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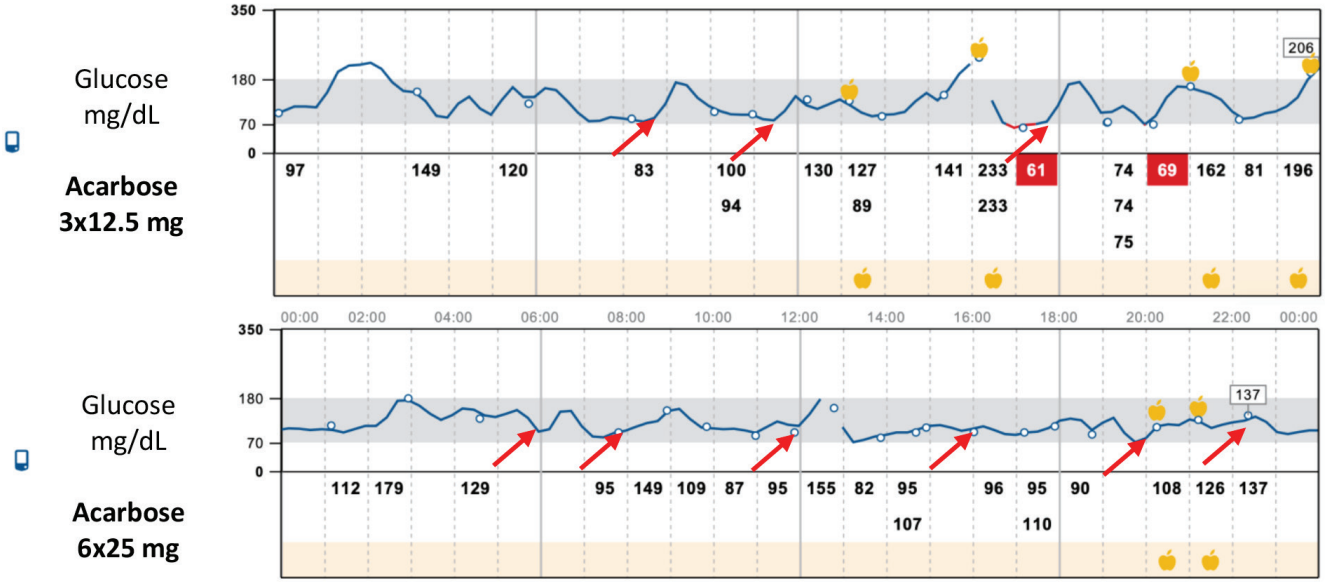

Continuous Glucose Monitoring Systems and the Efficacy of Acarbose Treatment in Cystic Fibrosis-related Dysglycemia

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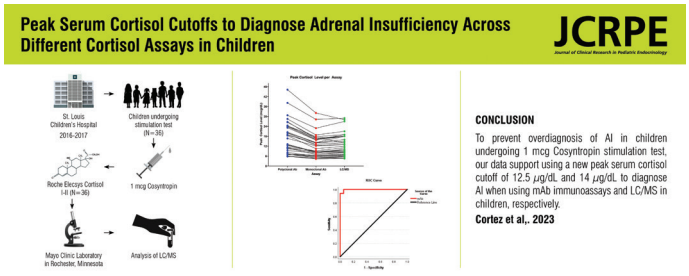

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REVIEW

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J Clin Res Pediatr Endocrinol 2025;17(1):1-8

Rationale for Long-acting Growth Hormone Therapy and Future Aspects

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Abstract

Recombinant growth hormone (GH) is administered as daily subcutaneous injections. Daily treatment can be challenging for children/adolescents, as well as for parents and/or caregivers, such as legal representatives or guardians of children in institutional care. Challenges associated with daily treatment may result in missing several doses but non-adherence with treatment leads to inadequate growth response. As an inadequate growth response does not meet criteria for continuing treatment, payers (commercial or public) may decide to end reimbursement. Novel long-acting GH (LAGH) formulations with extended half-life may be administered less frequently and aim to improve patient convenience and consequently to improve adherence and responses to treatment. LAGH formulations can restore growth velocity and body composition as effectively as daily treatment, without unexpected adverse effects, as reported in randomized clinical trials.

Keywords: Recombinant growth hormone, long-acting growth hormone, treatment adherence, review, future aspects

Introduction

History of Growth Hormone Therapy

In 1921, Evans and Long demonstrated the efficacy of growth hormone (GH) from bovine pituitary gland on growth in rats (1). Until the 1930s, GH was investigated for its effects not only on growth but also on glucose metabolism, proteins, minerals, and lipids. In 1944, Li and Evans isolated GH from bovine and human pituitary glands and identified GH as a protein of 191 amino acids (2). During the 1940s and 1950s, GH was purified from various species and tested in animal and human subjects. In 1979, human GH (hGH) could be expressed by recombinant DNA technology (3). In 1985, the United States (US) Food and Drug Administration (FDA) approved recombinant human GH (rhGH) produced in *E. coli*. Long-term effects of rhGH were monitored in several studies, including the National Collaborative Growth Study and Kabi International Growth Study. Over a period of more than 25 years, data from nearly two hundred thousand patients treated with rhGH, and studies on long-term efficacy and safety have been presented (4,5).

Daily Growth Hormone Therapy

The first study on GH dosing used pituitary hGH administered twice weekly. Later, further increase was shown in growth velocity when the three times weekly pit-hGH regimen switched to once daily injections (6,7). Current recommendation for GH therapy involves daily rhGH injections. Daily dose of GH may vary from 25 to

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43 µg/kg/body weight while the dose can be increased in puberty or syndromic disorders with short stature (8).

Adherence to Growth Hormone Therapy

Adherence to GH therapy is critical to treatment success. Poor adherence is the leading cause of inadequate growth velocity in patients receiving GH therapy (9). In 2022, 12-month treatment adherence rates were reported to vary from 73.3 to 95.3% with a mean adherence rate of 79.3% in a systematic review of 11 eligible studies (10). In studies conducted in Türkiye, the adherence rate was 92% in a series of 689 cases; a multicenter study in 216 patients assessed 1-year adherence rate and reported that poor adherence correlated with lower height velocity and lower insulin-like growth factor-1 (IGF-1) response (11,12). Poor adherence rates increase over timeand correlate with duration of GH therapy (13). The national survey of GH in New Zealand concluded that linear growth could decrease significantly in patients missing more than one dose in a week (14).

