JA 3 rd. GH1 gene deletions and IGHD type 1A. Pediatr Endocrinol Rev 2006;3(Suppl 3):480-488.
4. Laron Z, Kelijman M, Pertzelan A, Keret R, Shoffner JM, Parks JS. Human growth hormone gene deletion without antibody formation or growth arrest during treatment--a new disease entity? Isr J Med Sci 1985;21:999-1006.
5. Baumann G. Genetic characterization of growth hormone deficiency and resistance: implications for treatment with recombinant growth hormone. Am J Pharmacogenomics 2002;2:93-111.

6. Wit JM, Oostdijk W, Losekoot M, van Duyvenvoorde HA, Ruivenkamp CA, Kant SG. MECHANISMS IN ENDOCRINOLOGY: Novel genetic causes of short stature. Eur J Endocrinol 2016;174:145-173. Epub 2015 Nov 17
7. Price SM, Stanhope R, Garrett C, Preece MA, Trembath RC. The spectrum of Silver-Russell syndrome: a clinical and molecular genetic study and new diagnostic criteria. J Med Genet 1999;36:837-842.
8. Laron Z. Deficiencies of growth hormone and somatomedins in man. Spec Top Endocrinol Metab 1983;5:149-199.
9. Smuel K, Kauli R, Lilos P, Laron Z. Growth, development, puberty and adult height before and during treatment in children with congenital isolated growth hormone deficiency. Growth Horm IGF Res 2015;25:182-188. Epub 2015 May 12
10. Hawkes CP, Grimberg A. Insulin-Like Growth Factor-I is a Marker for the Nutritional State. Pediatr Endocrinol Rev 2015;13:499-511.
11. Aguiar-Oliveira MH, Souza AHO, Oliveira CRP, Campos VC, Oliveira-Neto LA, Salvatori R. MECHANISMS IN ENDOCRINOLOGY: The multiple facets of GHRH/GH/IGF-I axis: lessons from lifetime, untreated, isolated GH deficiency due to a GHRH receptor gene mutation. Eur J Endocrinol 2017;177:85-97. Epub 2017 Apr 20
12. Kale S, Budyal S, Kasaliwal R, Shivane V, Raghavan V, Lila A, Bandgar T, Shah N. A novel gross indel in the growth hormone releasing hormone receptor gene of Indian IGHD patients. Growth Horm IGF Res 2014;24:227-232. Epub 2014 Aug 7
13. Corazzini V, Salvatori R. Molecular and clinical aspects of GHRH receptor mutations. Endocr Dev 2013;24:106-117. Epub 2013 Feb 1
14. Faro ACN, Pereira-Gurgel VM, Salvatori R, Campos VC, Melo GB, Oliveira F, Oliveira-Santos AA, Oliveira CRP, Pereira FA, Hellströem A, Oliveira-Neto LA, Valenca EHO, Aquiar-Oliveira MH. Ocular findings in adult subjects with an inactivating mutation in GH releasing hormone receptor gene. Growth Horm IGF Res 2017;34:8-12. Epub 2017 Apr 21
15. Gregory LC, Alatzoglou KS, McCabe MJ, Hindmarsh PC, Saldanha JW, Romano N, Le Tissier P, Dattani MT. Partial Loss of Function of the GHRH Receptor Leads to Mild Growth Hormone Deficiency. J Clin Endocrinol Metab 2016;101:3608-3615. Epub 2016 Aug 8
16. Marui S, Trarbach EB, Boguszewski MC, França MM, Jorge AA, Inoue H, Nishi MY, de Lacerda Filho L, Aguiar-Oliveira MH, Mendonca BB, Arnhold IJ. GH-releasing hormone receptor gene: a novel splice-disrupting mutation and study of founder effects. Horm Res Paediatr 2012;78:165-172. Epub 2012 Oct 10
17. Sıklar Z, Berberoğlu M, Legendre M, Amselem S, Evliyaoğlu O, Hacıhamdioğlu B, Savaş Erdeve S, Oçal G. Two siblings with isolated GH deficiency due to loss-of-function mutation in the GHRHR gene: successful treatment with growth hormone despite late admission and severe growth retardation. J Clin Res Pediatr Endocrinol 2010;2:164-167. Epub 2010 Nov 6
18. Inoue H, Kangawa N, Kinouchi A, Sakamoto Y, Kimura C, Horikawa R, Shigematsu Y, Itakura M, Ogata T, Fujieda K; Japan Growth Genome Consortium. Identification and functional analysis of novel human growth hormone-releasing hormone receptor (GHRHR) gene mutations in Japanese subjects with short stature. Clin Endocrinol (Oxf) 2011;74:223-233.
19. Inamdar AS, Croucher RE, Chokhandre MK, Mashyakhy MH, Marinho VC. Maternal Smokeless Tobacco Use in Pregnancy and Adverse Health Outcomes in Newborns: A Systematic Review. Nicotine Tob Res 2015;17:1058-1066. Epub 2014 Dec 22
20. Shenassa ED, Wen X, Braid S. Exposure to Tobacco Metabolites via Breast Milk and Infant Weight Gain: A Population-Based Study. J Hum Lact 2016;32:462-471. Epub 2015 Dec 7
21. Muraro AP, Gonçalves-Silva RM, Moreira NF, Ferreira MG, Nunes-Freitas AL, Abreu-Villaça Y, Sichieri R. Effect of tobacco smoke exposure during pregnancy and preschool age on growth from birth to adolescence: a cohort study. BMC Pediatr 2014;14:99.

CASE REPORT

DOI: 10.4274/jcrpe.5374

J Clin Res Pediatr Endocrinol 2018;10(3):289-293

Peripheral Neuropathy as a Complication of Diabetic Ketoacidosis in a Child with Newly Diagnosed Diabetes Type 1: A Case Report

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What is already known on this topic?

Neurological complications of ketoacidosis in diabetes mellitus type 1 are serious clinical problems. Neuropathy after ketoacidosis in children is extremely rare. Only a small number of cases with this complication have been reported.

What this study adds?

This paper presents the current state of knowledge about peripheral neuropathy in pediatric patients with new-onset type 1 diabetes and includes clinical presentation, pathophysiology and available treatment for this rare complication.

Abstract

Neurological complications of diabetic ketoacidosis are considered to be a serious clinical problem. The most common complication is cerebral edema. However, these neurological complications also include less common entities such as ischemic or hemorrhagic stroke, cerebral venous and sinus thrombosis or peripheral neuropathy.

We present a case of a 9-year old girl admitted to our intensive care unit with new onset type 1 diabetes, diabetic ketoacidosis, cerebral edema, multifocal vasogenic brain lesions and bilateral lower limb peripheral paresis. The patient developed polydipsia and polyuria one week before admission. The initial blood glucose level was 1136 mg/dL and severe acidosis was present (pH 7.1; BE-25.9). Computed tomography scan showed brain edema and a hypodense lesion in the left temporal region. Brain magnetic resonance imaging revealed more advanced multifocal brain lesions. Nerve conduction studies demonstrated damage of the motor neurons in both lower limbs with dysfunction in both peroneal nerves and the right tibial nerve. With treatment and physiotherapy, the patient's health gradually improved.