Paradigms Improving Adherence with Growth Hormone Therapy

Treatment adherence can be affected by a variety of factors including needle phobia (fear, reasons associated with injections), forgetfulness, treatment duration, low socioeconomic status, type of injection device used, unmet treatment expectations, and poor understanding of consequences of missed doses (15). Treatment adherence was assessed during the first 2-years in a study conducted in 110 patients and negative correlations were found between treatment adherence and age, pretreatment growth velocity and treatment duration while treatment adherence was positively correlated with parents' educational attainment (9). Treatment adherence is further affected by access to medicines, patients', and parents' motivation, and having received necessary training. Other significant factors include individual differences in response to GH therapy, diagnostic differences, age at diagnosis, current age, and dose of medication (16).

Another factor reducing treatment adherence was defined as injection refusal among adolescents and the importance of family support was underlined (17). Regional differences may affect adherence with treatment. Medication costs, inability to access medicine, concerns about long-term complications, treatment fatigue due to long-term injections, dissatisfaction with treatment outcomes, and painful injections were highlighted as reasons for non-adherence with treatment among 169 patients included in a study conducted in Iran (18).

The most remarkable reason for treatment discontinuation is treatment fatigue and dosing interval lengthening is followed by discontinuation over time. Treatment duration negatively correlates with adherence as daily injections may become more challenging either for GH-deficient patients or for their parents, over time. Due to the challenges associated with daily treatments, once weekly, long-acting GH (LAGH) therapy is expected to improve adherence with treatment and convenience for patients.

Long-Acting Growth Hormone Formulations in Use

LAGH analogues approved in Asia include valtropin/declage (Eutropin Plus®- South Korea) and PEG-rhGH (Jintrolong®- China). The LAGH analogue Eutropin Plus® was previously approved but not marketed in Europe, whereas the LAGH analogue somapacitan-beco (Sogroya®) was approved in US and European Union (EU), Canada, Japan, the LAGH analogue lonapegsomatropin-tcgd, Skytrofa® was approved in US and EU, and LAGH analogue Somatrogon (Ngenla®) was approved in EU, Australia, Canada, Japan, United Kingdom, Brazil, India, and US, and most recently in Türkiye and Saudi Arabia. Other LAGH analogues are in various stages of clinical development. This article is focused on somatrogon (Ngenla®), lonapegsomatropin-tcgd (Skytrofa®), and somapacitan-beco (Sogroya®) considering that these LAGH formulations have been approved by the US FDA and European Medicines Agency (EMA) for use in children and adolescents (Table 1) (19).

Pharmacological Characteristics of Long-acting Growth Hormone Formulations

hGH is a protein containing 191 amino acids with a molecular weight of 22 kDa and an isoelectric pH of 5.8. Currently available rhGH formulations have similar molecular weight and characteristics to hGH although not identical. rhGH has a half-life of 3 to 4 hours following subcutaneous injection and 0.36 hours following intravenous injection with an effect duration of less than 24 hours. Blood GH levels vary depending on age, sex, physiological state, and environmental conditions. GH secretion surges and several peaks occur throughout the day and shows an episodic and pulsatile pattern with increasing frequency during sleep (particularly in the second half of the night) in humans. Therefore, treatment with once daily rhGH injections does not mimic the normal biological pattern of hGHrelease and provide a unimodal blood level pattern. However, rhGH may provide an adequate growth response in children and adolescents with GH deficiency (20,21). As with once-daily rhGH formulations, the pharmacodynamics of LAGH formulations may not be identical with hGH secretion, but treatment responses are not inferior to those induced by once-daily formulations (19).

Table 1. In use long-acting growth hormone formulations by Food and Drug Administration and European Medicines Agency of approval

	Somatrogon	Lonapegsomatropin	Somapacitan
Approval	US, EU, Canada, Japan, Australia, Brazil, Taiwan, UAE, India, KSA, Türkiye	US, EU	US, EU, KSA, Canada, Japan

US: United States, EU: European Union, KSA: Kingdom of Saudi Arabia, UAE: United Arab Emirates

Several techniques, including depot formulations, PEGylated formulations, pro-drug formulations, non-covalent albumin binding GH formulations, and GH fusion proteins have been used in the development process of LAGH analogues to extend the half-life of the formulation. Approved LAGH formulations are presented in Table 2 (19).

Long-acting therapies have been previously developed for several medical conditions including hemophilia and type 2 diabetes and such therapies have proved to be safe and are associated with higher treatment adherence, greater patient satisfaction and improved quality of life (10,22,23).

The prodrug formulation ACP-001 (Skytrofa®, lonapegsomatropin-tcgd) is an unmodified rhGH transiently conjugated with a methoxy-PEG containing carrier molecule which is hydrolysable depending on pH and temperature. ACP-001, was approved by the US FDA and EMA in 2021 for use in pediatric patients (aged > 1 year with a body weight of > 11.5 kg) (24).

The non-covalent albumin binding GH formulation NNC0195-0092 (Sogroya®, Somapacitan-beco) was approved by the FDA in August 2020 for use in adults with GH deficiency. Non-covalent binding of albumin to GH with single point mutation, by a terminal fatty acid linker resulted in a reduced clearance rate and a longer half-life. The phase 3 pediatric study REAL-4 started in 2019 (25,26).

Somatrogon is a chimeric product consisting of the fusion of rhGH with three copies of carboxyl-terminal peptide of human chorionic gonadotropin β-subunit (molecular weight: 47.5 kDa). In historical process, as shownin Table 3, the development of LAGH formulations is a long process that will require accumulated experience and allocation of a large budget. Any approved GH formulation is obviously a product of a challenging process and experience. Nonetheless, further efforts are still needed (27,28).