Acute neuropathy after ketoacidosis is a rare complication and its pathogenesis is not clear. Patients with diabetic ketoacidosis require careful monitoring of neurological function, even after normalization of their glycemic parameters.

Keywords: Polyneuropathy, ketoacidosis, diabetes mellitus type 1, children

Introduction

Neurological complications of ketoacidosis in diabetes mellitus type 1 (DM1) present a serious clinical problem. Brain edema is the most common central nervous system (CNS) complication which occurs in approximately 0.5-1% of cases with diabetic ketoacidosis (DKA) and has a 20% mortality rate (1,2). Ischemic and hemorrhagic strokes are less common and account for 10% of intracerebral complications of DKA (3). Cerebral vein and sinus thrombosis are also less frequent than brain edema while neuropathy after DKA is extremely rare.

Case Report

We present a 9-year-old girl with newly diagnosed DM1, DKA, brain edema, multifocal vasogenic brain lesions and lower limb paresis.

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Conflict of interest: None declared
Received: 27.09.2017
Accepted: 09.12.2017

J Clin Res Pediatr Endocrinol
2018;10(3):289-293

The patient was reported to have polyuria and polydipsia over the past week and a weight loss of 3 kg over the previous month. The patient was admitted to the district hospital in a serious clinical condition with severe dehydration. Initial intravenous fluid therapy included infusion of 15 mL/kg of 0.9% sodium chloride during the first 90 minutes. The total volume of fluids administered during the first 12 hours and consisting of 1250 mL 0.9% sodium chloride (NaCl) and 1000 mL of 5% dextrose with 0.9% NaCl (2:1 proportion, sodium concentration-51.34 mEq/L) was 65 mL/kg (patient's weight-34.6 kg). Intravenous insulin therapy was introduced in an initial dose of 0.05 units/kg/hour in order to prevent a rapid decrease of glycaemia. After three hours, the patient's medical state and neurological condition was reported to deteriorate. She experienced motor restlessness and agitation followed by upper limb spasms. At the end of the first day of treatment the patient was transferred to the Intensive Care Unit (ICU) of the Children's Memorial Health Institute in Warsaw with a Glasgow Coma Scale (GCS) score of 13 points. Results of laboratory tests are shown in Table 1.

Six hours after admission to the ICU, her clinical state was deteriorating rapidly and the GCS score had decreased to 7 points. Computed tomography scan revealed brain edema and a 13-mm hypodense lesion in the left temporal region (Figure 1).

The patient was sedated and intubated. Insulin infusion was continued and intravenous fluid administration was diminished. Anti-edematous treatment was introduced (Mannitol 0.3 g/kg/dose, three times per day). The patient's state showed a gradual improvement. After four days, she was extubated. Subsequently the patient was transferred to the Department of Endocrinology and Diabetology. Despite improvement in her clinical condition, the patient was found to have developed symmetric lower limbs paresis. Brain magnetic resonance imaging (MRI) revealed numerous, diffuse lesions (Figure 2, 3). Presence of infection and neoplasm of CNS were ruled out

Lower limb nerve conduction studies (NCS) revealed damage to the motor neuron in both lower extremities with

Table 1. Results of laboratory tests during admission to the district hospital and to the Intensive Care Unit of the Children's Memorial Health Institute

Parameters	District hospital	Intensive care unit	Reference ranges
Glucose (mg/dL)	1136	308	-
Osmolarity (mOsm/kg H_2O)	328	290	275-295
pH	7.1	7.2	7.35-7.43
Base excess mEq/L	-25.9	-20	-2 to +2
Corrected sodium level (mmol/L)	152	144	136-145
Potassium (mmol/L)	5.5	3.8	3.5-5.1
Phosphate (mmol/L)	0.54	-	1.05-1.85
CRP (mg/dL)	1.03	0.8	<0.5
D-dimer (ug/L)	-	1284	<500
Fibrinogen (g/L)	-	3.16	1.9-3.6

pH: potential of hydrogen, CRP: c-reactive protein

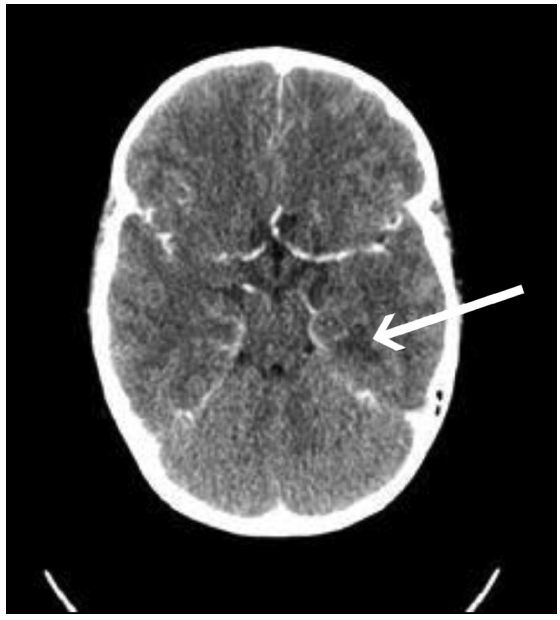

Figure 1. Brain computed tomography scan. Thirteen mm hypodense lesion in the left temporal lobe, not visible after contrast injection-ischemic lesion? The supratentorial ventricular system is narrow and symmetrical. Cerebral sulci are not distinct

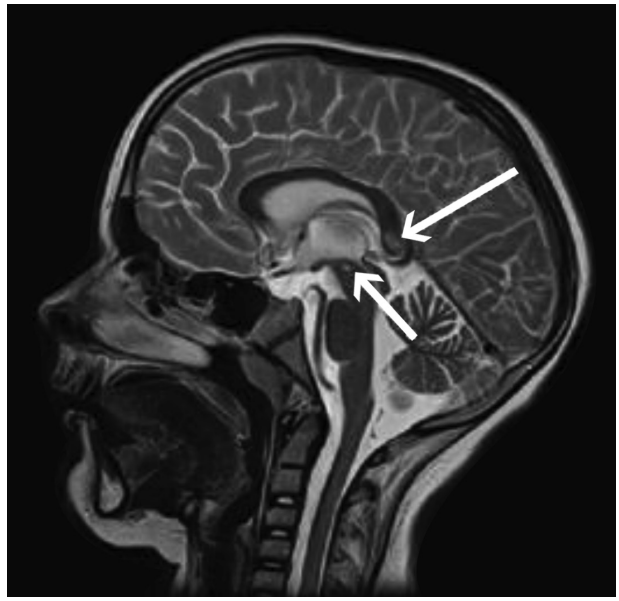

Figure 2. Brain magnetic resonance imaging. T1-weighted scan. Lesions located in the corpus callosum and the midbrain

dysfunction in both peroneal nerves and in the right tibial nerve. Neurological opinion was that the etiology of the multifocal brain lesions was vasogenic. However, the cause of neuropathy was not fully clear. Presence of DKA and peripheral ischemia were given as the probable factors leading to development of the neuropathy.

Alpha lipoic acid and vitamins B1, B6 and B12 were introduced to the therapeutic regimen and the patient underwent intensive physiotherapy, which led to improvement of left lower limb motor function.

Brain MRI was performed three months later in which no progression in size and number of the brain lesions was observed. NCS revealed normalization of the left peroneal nerve parameters. However, findings indicative of deep motor neuropathy of the right lower limb was found to persist. An informed consent form for publication was given by the parents.