Long-acting Growth Hormones

In theory, clinical indications for the use of LAGH formulations include needle phobia in children, non-adherence in adolescents, pediatric patients without a consistent caregiver/guardian, children in institutional care, treatment fatigue in patients on long-term therapy when compared with once-daily GH formulations. In addition, the ability to administer LAGH at any time in a given day may be considered as an advantage of once weekly-formulations over once-daily formulations. LAGH preparations may improve patient adherence, quality of life and clinical outcomes (29).

LAGH formulations approved by the US FDA and EMA for use in children and adolescents include somatrogon (Ngenla®), lonapegsomatropin-tcgd (Skytrofa®) and somapacitan-beco (Sogroya®). In standard 52-week phase 3 clinical trials, once weekly lonapegsomatropin, somatrogon and somapacitan have been found to yield non-inferior height velocities. These three LAGH formulations have similar safety profiles to daily GH in children with pediatric GH deficiency (29).

Somatrogon (Ngenla®)

Somatrogon is the first LAGH formulation approved in Türkiye. Somatrogon significantly reduces treatment burden compared to daily GH (Genotropin®) therapy and its effectiveness is non-inferior (30). Somatrogon is produced by recombinant DNA technology and administered subcutaneously. Since somatrogon is a fusion protein, its half-life is long, its renal clearance is low, and its diffusion into the growth plate is good (31). Somatrogon is indicated for the treatment of children from three years of age with GH deficiency. In a randomized controlled phase 2 study in which somatrogon (at doses of 0.25, 0.48, 0.66 mg/kg/week) or daily GH (Genotropin® at a dose of 0.034 mg/kg/day), was administered to 53 prepubertal GH deficient children, the growth responses of somatrogon at doses of 0.25, 0.48, 0.66

Table 2. Characteristics of LAGH formulations by Food and Drug Administration and European Medicines Agency of approval

	Somatrogon	Lonapegsomatropin	Somapacitan
Brand name	Ngenla®	Skytrofa®	Sogroya®
Mechanism	Fusion protein	Prodrug	Increased albumin-binding

LAGH: long-acting growth hormone

Table 3. Long-acting growth hormone formulations (in development, approved/not approved)

Product	Mechanism	Frequency of administration	Current status
ALTU-238	Depot	7 days	No longer being developed
Nutropin depot	Depot	14 days	Removed from market
Eutropin plus	Depot	7 days	Approved in South Korea, EMA
ARX201	PEGylation	7 days	No longer being developed
BBT-031	PEGylation	7 days	Developing stopped at preclinical studies
PHA-794428	PEGylation	7 days	No longer being developed
NNC126-0083	PEGylation	7 days	No longer being developed
Jintrolong	PEGylation	7 days	Approve in China
Lonapegsomatropin	Prodrug	7 days	Approved in USA, EU
Somatrogon	Fusion protein	7 days	Approved in USA, EU, Canada, Australia, Türkiye, Japan, Kingdom of Saudi Arabia
AG-B1512	Fusion protein	14 or 28 days	Pre-clinical studies
ALT-P1	Fusion protein	Unknown	Developing stopped at phase 2
Profuse	Fusion protein	1 month	Developing stopped at preclinical studies
GX-H9	Fusion protein	7-14 days	Phase 3 studies
HM10560A	Fusion protein	7-14 days	Phase 3 studies
JR-142	Fusion protein	7 days	Phase 2 studies
Albutropin	Fusion protein	7 days	No longer being developed
Somavaratan	Fusion protein	7, 14 or 28 days	No longer being developed
Somapacitan	Increased albumin binding	7 days	Approved in USA, EU, KSA, Canada, Japan

US: United States, EU: European Union, EMA: European Medicines Agency, KSA: Kingdom of Saudi Arabia

mg/kg/week were 7.73 ± 1.89, 7.54 ± 1.28 and 8.81 ± 1.12 cm/year respectively (32). In the phase 3 study in which somatrogon (0.66 mg/kg/week) and somatropin (0.24 mg/kg/week, Genotropin®) were administered to 228 children with GH deficiency, the annual change in height standard deviation score (SDS) was similar (33). These studies have shown that long-acting somatrogon is well tolerated and causes mild to moderate side effects similar to daily GH, such as myositis, injection side pain, water retention including edema, arthralgia, carpal tunnel syndrome, and benign intracranial hypertension. These studies suggested that mean/average IGF-1 levels should be taken at four days post somatrogon administration. The authors reported that this sampling time for IGF-1 level was a more useful and representative time for overall systemic exposure to IGF-1 levels. Somatrogon has been found to have similar safety and tolerability to daily GH. The currently recommended/approved dosage of somatrogon in our Türkiye is 0.66 mg/kg body weight administered once weekly by subcutaneous injection (33).

When switching from daily GH therapy, somatrogon may be administered subcutaneously at a weekly dose of 0.66 mg/kg body weight on the day following the last daily injection. In the phase 3 study in which somatrogon (0.66 mg/kg/week) was given to children with pituitary GH deficiency, the average IGF-1 SDS value was 0.66, while this value for daily GH was -0.69 (29). Serum IGF-1 concentrations should be monitored regularly and blood samples should be collected four days after the prior dose. It is recommended to maintain IGF-1 concentrations within upper normal range without exceeding +2 SDS. If serum IGF-1 concentrations exceed the mean reference value by >2 SDS, the dose of somatrogon should be reduced by 15%. Higher dose reductions may be required in some patients. Height velocity should be monitored particularly during the first year of treatment and treatment adherence should be supervised. Zadik et al. (32) found treatment compliance to be >90% in the patient group followed for five years with somatrogon treatment.

When needed, the day of weekly injection can be changed if time from the last injection is more than 72 hours. If a dose is missed, the missed dose can be administered as soon as possible, if the delay is less than three days. If the delay is >3 days, the missed dose should be skipped and the next dose should be administered on the scheduled day. Underdose and overdose should be managed based on the experience with daily GH therapy. At recommended doses, significant changes have not been reported in insulin sensitivity and glucose metabolism during treatment with somatrogon. Other effects on glucose metabolism are similar to those of daily GH therapy (32).

Somatrogon is not recommended in pediatric patients with multiple pituitary hormone deficiency below three years of age due to the challenges associated with the management of the risk for hypoglycemia. There is a scarcity of research into this specific issue (32).

A survey of 24 pediatric endocrinologists from 12 countries with experience in GH therapy was undertaken on topics such as GH adherence monitoring, device use, injection regimen, and disclosure of missed injections to address concerns of the patient's family or caregiver. In general, 75% of pediatric endocrinologists preferred weekly somatrogon, 79.2% found it more useful, 83.3% stated that they would prefer to prescribe somatrogon in the future, and 50% stated that they thought it was beneficial for patients. It was also observed that somatrogon provided 62.5% satisfaction among physicians in reducing the frequency of injections and reducing the burden on family and caregivers (34). In a survey conducted on the families and caregivers of 87 GH-deficient pediatric patients, somatrogon was reported to be the more preferred treatment method with a lower treatment burden than daily GH therapy (35). Anti-drug antibodies developed against the drug have not been shown to have any effect on growth when using somatrogon (29). In a meta-analysis, it was predicted that somatrogon provided higher near-final height compared to daily GH in pediatric GH deficiency cases, improved the quality of life, and reduced the cost per cm (36).

Zelinska et al. (37) reported that there was no significant change in glucose and HbA1c levels in patients using somatrogon.

Somapacitan-beco (Sogroya®)

Somapacitan is a LAGH with an extended half-life because of reversible non-covalent binding to albumin. Somapacitan was approved for the treatment of patients aged 2.5 years and older. Somapacitan is the second LAGH approved in Türkiye. Somapacitan is produced by recombinant DNA technology and is administered by subcutaneous injections. While somapacitan provided annual growth of 7.5, 9.7, and 11.7 cm/year at doses of 0.04, 0.08, and 0.16 mg/kg/week, respectively, daily GH (Norditropin®) provided 9.9 cm/year growth (38). In Türkiye, the suggested dose for GH deficient pediatric patient is 0.16 mg/kg/week. In the phase 3 REAL-4 study, in 200 GH deficient children aged 2.5-11 years, somapacitan (0.16 mg/kg/week) provided growth non inferior to daily GH (0.034 mg/kg/day) (11.2 cm/year vs. 11.7 cm/year, respectively). Side effects, such as nasopharyngitis, fever, headache, and injection site pain were seen in 5% of the cases (26). In the study where somapacitan and daily GH (Norditropin®) treatment was given for three years, the growth velocity SDS change was 2.9, 2.3, and 2.4 for somapacitan and 2.1 for daily GHby year (39). In phase 1, phase 2 (REAL-3), and phase 3 (REAL-4) studies, 1473 pharmacokinetic samples (210 treated with somapacitan) were taken from 210 GH deficient children and IGF-1 SDS values were determined. While the IGF-1 SDS value did not exceed +3 in those receiving somapacitan, it ranged between -2 and +2 in those receiving daily GH (40). In a study, it was also reported that while the adverse effect rate was 71.1% in those receiving somapacitan, it was 71.4% in those receiving daily GH (39). In a 3-year study comparing somapacitan with daily GH (Norditropin®) treatment, no significant changes in glucose and HBA1c were detected (40). In a study with a small sample size, patients using somapacitan or daily GH were compared in terms of their quality of life and no significant difference was found between them (41). The approved dose for initiating treatment with Somapacitan or switching from daily GH therapy is 0.16 mg/kg once weekly (39).

Lonapegsomatropin-tcgd (Skytrofa®)

Lonapegsomatropinwas the first FDA-approved LAGH formulation. Lonapegsomatropin is a preservative-free, reversible PEGylated rhGH preparation. Therefore, the treatment cost of lonapegsomatropin was calculated to be 20-40% higher than the preservative-free Genotropin® treatment (42). FDA approval was given for use in patients aged one year and older or with a weight more than 11.5 kg. In the study comparing lonapegsomatropin (0.24 mg/kg/week) and daily GH (0.24 mg/kg/week, Genotropin®), the annual growth rate was found to be 11.2 and 10.3 cm, respectively (43).

In the 104-week heiGHt, fliGHt and continued enliGHten study comparing lonapegsomatropin and daily GH, it was shown that the height SDS value improved from -2.89 to -1.37 and from -3 to -1.5, respectively. In this study, no adverse effects were reported except fever and local reaction. In this study, mean IGF-I value five days after lonapegsomatropin injection was +1.46 SDS (44).

The recommended dose for starting anddose for switching from daily GHis 0.24 mg/kg body weight administered subcutaneously once weekly. In addition to adverse effects associated with other formulations, lonapegsomatropin-tcgdalso included a higher risk for pancreatitis. Follow-up recommendations for lonapegsomatropin-tcgdinclude routine monitoring of serum phosphate, alkaline phosphatase and parathormone levels in addition to other recommendation for LAGH formulations. Missed dose should be administered as soon as possible and within less than 2 days. Dosing intervalsshould be at least five

days. Neutralizing anti-drug antibodies were not detected against this active substance during the treatment period of 72 weeks. Of note, these recommendations were presented in the prospectus but have not been reported (38).

Treatment Adherence and Other Expectations with LAGH Formulations

Efficacy and safety, treatment adherence, child's and parents' quality of life, and cost-effectiveness analyses were conducted in a recently published meta-analysis on LAGH analogues vs. daily rhGH therapy. Based on these analyses, treatment adherence varied between 87.2% and 99.7% with daily rhGH therapy and between 99.2% and 99.4% with LAGH analogues.

Although the efficacy and safety of LAGH analogues were comparable to those of daily rhGH formulations, well-designed, medium to long-term studies on quality of life of the child and parents and cost-effectiveness studies are still needed (38).

In a recent online article about somatrogon, non-adherence rates were reported to be as low as 4% for the first year of treatment (adherence rates reported for daily GH formulations in the literature varybetween 65% and 95.3%). A scenario analysis emphasized the improved quality of life and lower costs for cm gained with somatrogon (28). Analyses oflong-term treatment responses, adverse effects, treatment costs, effects on lipid and glucose metabolism, follow-up parameters and safety and efficacy are becoming increasingly important as LAGH formulations are reimbursed, currently.