Discussion

Diabetic neuropathy (DN) refers to the presence of symptoms and/or signs of peripheral nerve dysfunction due to diabetes. In order to make a diagnosis of DN, other neuropathic etiologies should be excluded which include vitamin deficiency, infection, inflammatory causes, toxic, autoimmune, paraneoplastic and genetic causes (4). Neuropathy is the most common complication of diabetes and is encountered in approximately 45% patients with DM2 and 54-59% patients with DM1 (5). Due to different symptoms, clinical courses and pathogenic mechanisms, DN is considered a heterogeneous entity which includes many types of nerve dysfunction. More than 80% of patients with symptomatic DN suffer from generalized symmetric, chronic polyneuropathy including motor, sensory and/or autonomic nerve dysfunctions. In most cases DN develops over the years in patients with long standing hyperglycemia (6). However, there are also few types of neuropathy related to newly diagnosed DM1, which are extremely rare and there is scarce data on this issue (7). They can be classified as acute painful DN, hyperglycemic neuropathy and neuropathy after ketoacidosis. It is interesting that not only symptomatic but also asymptomatic changes in nerve function can be seen at the time of DM1 diagnosis. Lee et al (8) examined nerve conduction in children with newly diagnosed DM1 and periodically during their 5-year follow-up. This prospective study included patients aged 3-19 years (n = 37), who underwent bilateral NCS of median, ulnar, posterior tibial, peroneal and sural nerves annually for five years. In 32.4% of the patients the examination revealed electrophysiological evidence of polyneuropathy in at least two different nerves at the time of diagnosis of DM1. Dayal et al (9) presented a case report of a 12-year-old girl who developed acute, asymmetric, sensorimotor neuropathy during the first month after diagnosis of DM1. During this time, the authors observed reduction of hemoglobin A1c (HbA1c) from 14.2% to 10.4% which was described as a potential trigger factor for neuropathy. Wilson et al (10) presented a similar case of DN. A 14-year-old boy was diagnosed with DM1. After nine weeks of diabetes treatment the authors observed HbA1c level reduction from 14.1% to 7.6%. These two cases present clinical features of acute painful DN, also known as a treatment-induced neuropathy (insulin neuritis). This is a reversible disorder and affects diabetes patients who show a rapid improvement of metabolic control and have a favorable clinical outcome (11). In our patient, this diagnosis was taken into consideration however the decrease of the HbA1c level was not that significant as in two cases above (HbA1c level at diagnosis was 11.5% and one month later it was 9.6%).

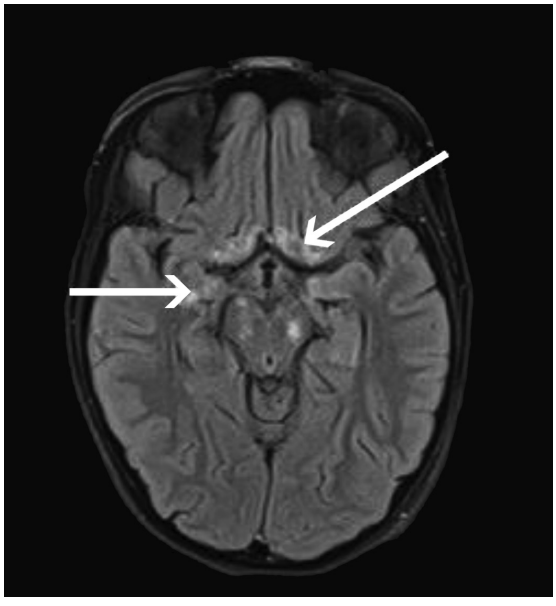

Figure 3. Brain magnetic resonance imaging. Fluid-attenuated inversion recovery sequence. Lesions located in the medial parts of the temporal lobes and in the lower-medial area of the frontal lobes and the ventricles

Another type of DN is hyperglycemic neuropathy. Its clinical presentation includes temporary limb hyperalgesia which is potentially reversible after glycemic normalization (12).

Rangel et al (7) described a 10-year-old patient with new onset DM1 and concomitant acute mononeuropathy which manifested in difficulty in flexing the right foot and hyperalgesia, extending from the dorsum of the right foot to the ankle. According to these authors, DM was responsible for this mononeuropathy as its onset was simultaneous with

the onset of DM1. Motor dysfunction rapidly improved after adequate glycemic control.

Our patient suffered from acute motor peripheral neuropathy, which was probably caused by DKA. This neuropathy can be a consequence of the peripheral ischemia or hemodynamic and metabolic changes linked to the ketoacidosis (13). One hypothesis is that the procoagulant state which occurs during DKA can cause nerve damage through vascular endothelial dysfunction, which is the first line of defense against thrombosis. Endothelial damage also leads to platelet and coagulation factor activation (14,15). The plasma levels of fibrinogen, factors VII, VIII, XI, XII and von Willebrand are elevated in DKA. A procoagulant state is worsened by disrupted anticoagulant mechanisms, such as a low protein C level. Fibrinolysis is also impaired due to different factors such as more difficult degradation of the thrombi or an increased concentration of plasminogen activator inhibitor type 1 (14,15). The patient in our report had slightly elevated d-dimer concentrations. However, further diagnostic tests were not conducted. In our case other etiologies of neuropathy, such as hypophosphatemia, which was observed during admission to the district hospital, were also taken into consideration. Hypophosphatemia is generally asymptomatic. Nonetheless, its severe form (<0.32 mmol/L) can lead to peripheral polyneuropathy which may be both motor and sensory (16). In our patient, neurological symptoms were present even after normalization of the phosphate level suggesting an alternative mechanism. Differential diagnosis also included Guillain-Barré syndrome, but no typical pathology in cerebrospinal fluid was seen.

The treatment of DN includes use of strong antioxidants such as alpha lipoic acid. The effectiveness of this therapy was proven in meta-analyses (17,18). Benfotiamine, a derivative of vitamin B1, was also shown to increase the utilization of active glycolysis products, although less effectively than alpha lipoic acid (19).

In conclusion, the findings in this patient indicate that neuropathy is not only seen as a late complication of diabetes mellitus, but that it can develop any time after, or even before DM1 diagnosis (6). However, acute neuropathy after ketoacidosis is a rare complication and its pathogenesis is not clear. Patients with DKA require careful monitoring of neurological functions even after normalization of glycemic parameters (20).

Ethics

Informed Consent: Written consent was obtained from the patient's parents.

Peer-review: Externally peer-reviewed.