Theoretical Concerns About LAGH Formulations

The issues of theoretical concern are the effect of LAGH analogues on fat and glucose metabolism, their effectiveness in correcting hypoglycemia in infants with hypoglycemia associated with severe GH deficiency, and their different therapeutic efficacy profiles in different tissues, especially due to the large size of the fusion proteins. When IGF-1 levels above the physiological value are obtained for a very long time; risk statuses for iatrogenic acromegaly, neoplasia and glucose intolerance are unclear. Elevated and high-normal serum IGF-1 levels in early epidemiological studies raised concerns about the potential of an increased risk of malignancies. A safe serum IGF-1 cut-off level is another area requiring further investigation (45).

Future Goals for LAGH Therapy

Theoretical concerns associated with the use of LAGH analogues suggestthe importance of establishing thesafety of various LAGH formulations. Dosages in treatment-naïve patients, dosages in patients switching from daily recombinant therapy to LAGH therapy, potential differences in starting doses, dose adjustments and methodology to be used in dose adjustments, timing of serum IGF-1 measurements, safety, sustainable efficacy, cost-effectiveness, and effects on the quality of life and treatment adherence should be assessed further. There are registries, such as PROGRES and GloBE-Reg. National registries will alsobe useful to collect and analyze data from these patients on a yearly basis and the results should be communicated (46,47).

Reliability, Follow-up Parameters and Unknown Factors in LAGH Therapy

It is important to establish a Future Research Agenda for LAGH therapy to compare weekly and daily GH therapy in long-term treatment responses, to conduct analyses on adverse effects, treatment costs, effects on lipid and glucose metabolism, follow up parameters and safety and efficacy, effects on quality of life and treatment adherence and to update follow-up plans based on data collected from these analyses.

Studies have shown that day four is recommended for optimum IGF-1 evaluation but longitudinal studies are needed to determine IGF-Ilevels after dosing, how to make dose reductions in case of an adverse effect, and risk for developing acromegalia, neoplasia or glucose intolerance. The dose, efficacy and reliability of treatment with LAGH therapies in Turner syndrome, born small for gestational age, Prader-Willi syndrome, Silver-Russell syndrome, intracranial malignancies or other cancersurvivors, the use in severe GH deficiency presenting with neonatal hypoglycemia and the use in patients younger than three years remains to be determined. Further areas requiring additional research include dosing in obese patients, the level of growth response in each individual organ and tissue, neutralizing antibody production and effect for each individual formulation. Several other parameters, including long-term (decades) adherence, treatment costs and growth response also require much more data.

Conclusion

New LAGH with long half-lives provide significant advantages for children and adolescents with treatment incompliance, those receiving multiple treatments, those with additional problems, those with injection fear, those studying in boarding schools, those not raised by their parents, those with low family health literacy, and those >3 years of age diagnosed with GH deficiency. There are uncertainties regarding LAGH therapy in cases diagnosed other than GH deficiency (such as panhypopituitarism, Turner syndrome),

those <3 years of age, those requiring GH therapy after intracranial tumor treatment, and those with elevated IGF1 under treatment. Registry studies with long-term follow-up data are needed.

Footnotes

Authorship Contributions

Concept: Semra Çetinkaya, Erdal Eren, Furkan Erdoğan, Feyza Darendeliler, Design: Semra Çetinkaya, Erdal Eren, Furkan Erdoğan, Literature Search: Semra Çetinkaya, Erdal Eren, Furkan Erdoğan, Feyza Darendeliler, Writing: Semra Çetinkaya, Erdal Eren, Furkan Erdoğan.

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Gender Difference and Changes in the Prevalence of Obesity Over Time in Children Under 12 Years Old: A Meta-analysis

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What is already known on this topic?

Childhood obesity is a global epidemic with an alarming increase. A comprehensive approach including diet, exercise, behavior modification, and psychological support is vital in combating obesity in children and adolescents. There is ongoing debate surrounding the relationship between obesity and gender among children, particularly across various regions.

What this study adds?

There was no difference in obesity rates among children under the age of 12 based on gender or time trends in this meta-analysis. Comprehensive interventions are necessary in order to control obesity among children.

Abstract

Objective: Evaluating changes over time for the odds of developing obesity according to sex.

Methods: PubMed, Embase, Cochrane Library, and China National Knowledge Database were searched for relevant studies. Full-text studies evaluating the influence of sex on obesity were analyzed. R 3.4.3 was used to assess the impact of results in the selected studies, calculated pooled prevalence and odds ratio (OR) with their respective 95% confidence intervals (CIs). A $p < 0.10$ and $I2 > 50\%$ indicated high heterogeneity, and the random-effects model was used, otherwise, the fixed-effects model was used.

Results: The included studies reported the prevalence of obesity in children covering 1987-2017. The pooled prevalence of obesity in boy and girl groups were 0.13 (95% CI: 0.08, 0.20) and 0.10 (95% CI: 0.07, 0.13). In the analysis of the boy group, the pooled OR in earlier time vs. recent time was 0.98 (95% CI: 0.76, 1.26). The estimated OR for girls in earlier vs. recent time was 1.01 (95% CI: 0.80, 1.28). In the analysis of studies with follow-up period ≥10 years, the pooled OR for obesity in earlier vs. recent time period was 0.99 (95% CI: 0.76, 1.30). For those with follow-up period < 10 years, the pooled OR in earlier vs. recent time period was 0.94 (95% CI: 0.57, 1.54).

Conclusion: Comprehensive measures are required to control obesity among children, albeit with non-significant gender difference and time trend for obesity rates in children.

Keywords: Children, obesity, trend, gender, meta-analysis

Introduction

Childhood obesity has become a global epidemic. The World Health Organization (WHO) estimated that, in 2000, the global overweight rate for children aged 5-17 years was 10%, and the obesity rate was 2-3% (1,2). In 2016, the worldwide prevalence of obesity was 5.6% in girls and 7.8% in boys aged 5-19 years, with prevalence >20% in many regions (3). Another study estimated the prevalence of childhood obesity in 2013 at 23% in developed countries and 13% in developing countries (4). Developed countries

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report higher prevalence rates compared to developing ones, where obesity is less common among children and adolescents (5,6,7). The occurrence of childhood obesity is the result of a combination of genetic, environmental, and other factors and is caused by the long-term imbalance of energy intake and energy consumption (8). A high-energy-density diet, low physical activity, sedentary lifestyle, and unhealthy eating behaviors are generally considered to be important risk factors for the development of obesity. Comprehensive dietary interventions, exercise, behavior, and psychology at different times and different levels are necessary to develop an effective strategy for curbing the spread of obesity among children and adolescents (9).