Authorship Contribution

Concept: Marta Baszyńska-Wilk, Mieczysław Szalecki. Design: Marta Baszyńska-Wilk. Data Collection and Processing: Marta Baszyńska-Wilk, Magdalena Marszał, Marta Wysocka-Mincewicz, Anna Świercz, Jolanta Świderska. Analysis and Interpretation: Marta Baszyńska-Wilk, Marta Wysocka-Mincewicz, Mieczysław Szalecki. Literature Research: Marta Baszyńska-Wilk. Writing: Marta Baszyńska-Wilk, Marta Wysocka-Mincewicz, Mieczysław Szalecki.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Edge JA, Hawkins MM, Winter DL, Dunger DB. The risk and outcome of cerebral oedema developing during diabetic ketoacidosis. Archives of Disease in Childhood 2001;85:16-22.
2. Dunger DB, Sperling MA, Acerini CL, Bohn DJ, Daneman D, Danne TP, Glaser NS, Hanas R, Hintz RL, Levitsky LL, Savage MO, Tasker RC, Wolfsdorf JI;ESPE; LWPES. ESPE/LWPES consensus statement on diabetic ketoacidosis in children and adolescents. Arch Dis Child 2004;89:188-194.
3. Bialo SR, Agrawal S, Boney CM, Quintos JB. Rare complications of pediatric diabetic ketoacidosis. World J Diabetes 2015;6:167-174.
4. Meh D, Denislic M. Subclinical neuropathy in type I diabetic children. Electroencephalogr Clin Neurophysiol 1998;109:274-280.
5. Zilliox L, Russell JW. Treatment of Diabetic Sensory Polyneuropathy. Curr Treat Options Neurol 2011;13:143-159.
6. Tesfaye S, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, Lauria G, Malik RA, Spallone V, Vinik A, Bernardi L, Valensi P; Toronto Diabetic Neuropathy Expert Group. Diabetic Neuropathies: Update on Definitions, Diagnostic Criteria, Estimation of Severity, and Treatments. Diabetes Care 2010;33:2285-2293.
7. Rangel MA, Baptista C, Santos F, Real MV, Campos RA, Leite AL. Acute mononeuropathy in a child with newly diagnosed type 1 diabetes mellitus. J Pediatr Endocrinol Metab 2015;28:341-344.
8. Lee SS, Han HS, Kim H. A 5-yr follow-up nerve conduction study for the detection of subclinical diabetic neuropathy in children with newly diagnosed insulin-dependent diabetes mellitus. Pediatr Diabetes 2010;11:521-528. Epub 2010 Aug 15
9. Dayal D, Jayaraman D, Sankhyan N, Singhi P. Acute Painful Neuropathy in a Girl with Type 1 Diabetes: Long Term Follow-Up. J Clin Diagn Res 2016;10:1-2.
10. Wilson JL, Sokol DK, Smith LH, Snook RJ, Waguespack SG, Kincaid JC. Acute painful neuropathy (insulin neuritis) in a boy following rapid glycemic control for type 1 diabetes mellitus. J Child Neurol 2003;18:365-367.
11. Tran C, Philippe J, Ochsner F, Kuntzer T, Truffert A. Acute painful diabetic neuropathy: an uncommon, remittent type of acute distal small fibre neuropathy. Swiss Med Wkly 2015;145:14131.
12. Ahmad S, Manish Mittal. Diabetic Neuropathies. JIMSA 2015;28.
13. Tracy JA, Dyck PJ. The Spectrum of Diabetic Neuropathies. Phys Med Rehabil Clin N Am 2008;19:1-26.

14. Carr ME. Diabetes mellitus - A hypercoagulable state. J Diabetes Complications 2001;15:44-54.

15. Foster JR, Morrison G, Fraser DD. Diabetic Ketoacidosis-Associated Stroke in Children and Youth. Stroke Res Treat 2011;2011:219706.

16. Iguchi Y, Mori K, Koike H, Mano K, Goto Y, Kato T, Nakano T, Furukawa D, Sobue G. Hypophosphataemic neuropathy in a patient who received intravenous hyperalimentation. J Neurol Neurosurg Psychiatry 2007;78:1159-1160.

17. Ziegler D, Nowak H, Kemplert P, Vargha P, Low PA. Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a meta-analysis. Diabetic Med 2004;21:114-121.

18. Mijnhout GS, Kollen BJ, Alkhalaf A, Kleefstra N, Bilo HJ. Alpha Lipoic Acid for Symptomatic Peripheral Neuropathy in Patients with Diabetes: A Meta-Analysis of Randomized Controlled Trials. Int J Endocrinol 2012;2012:456279.

19. Ang CD, Alviar MJ, Dans AL, Bautista-Velez GG, Villaruz-Sulit MV, Tan JJ, Co HU, Bautista MR, Roxas AA. Vitamin B for treating peripheral neuropathy. Cochrane Database of Syst Rev 2008:CD004573.

20. Dabelea D, Rewers A, Stafford JM, Standiford DA, Lawrence JM, Saydah S, Imperatore G, D'Agostino RB Jr, Mayer-Davis EJ, Pihoker C; SEARCH for Diabetes in Youth Study Group. Trends in the Prevalence of Ketoacidosis at Diabetes Diagnosis: The SEARCH for Diabetes in Youth Study. Pediatrics 2014;133:938-945. Epub 2014 Mar 31

CASE REPORT

DOI: 10.4274/jcrpe.4865

J Clin Res Pediatr Endocrinol 2018;10(3):294-298

Resolution of Consumptive Hypothyroidism Secondary to Infantile Hepatic Hemangiomatosis with a Combination of Propranolol and Levothyroxine

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What is already known on this topic?

Cutaneous infantile hemangiomas can be associated with significant hepatic involvement. Diffuse hepatic hemangiomatosis is associated with a unique and challenging form of hypothyroidism known as consumptive hypothyroidism.

What this study adds?

In cases of systemic compromise, infants with hepatic hemangiomatosis should be screened for hypothyroidism at an early stage, even in the absence of obvious cutaneous clues. We advocate propranolol as a single first line agent to treat diffuse infantile hepatic hemangioma with systemic decompensation. Coexisting consumptive hypothyroidism should be aggressively managed to prevent long-term intellectual and developmental sequelae.

Abstract

Infantile hepatic hemangiomas (IHH), particularly of the diffuse subtype can, in severe cases, be associated with hepatic and cardiac failure, compartment syndrome and consumptive hypothyroidism. Early recognition and treatment of these pathologies is paramount in order to minimise the risk of long-term sequelae.

We report an interesting case of a female infant who presented with systemic compromise, in the absence of large or obvious cutaneous infantile hemangiomas. Imaging identified innumerable hepatic hemangiomas, consistent with diffuse infantile hepatic hemangiomatosis. Subsequent to this, thyroid function tests confirmed an associated but comparatively rare form of hypothyroidism, known as consumptive hypothyroidism. Following joint consultation with dermatology and endocrinology she was promptly treated with oral propranolol and levothyroxine, with subsequent improvement in her clinical parameters.

This case reiterates the importance of aggressive investigation and management of consumptive hypothyroidism in any infant diagnosed with IHH, particularly when there is systemic compromise. We advocate propranolol as a single first line treatment for IHH, supported by thyroid replacement when appropriate.

Keywords: Hemangioma, consumptive hypothyroidism, type 3 iodothyronine deiodinase, propranolol

Introduction

Infantile hemangiomas (IH) are benign endothelial cell neoplasms and the most common tumours of infancy, occurring in 3-5% of infants (1). They are more common in preterm and low birth weight infants and have a distinct pattern of proliferation during the first year of life, followed by gradual involution (2,3). While most IH are cutaneous, extracutaneous involvement of the liver may also occur. Although histologically benign and frequently asymptomatic, infantile hepatic hemangioma (IHH) can manifest as congestive heart failure associated with vascular shunting, abdominal compartment syndrome and fulminant hepatic failure with consumptive hypothyroidism, leading to death in the most severe cases.