Some studies found sex-related differences in obesity. In many Western countries, the obesity rate is higher in girls than in boys, and in Asian countries, the trend is opposite (10,11). Among children aged 6-18 years in Taiwan and China, during 1991-2003, the overweight rate of boys increased from 5.7% to 14.2%, and the obesity rate increased from 7.9% to 17.4%. At the same time, the overweight rate of girls increased from 11.1% to 13.4%, and the obesity rate increased from 3.1% to 11.11% (12). Recent trends indicated a rise in obesity rates among both boys and girls, with boys experiencing a higher incidence. Over time, the disparity in obesity rates between genders has widened, particularly in urban areas where boys are more affected than girls. This urban predominance in boys significantly influences the global increase in childhood obesity and overweight cases (13). A meta-analysis showed that childhood obesity increases the prevalence of prediabetes and non-alcoholic fatty liver disease (14).

Considerable debate continues on the trend of obesity among children. Ogden et al. (15) stated that the prevalence of obesity in children aged 2-5 years increased until 2003-2004 and then decreased, while Skinner et al. (16) found no evidence of a decline in obesity prevalence at any age. From 1999 to 2016, in Europe, the prevalence of childhood obesity increased in the Mediterranean region, decreased in the Iberic region, and remained stable in Atlantic or Central Europe (17). In addition, whether biological sex has an impact on eventual differences is unknown. The present study aimed to evaluate changes over time in the odds of obesity according to sex and follow-up. The results provided a trend of obesity over time according to sex and follow-up period.

Materials and Methods

This article describes a meta-analysis. The data comes from published articles and does not require ethical approval and written informed consent.

Literature Search and Study Selection

This study was conducted in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis guideline (18). Databases, including PubMed, Embase, the Cochrane Library, and China National Knowledge Database, were searched for studies published up to March 13, 2024 on a comparison between boys and girls in terms of obesity. The following keywords were used for the search: (1) obesity or overweight; and (2) child* or adolescent*. All these words were combined with the Boolean operators "AND" and "OR" in the strategy: (obesity OR obese OR overweight) AND (child* OR children*). No restrictions were imposed on the language of publication in document retrieval. The reference lists of the retrieved studies were further screened to find other relevant studies that were not identified by the retrieval strategy to maximize the specificity and sensitivity of retrieval. The definition of obesity was based on the WHO's, i.e., body mass index (BMI) >30 kg/m^2 (http://www.emro.who.int/health-topics/obesity/). Meanwhile, the included samples were children under 12 years old.

After the primary selection, the full text of the potentially relevant studies was reviewed to ensure that they met the following inclusion criteria:

1. Comparison between boys and girls, i.e., separate effect estimates for boys and girls;

2. Children with obesity;

3. Containing prevalence of obesity in different gender groups, and/or in recent and earlier time periods, "earlier time period" is referred to as the prevalence rates in the first time period reported, and "recent time period" is referred to as the prevalence rates in the latest time period reported;

4. Available in full text;

5. In the case of overlapping samples from the same organization, only the most recent ones were selected.

The exclusion criteria were as follows:

1. Studies on health problems other than obesity;

2. Studies that only included adults;

3. Studies lacking available data;

4. Other study types such as reviews, letters or case reports, and in vitro or in vivo studies.

Database search and study identification were performed by two independent authors and discrepancies were resolved through discussion.

Data Extraction and Quality Assessment

Two commentators independently screened the full text of the manuscript and extracted the following data from each eligible study: first author's name, patient's age and sex, country of origin, year of publication, sample size, and duration of each study. Two authors assessed related studies independently, complying with inclusion and exclusion criteria. In case of disagreement between two evaluators, a third evaluator was consulted to resolve the issue. The Newcastle-Ottawa Scale (NOS) table was used to evaluate the methodological quality of the study.

Statistical Analysis

R (version 3.4.3; Comprehensive R Archive Network), package meta was used for data analysis. Pooled prevalence with 95% confidence interval (CI) was calculated. Pooled estimate of odds ratio (OR) was used to compare change in prevalence rates from the earliest time periods to the most recent time periods in included studies. Heterogeneity was evaluated by I2 statistics, a quantitative measure of inconsistency in studies; 25-50% of the studies with I2 were considered to have low heterogeneity, 50-75% of the studies with I2 were considered to have medium heterogeneity, and 75% of the studies with I2 $>$ 75% were considered to have high heterogeneity (19). If I2 $>$ 50%, the potential sources of heterogeneity were examined by sensitivity analysis, which omitted one study in each round and investigated the impact of a single study on portfolio estimation (19). In addition, when heterogeneity was observed, the random-effects model was used, and when it did not exist, the fixed-effects model was used (19). Egger's test ,along with funnel plot ,were adopted to detect the publication bias. A $p < 0.05$ was considered to be statistically significant.

Results

Search Process

Since the number of selected results from several databases was huge, the electronic search ended with 4,113 most relevant studies. After careful reading, 175 studies reached the preliminary standard. After further screening, 165 studies were excluded because of improper research type (n = 74), insufficient data (n = 71), and study type (n = 20). Finally, ten studies were included for analysis. Figure 1 shows the flowchart of identification, inclusion, and exclusion of the studies, reflecting the search process and the reason for exclusion.

Characteristics of Included Studies

Table 1 summarized the types of studies reported and the total number of patients associated with each group. It included the author, year of publication, country, age, sex,

Table 1. Characteristics of studies included in the meta-analysis

Study	Year	Language	Type	Country	Age range (mean)	Groups	n	Years of onset
Keß et al. (20) 2017	2017	English	Cross-sectional study	Germany	8.2 ± 5.3	Boys	28,691	2005-2010
						Girls	26,569	
Chen et al. (21) 2019	2019	English	Cross-sectional study	China	4.9 ± 2.5	Boys	2,542	2011-2017
						Girls	2,071	
de Ruiter et al. (22) 2017	2017	English	Cross-sectional study	Spain	8.5 ± 4.5	Boys	3,908	1987-2011
						Girls	3,376	
Skinner et al. (23) 2018	2018	English	Cross-sectional study	America	6.5 ± 5.5	Boys	1,962	2015-2016
						Girls	1,872	
Çelmeli et al. (24) 2019	2019	English	Case-control study	Turkey	7.4 ± 6.6	Boys	308	2003-2015
						Girls	249	
Ogden et al. (25) 2018	2018	English	Cross-sectional study	America	8.6 ± 7.5	Boys	2,584	2013-2016
						Girls	2,540	
Vanhelst et al. (26) 2017	2017	English	Case-control study	France	8.4 ± 6.5	Boys	173	2009-2013
						Girls	200	
Zhang (Zhang et al. 2018)	2018	English	Cross-sectional study	China	10.5 ± 7.5	Boys	1,297	2011-2015
						Girls	1,245	
Decyk and Kolanowski (27) 2020	2020	English	Cross-sectional study	Poland	9.1 ± 1.8	Boys	181	2017-2018
						Girls	269	
Mai et al. (28) 2024	2024	English	Cross-sectional study	Australia	10.6 ± 0.5	Boys	101	2020
						Girls	120	

group, sample size, and recruitment time. This meta-analysis included studies from a variety of countries, including Germany, China, Spain, the USA, Türkiye, France, Poland, Australia. Ten studies (16,20,21,22,23,24,25,26,27,28) published from 2017 to 2024, with a sample size between 221 and 55,260, were included in the analysis.

Results of Quality Assessment

The NOS table (Supplementary Table 1) was used to evaluate the risk of study quality of the ten included trials. On a maximum of nine points (indicating the highest quality), six studies scored 8 points, and four scored 9 points.

Prevalence of Obesity in Different Gender Groups

The included studies reported the prevalence of obesity in children covering 1987-2017 intervals. The overall prevalence in both genders was 0.11 (95% CI: 0.08, 0.15). The pooled prevalence in boy and girl groups were 0.131 (95% CI: 0.08, 0.20) and 0.10 (95% CI: 0.07, 0.13) (p = 0.37), respectively (Figure 2).

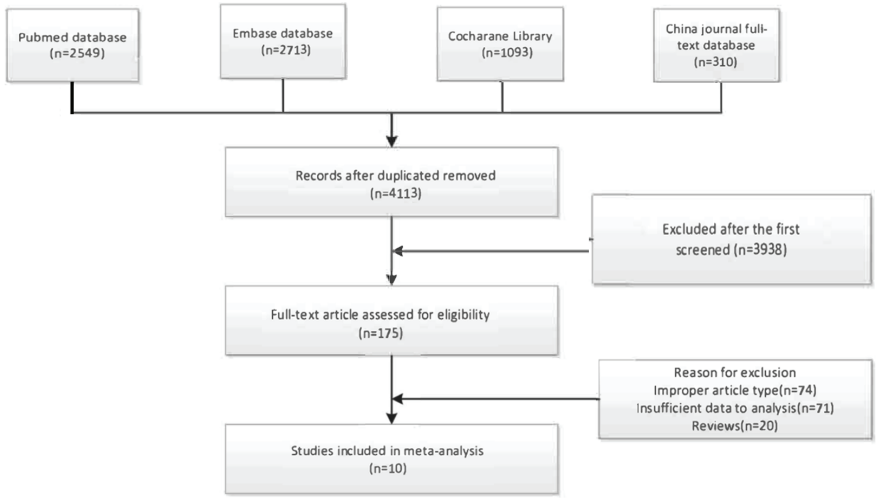

Figure 1. Flow diagram of the study selection

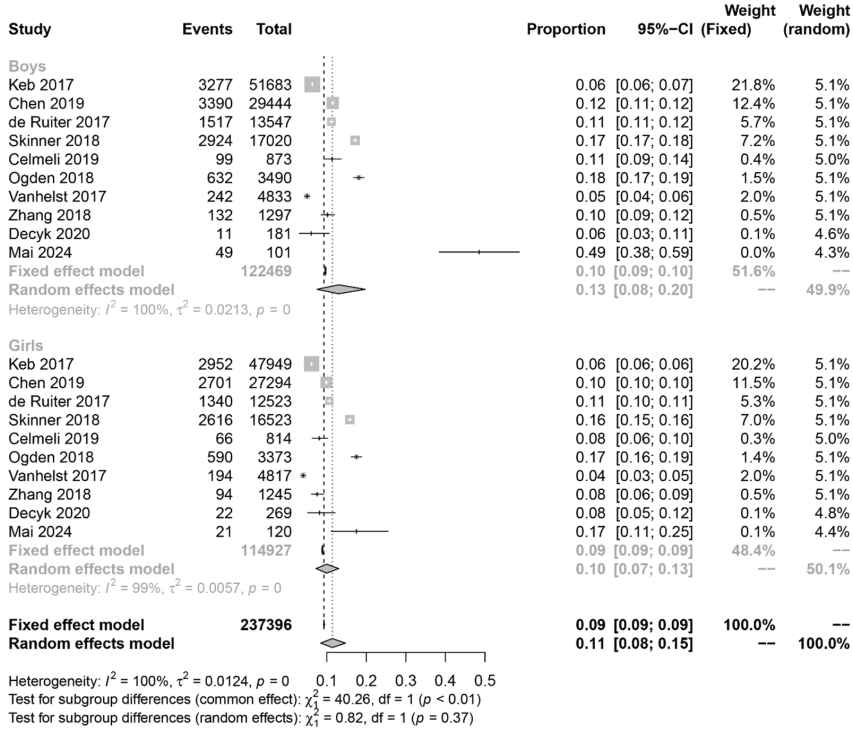

Figure 2. Forest plots of obesity rates in boys and girls

CI: confidence interval

Time Trends in the Prevalence of Severe Obesity

Six studies revealed time trends of obesity over a period of time. Results showed pooled OR of 1.00 (95% CI: 1.53, 1.90) for obesity in the earlier time period, than during the recent time period. In the analysis regarding boys, the pooled OR in earlier vs. recent time was 0.98 (95% CI: 0.76, 1.26). The estimated OR for girls in earlier vs. recent time was 1.01 (95% CI: 0.80, 1.28) (Figure 3).