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Conflict of interest: None declared
Received: 21.08.2017
Accepted: 28.12.2017

In 2007 Christison-Lagay et al (4) divided IHH into three groups-focal, multifocal, and diffuse-based on the pattern and extent of liver involvement, correlated with clinical risk and outcomes. Focal lesions are predominantly glucose transporter (GLUT)-1 negative, and since they are formed *in utero* are amenable to antenatal diagnosis using ultrasound. They often lack associated cutaneous lesions, and as such may be missed if the presence of cutaneous hemangiomas is the sole stimulus to screen for hepatic involvement (5). Focal lesions have the potential to regress rapidly, behaviour akin to cutaneous, rapidly involuting congenital hemangiomas. In contrast, multifocal hepatic lesions are typically associated with multiple, small cutaneous IH, and are GLUT-1 positive. Most remain asymptomatic, and spontaneously resolve without sequelae. A minority have been associated with congestive cardiac failure (6).

Diffuse hepatic hemangiomas are associated with the highest risk of morbidity and mortality, secondary to massive infiltration of the hepatic parenchyma with innumerable hemangiomas. The diffuse subtype is associated with the rare entity of consumptive hypothyroidism, first described by Huang et al (7) in 2000.

The three types of iodothyronine deiodinases that regulate thyroid hormone activity are classified as types 1, 2 and 3. Type 3 iodothyronine deiodinase (D3) is a selenoenzyme, normally present in brain, placenta and fetal liver, and works by catalysing the conversion of thyroxine (T4) to reverse triiodothyronine (rT3) and the conversion of triiodothyronine (T3) to 3,3'-diiodothyronine, both of which are biologically inactive. High levels of D3 have been reported in hemangioma tissue (7). Consumptive hypothyroidism is characterised by low free T3 (fT3) and normal or low free T4 (fT4), despite elevated thyroid stimulating hormone (TSH) (8,9). Patients have elevated serum rT3 levels as a result of increased T4 and T3 degradation by D3 (7,10).

In a review of 30 published cases of diffuse IHH, Yeh et al (6) reported that more than 70 % were hypothyroid, with eleven requiring treatment. They postulated that hypothyroidism may have been occult in the remaining cases. Thyroid hormones are crucial for growth and neurodevelopment during early childhood, with three to five IQ points lost for each month in which hypothyroidism remains untreated in the first year of life (11). This developmentally critical period parallels the proliferative phase of hemangiomas and highlights a window of opportunity to screen for and aggressively treat hypothyroidism in the context of diffuse hepatic hemangiomas.

Here we report a female infant with diffuse IHH and consumptive hypothyroidism, successfully managed with propranolol and levothyroxine.

Case Report

(Parental informed consent was obtained prior to writing and publication of this case, inclusive of images).

A female twin conceived through in vitro fertilization was born via normal vaginal delivery at 34 + 3 weeks to non-consanguineous parents, weighing 1.98 kg. The antenatal and perinatal periods were reported to be uneventful. Her older brother and twin are both well, and there was no relevant family history. The patient presented to our hospital with complaints of poor feeding and pallor at age eleven days. Her initial C-reactive protein (CRP) was elevated at 55 mg/L, and she was treated with antibiotics. She presented again at age three weeks in extremis with reduced consciousness, pallor, tachycardia, tachypnoea, epistaxis after feeding and abdominal distension. Petechiae were noted on her lower limbs. She was intubated and transferred to intensive care, where a chest X-ray suggested infection. She was again treated for possible sepsis with intravenous amoxicillin and cefotaxime. Ventilatory support was weaned and she was extubated after twenty-four hours. She received a unit of blood for anaemia (haemoglobin 6.4 g/dL prior to transfusion).

She was again readmitted at age eight weeks following an unresponsive episode, ongoing feeding difficulties with vomiting, and a distended, tense abdomen. On this occasion, an abdominal X-ray revealed hepatomegaly. Ultrasound of the abdomen showed innumerable hypoechoic nodules and increased vascularity within the liver, confirmed on computed tomography and magnetic resonance imaging. Alpha fetoprotein was markedly elevated at 1165 KU/L (normal range 0-10 KU/L), with associated derangement of her liver function tests and coagulation profile. High output cardiac failure was diagnosed, with a N-terminal pro-brain natriuretic level of 1492 ng/L (normal range < 115 ng/L). Diuretics were commenced with good effect. A baseline echocardiogram indicated a mildly dilated left heart.

Incidentally, a small (3 mm) cutaneous haemangioma at the right lateral thigh was noted during abdominal ultrasound. Following a dermatology review, two further small cutaneous hemangiomas were identified at the left lateral canthus and left axilla (Figure 1a, 1b and 1c).

In view of the combined cutaneous and radiological findings, thyroid function tests were checked and found to be grossly abnormal, with an initial fT4 of 7.1 pmol/L (normal range 9-20 pmol/L) and a TSH of 115.4 mU/L (normal range 0.35-4.94 mU/L). They were repeated a day later, showing a fT4 of < 5.0 pmol/L, a fT3 of 2.3 pmol/L (normal range 3.0-9.28 pmol/L) and a TSH of 102.5 mU/L, in keeping with

consumptive hypothyroidism. Following consultation with the pediatric endocrinologists, levothyroxine at a dose of 9.6 µg/kg once daily was commenced. Eleven days later, TSH had normalised to 5.33 mU/L and fT4 was appropriately elevated at 29.6 pmol/L (Figure 2).

Following discussion with colleagues in dermatology and cardiology, the patient was started on propranolol 1 mg/kg once daily, in two divided doses (6,12,13,14,15). This was escalated to 2 mg/kg after five days, with close monitoring of blood pressure, heart rate and capillary glucose levels. Treatment was well tolerated with no documented side effects, and within two days of commencing propranolol gamma-glutamyl transpeptidase had decreased from 522 to 426 U/L (normal range 6-42 U/L) and continued to do so in a linear fashion (Figure 3). This coincided with clinical improvement and a subsequent ultrasound at eighteen weeks of age confirmed improvement in the hepatomegaly, with a reduction in the size and number of lesions. This correlated with involution of the cutaneous hemangiomas. Post-treatment the child is well, with normal developmental milestones.

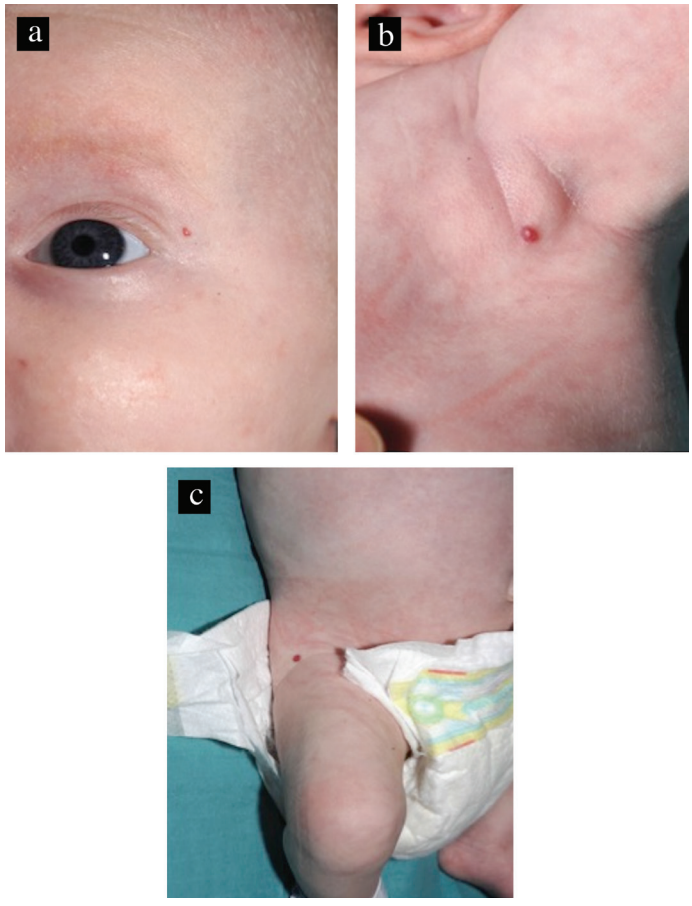

Figure 1. Cutaneous infantile hemangiomas a) at the left lateral canthus, b) left axilla, c) and right lateral thigh

Discussion

The potential for consumptive hypothyroidism, hepatic and cardiac failure, and abdominal compartment syndrome prompted Dickie et al (16) to recommend that an abdominal ultrasound should be obtained to assess for IHH in any infant (symptomatic or asymptomatic) younger than six months of age who presents with five or more cutaneous IH. This recommendation is in line with Horii et al (5), who confirmed the trend for a greater risk of IHH with increasing numbers of cutaneous IH. However, it has also been reported that IHH can cause liver disease in the absence of any cutaneous lesions and this case, where only three small and easily missed cutaneous IH were identified, highlights the importance of having a low threshold to perform abdominal ultrasound in a child with any cutaneous lesions and systemic compromise (16). The signs of systemic compromise may be subtle, and include failure to thrive (secondary to underlying thyroid or cardiac dysfunction) and feeding difficulties. The presence of hepatomegaly on clinical examination should expedite radiological investigation.

Yeh et al (6) recognised that cutaneous IH are heterogeneous in morphology, varying from small papules to large

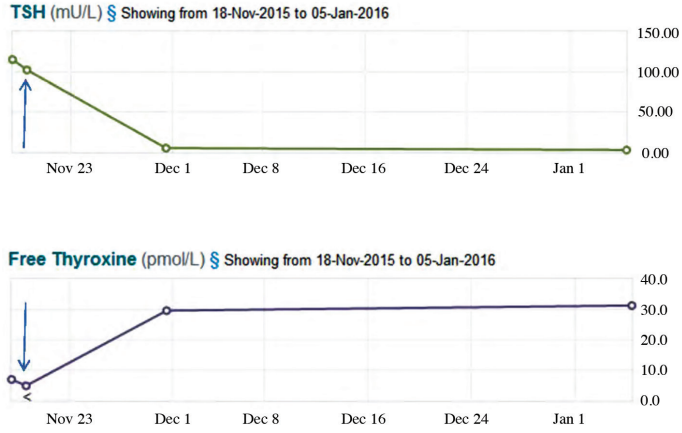

Figure 2. Trends in thyroid stimulating hormone and free thyroxine over time after treatment with levothyroxine at 9.6 micrograms/kg/day

TSH: thyroid stimulating hormone

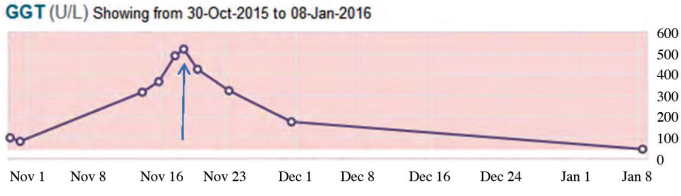

Figure 3. Trend in gamma-glutamyl transpeptidase over time. The arrow indicates when propranolol was commenced

GGT: gamma-glutamyl transpeptidase

segmental areas of involvement. In their case series of four infants with diffuse IHH, all the cutaneous IH were firm, thick, dome-shaped nodules. They recognised that further reports on the morphology of cutaneous IH in the setting of diffuse IHH would be of interest to determine if this could be used as a predictor for the diffuse pattern of hepatic disease (6). Again, our case emphasises that not just the number, but also the morphology of cutaneous IH cannot always be reliably used as an indicator of internal and systemic involvement.

The importance of consumptive hypothyroidism as a diagnosis mandates screening for thyroid abnormalities in those infants with identified IHH, particularly those with the diffuse subtype (5,6). Consultation with endocrinology for prompt and specialist management of hypothyroidism is imperative if growth and irreversible intellectual retardation are to be prevented (6,10). In 2000, a report on severe hypothyroidism in the context of IHH suggested that "given the adaptive capacity of the thyroid gland, it is likely that only patients with both high levels of D3 activity and large tumour burdens are at risk for hypothyroidism" (7). This statement underpins the rationale as to why consumptive hypothyroidism is most prevalent in the diffuse subtype of IHH. The aetiology of elevated D3 in IHH is not fully understood, but some postulate that it is due to similarities between the endothelial cells in hemangiomas and those in placenta, which share certain immunohistochemical markers such as GLUT-1. Furthermore, it has been proposed that IH could be derived from placental angioblasts, and would explain the placenta-like characteristics of IHH such as high D3 activity and self-limited growth (10,17,18). Whatever the cause of consumptive hypothyroidism, clinicians must be mindful of the sometimes recalcitrant nature of this specific form of hypothyroidism and be willing to quickly escalate to higher than usual doses of levothyroxine and/or liothyronine in order to minimise the risk of long term sequelae. The dose required varies on an individual basis; in this case there was a rapid and sustained response to a dose of 9.6 µg/kg levothyroxine once daily (equivalent to a total dose of 37.5 µg/day). Much higher doses have been reported in the literature, with Emir et al (19) reporting the use of levothyroxine 75 µg/day in a female infant with IHH and associated consumptive hypothyroidism, and most recently Al Tasseh et al (20) documenting a dose of levothyroxine 25 µg/kg/day in order to achieve a euthyroid state in a 3.5-month-old male with diffuse IHH.

Although a percentage of patients with IHH may experience spontaneous regression, the development of systemic and life-threatening complications merits prompt treatment (21). Propranolol (a nonselective beta-blocker) has evolved to become a well accepted treatment option for cutaneous IH since its serendipitous discovery in 2008 (22). Propranolol has the combined advantage of promoting more rapid involution of the hemangioma, in addition to halting its growth.

More recently, there has been a growing body of evidence suggesting the benefit of propranolol in IHH (6,12,13,15). Traditional treatments for IHH have included systemic steroids, interferon and vincristine, all of which are associated with potentially severe or dangerous side effects (15). Conversely, propranolol is regarded as a well-tolerated treatment with a favourable risk-benefit ratio. In a case series of eight infants with IHH and diffuse neonatal hemangiomatosis, Mazereeuw-Hautier et al (14) reported rapid and dramatic efficacy of propranolol in all cases, both in the presence and absence of heart failure, and irrespective of whether it was used as a single agent or in combination with other therapies. No side effects of the drug were reported. These findings led them to conclude that propranolol is a valid first line treatment for IHH.

Yeh et al (6) were more cautious in their recommendations having reported four cases of diffuse IHH, and advocated early treatment with combined corticosteroids and propranolol, whilst acknowledging that the use of propranolol in infants with symptomatic IHH merits further study to elucidate if propranolol alone or in combination with steroids is most optimal.

Here we report a further case of diffuse IHH successfully managed with propranolol as a single, first line agent, well tolerated and with no adverse effects. Early recognition of coexisting consumptive hypothyroidism and cardiac failure, coupled with careful dermatological examination in the absence of obvious or numerous cutaneous clues, resulted in prompt involvement of the relevant specialties and timely treatment. More research is needed to fully understand the pathophysiology underlying systemic decompensation in diffuse IHH and to understand the exact mechanism of action of propranolol when used as a first line treatment in this context.

Ethics

Informed Consent: Written parental consent for both case report and images obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Victoria Campbell, Rachel Beckett, Noina Abid, Susannah Hoey, Concept: Victoria Campbell, Susannah Hoey, Design: Victoria Campbell, Susannah Hoey, Data Collection or Processing: Victoria

Campbell, Analysis or Interpretation: Victoria Campbell, Literature Search: Victoria Campbell, Writing: Victoria Campbell, Rachel Beckett, Noina Abid, Susannah Hoey.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Kilcline C, Frieden IJ. Infantile hemangiomas: how common are they? A systematic review of the medical literature. Pediatr Dermatol 2008;25:168-173.
2. Drolet BA, Esterly NB, Frieden IJ. Hemangiomas in children. New Engl J Med 1999;341:173-181.
3. Chen XD, Ma G, Chen H, Ye XX, Jin YB, Lin XX. Maternal and perinatal risk factors for infantile hemangioma: A case-control study. Pediatr Dermatol 2013;30:457-461. Epub 2012 Dec 26
4. Christison-Lagay ER, Burrows PE, Alomari A, Dubois J, Kozakewich HP, Lane TS, Paltiel HJ, Klement G, Mulliken JB, Fishman SJ. Hepatic hemangiomas: subtype classification and development of a clinical practice algorithm and registry. J Pediatr Surg 2007;42:62-68.
5. Horii KA, Drolet BA, Frieden IJ, Baselga E, Chamlin SL, Haggstrom AN, Holland KE, Mancini AJ, McCuaig CC, Metry DW, Morel KD, Newell BD, Nopper AJ, Powell J, Garzon MC; Hemangioma Investigator Group. Prospective study of the frequency of hepatic hemangiomas in infants with multiple cutaneous infantile hemangiomas. Pediatr Dermatol 2011;28:245-253. Epub 2011 Apr 26
6. Yeh I, Bruckner A, Sanchez R, Jeng MR, Newell BD, Frieden IJ. Diffuse infantile hepatic hemangiomas: a report of four cases successfully managed with medical therapy. Pediatr Dermatol 2011;28:267-275. Epub 2011 Apr 26
7. Huang SA, Tu HM, Harney JW, Venihaki M, Butte AJ, Kozakewich HP, Fishman SJ, Larsen PR. Severe hypothyroidism caused by type 3 iodothyronine deiodinase in infantile hemangiomas. New Eng J Med 2000;343:185-189.
8. Cho YH, Taplin C, Mansour A, Howman-Giles R, Hardwick R, Lord D, Howard NJ. Case report: consumptive hypothyroidism consequent to multiple infantile hepatic hemangiomas. Curr Opin in Pediatr 2008;20:213-215.
9. Mouat F, Evans HM, Cutfield WS, Hofman PL, Jefferies C. Massive hepatic hemangioendothelioma and consumptive hypothyroidism. J Pediatr Endocrinol Metab 2008;21:701-703.
10. Bessho K, Etani Y, Ichimori H, Miyoshi Y, Namba N, Yoneda A, Ooue T, Chihara T, Morii E, Aoki T, Murakami M, Mushiake S, Ozono K. Increased type 3 iodothyronine deiodinase activity in a regrown hepatic hemangioma with consumptive hypothyroidism. Eur J Pediatr 2010;169:215-221. Epub 2009 Jun 23
11. Fisher DA. Clinical review 19: Management of congenital hypothyroidism. J Clin Endocr Metab 1991;72:523-529.
12. Tan ST, Itinteang T, Leadbitter P. Low-dose propranolol for multiple hepatic and cutaneous hemangiomas with deranged liver function. Pediatrics 2011;127:772-776. Epub 2011 Feb 28
13. Marsciani A, Pericoli R, Alaggio R, Brisigotti M, Vergine G. Letter to the editor: massive response of severe infantile hepatic hemangioma to propranolol. Pediatric Blood & Cancer 2010;54:176.
14. Mazereeuw-Hautier J, Hoeger P, Benlahrech S, Ammour A, Broue P, Vial J, Ohanessian G, Leaute-Labreze C, Labenne M, Vabres P, Rössler J, Bodemer C. Efficacy of propranolol in hepatic infantile hemangiomas with diffuse neonatal hemangiomatosis. J Paediatr 2010;157:340-342. Epub 2010 May 20
15. Mhanna A, Franklin WH, Mancini AJ. Hepatic infantile hemangiomas treated with oral propranolol -- a case series. Pediatr Dermatol 2011;28:39-45. Epub 2011 Jan 25.
16. Dickie B, Dasgupta R, Nair R, Alonso MH, Ryckman FC, Tiao GM, Adams DM, Azizkhan RG. Spectrum of hepatic hemangiomas: management and outcome. J Pediatr Surg 2009;44:125-133.
17. Mo JQ, Dimashkieh HH, Bove KE. GLUT1 endothelial reactivity distinguishes hepatic infantile hemangioma from congenital hepatic vascular malformation with associated capillary proliferation. Hum Pathol 2004;35:200-209.
18. North PE, Waner M, Mizeracki A, Mrak RE, Nicholas R, Kincannon J, Suen JY, Mihm MC Jr. A unique microvascular phenotype shared by juvenile hemangiomas and human placenta. Arch Dermatol 2001;137: 559-570.
19. Emir S, Ekici F, Ikiz MA, Vidinlisan S. The association of consumptive hypothyroidism secondary to hepatic hemangioma and severe heart failure in infancy. Turk Pediatri Ars 2016;51:52-56.
20. Al Tasseh F, El-Khansa M, Abd O, Khalek AA, El-Rifai N. Diffuse hepatic hemangioma with single cutaneous hemangioma: an alerting occurrence. Clin Case Rep 2017;5:887-890.
21. Konrad D, Ellis G, Perlman K. Spontaneous regression of severe acquired infantile hypothyroidism with multiple liver hemangiomas. Pediatrics 2003;112:1424-1426.
22. Leaute-Labreze C, Dumas de la Roque E, Hubiche T, Boralevi F, Thambo JB, Taieb A. Propranolol for severe hemangiomas of infancy. New Eng J Med 2008;358:2649-2651.

DOI: 10.4274/jcrpe.5308

J Clin Res Pediatr Endocrinol 2018;10(3):299-300

The Importance of Gestation-Adjusted Birthweight Centile in Assessment of Fetal Growth in Metabolic Conditions

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Dear Editor,

While we welcome the intention of Li et al. (1) to assess the effect of intrahepatic cholestasis of pregnancy (ICP) upon newborn birthweight, we have concerns about the authors' interpretation of their findings because the birthweights in their meta-analysis were not corrected for gestational week or reported as a customised birthweight centile.

ICP is associated with delivery at an earlier gestational age (2), and this is reported in all of the cited manuscripts contributing to this meta-analysis (3,4,5,6,7). Using the data presented in Table 2 from the manuscript, the mean difference in gestational week of birth for the combined patient cohort of these 5 studies is 1.3 weeks (ICP 37.6 ± 1.9, control 38.9 ± 1.6) (1). The authors report a mean difference in birthweight of 175 g (95% confidence interval 48-301) between ICP and control babies. Between 36 and 40 weeks' gestation, the average weekly increase in birthweight typically exceeds this figuret (8), as shown by the Canadian population data which indicates that for female fetuses the average weekly weight gain is 183 g, whilst for male fetuses the weekly weight gain is 195.75 g.

An accurate birthweight centile should be adjusted taking into consideration the baby's sex, maternal height, weight, parity and ethnic group (9). These data were not reported in the meta-analysis by Li et al (1). However, the study by Martineau et al (6) reports customised birthweight centiles in ICP compared to normal pregnancies. This would be consistent with the reported increased ponderal index of ICP babies reported in the cited manuscript of Cheng et al (4). Thus, despite a lower absolute birthweight (at earlier gestations), the babies of ICP mothers in this study were unlikely to have features of intrauterine growth restriction.

This is consistent with multiple other studies reporting babies of ICP pregnancies to have increased birthweight centiles, including two large population cohorts reporting birthweight centiles of 6146 ICP babies compared with over 1.2 million controls (10,11); in contrast to the 198 (ICP) and 189 (control) babies reported in this meta-analysis (1).

This is of critical relevance to the management of women with ICP. The management of fetuses at risk of small for gestational age necessitates serial growth ultrasound scanning, for which there is no indication in ICP. Conversely, babies who are large for gestational age at birth have a longer-term risk of adverse metabolic health, as has been reported for babies of ICP pregnancies (12). Furthermore, the increased risk of larger fetuses in ICP will influence obstetric advice with regard to the risk of birth dystocia. It may also result in testing affected women for gestational diabetes, which is more prevalent in ICP (13).

We believe this manuscript could mislead affected women and their clinicians. Li et al's (1) findings reflect the fact that babies born to mothers with ICP are typically of a lighter weight secondary to being born at an earlier gestation. However, most studies demonstrate that they are of a greater birth weight centile for gestational week at delivery.

Keywords: Intrahepatic, cholestasis, pregnancy, birthweight, centile

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Conflict of interest: None declared
Received: 08.09.2017
Accepted: 28.12.2017

Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: Caroline Ovadia, Analysis or Interpretation: Caroline Ovadia, Hanns-Ulrich Marschall, Catherine Williamson, Literature Search: Caroline Ovadia, Writing: Caroline Ovadia, Hanns-Ulrich Marschall, Catherine Williamson

Financial Disclosure: Work was funded by Tommy's, ICP Support and the NIHR Biomedical Research Centre at Guy's and St Thomas' NHS Foundation Trust and King's College London. The views expressed are those of the authors and not necessarily those of the NHS, NIHR or the Department of Health.

References

1. Li L, Chen YH, Yang YY, Cong L. Effect of intrahepatic cholestasis of pregnancy on neonatal birth weight: a meta-analysis. J Clin Res Pediatr Endocrinol 2018;10:38-43. Epub 2017 Aug 21
2. Glantz A, Marschall HU, Mattsson LA. Intrahepatic cholestasis of pregnancy: Relationships between bile acid levels and fetal complication rates. Hepatology 2004;40:467-474.
3. Alsulyman OM, Ouzounian JG, Ames-Castro M, Goodwin TM. Intrahepatic cholestasis of pregnancy: perinatal outcome associated with expectant management. Am J Obstet Gynecol 1996;175:957-960.
4. Cheng XY, Zhang LJ, Lin L, Liu J, Ding YL. Relationship of fetal total bile acid and the change of fetal pancreas endocrine secretion and its impact on fetal growth and development in intrahepatic cholestasis of pregnancy. Zhonghua Fu Chan Ke Za Zhi 2009;44:23-26.
5. Kowalska-Kanka A, Maciejewski T, Niemiec KT. The concentrations of bile acids and erythropoietin in pregnant women with intrahepatic cholestasis and the state of the fetus and newborn. Med Wieku Rozwoj 2013;17:232-245.
6. Martineau MG, Raker C, Dixon PH, Chambers J, Machirori M, King NM, Hooks ML, Manoharan R, Chen K, Powrie R, Williamson C. The metabolic profile of intrahepatic cholestasis of pregnancy is associated with impaired glucose tolerance, dyslipidemia, and increased fetal growth. Diabetes Care 2015;38:243-248. Epub 2014 Dec 12
7. Ersoy AO, Kirbas A, Ozler S, Ersoy E, Ozgu-Erdinc AS, Ergin M, Erkaya S, Uygur D, Danisman N. Maternal and fetal serum levels of caspase-cleaved fragments of cytokeratin-18 in intrahepatic cholestasis of pregnancy. J Matern Fetal Neonatal Med 2016;29:562-566. Epub 2015 Feb 10
8. Kramer MS, Platt RW, Wen SW, Joseph KS, Allen A, Abrahamowicz M, Blondel B, Bréart G; Fetal/Infant Health Study Group of the Canadian Perinatal Surveillance System. A New and Improved Population-based Canadian Reference for Birth Weight for Gestational Age. Pediatrics 2001;108:35.
9. Gardosi J, Francis A. Customised Weight Centile Calculator. Gestation Network, www.gestation.net
10. Wikström Shemer E, Marschall HU, Ludvigsson JF, Stephansson O. Intrahepatic cholestasis of pregnancy and associated adverse pregnancy and fetal outcomes: a 12-year population-based cohort study. BJOG 2013;120:717-723. Epub 2013 Feb 19
11. Geenes V, Chappell LC, Seed PT, Steer PJ, Knight M, Williamson C. Association of severe intrahepatic cholestasis of pregnancy with adverse pregnancy outcomes: a prospective population-based case-control study. Hepatology 2014;59:1482-1491. Epub 2014 Feb 26
12. Papacleovoulou G, Abu-Hayyeh S, Nikolopoulou E, Briz O, Owen BM, Nikolova V, Ovadia C, Huang X, Vaarasmaki M, Baumann M, Jansen E, Albrecht C, Jarvelin MR, Marin JJ, Knisely AS, Williamson C. J Clin Invest 2013;123:3172-3181. Epub 2013 Jun 24
13. Martineau M, Raker C, Powrie R, Williamson C. Intrahepatic cholestasis of pregnancy is associated with an increased risk of gestational diabetes. Eur J Obstet Gynecol Reprod Biol 2014;176:80-85. Epub 2014 Jan 7

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27-29 September 2018, Athens, Greece

EASD 2018 (54th Annual Meeting of The European Association for the Study of Diabetes)

1-5 October 2018, Berlin, Germany

ISPAD 2018 (44th Meeting of The International Society for Pediatric and Adolescent Diabetes)

11-14 October 2018, Hyderabad, India