In the analysis of data limited to follow-up period ≥10 years, the pooled OR for obesity in earlier vs. recent time period was 0.99 (95% CI: 0.76, 1.30). For studies with follow-up period < 10 years, the pooled OR in earlier vs. recent time period was 0.94 (95% CI: 0.57, 1.54) (Figure 4).

Sensitivity Analysis and Publication Bias

Results of sensitivity analysis revealed the robustness of the meta-analysis. Specifically, the Egger's test results for the overall prevalence of obesity indicated an intercept of 0.2885, with a t-value of 1.14 and a p value of 0.27. In the analysis of time trends in different gender groups, the intercept was found to be -0.9874, with a t-value of 0.79 and a p value of 0.4540. Finally, for the time trend analysis in different follow-up periods, the intercept was 0.3660, with a

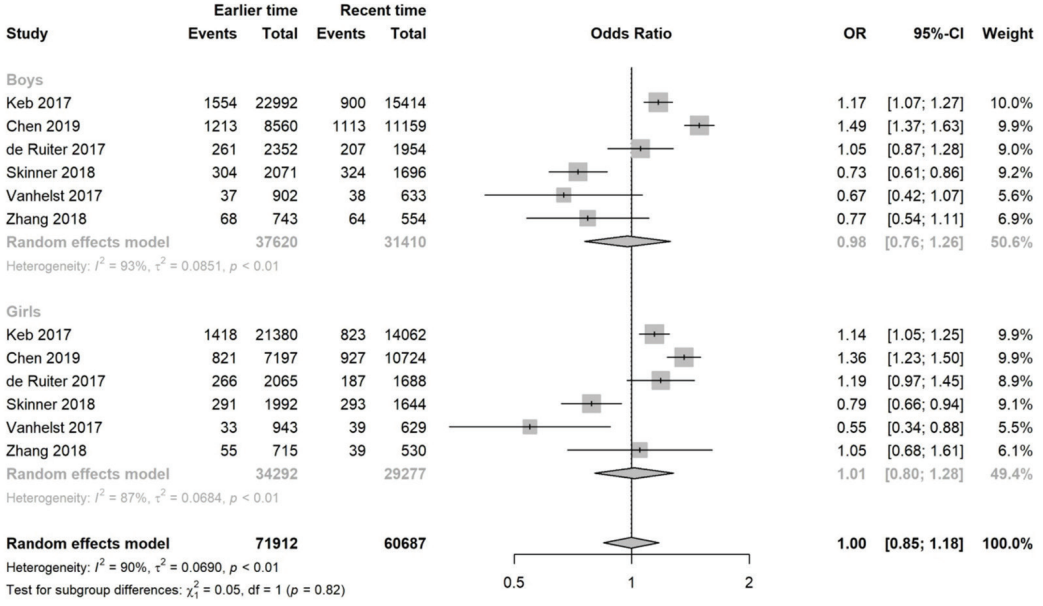

Figure 3. Time trend of obesity rates in boys and girls

CI: confidence interval

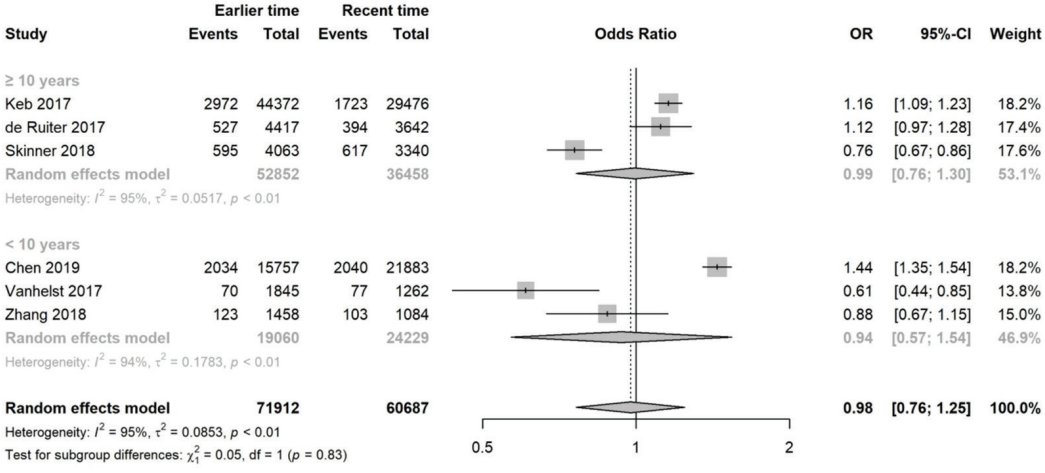

Figure 4. Time trend of obesity rates in different follow-up periods

CI: confidence interval, OR: odds ratio

t-value of -1.75 and a p-value of 0.1550. As shown in Figure 5, the symmetric funnel plot suggested a lack of publication bias in this meta-analysis.

Discussion

In this meta-analysis, we systematically reviewed and included a total of 10 studies to assess the prevalence and temporal trends of obesity among boys and girls. Our analysis reveals that the pooled prevalence of obesity in boys was 0.13 (95% CI: 0.08, 0.20), and in girls, it was slightly lower at 0.10 (95% CI: 0.07, 0.13). These findings suggest that while obesity is a significant concern in both genders, the prevalence rates are relatively similar.

Obesity has become a global public health concern. With the improvement in living conditions, abundant food is available for children, their growth and development levels have significantly improved, and the prevalence of malnutrition has declined significantly (29). The incidence of children being overweight and obese has increased rapidly in recent years. According to recent data released by the WHO, the number of overweight children aged less than 5 years has reached about 22,000.000 (30).

In the United States of America, two national health surveys conducted in the 1960s and four national health and nutrition surveys conducted during 1971-2000 provided information on childhood obesity. Childhood obesity has almost doubled, and the obesity rate has almost quadrupled, and this upward trend continues. According to the International Working Group on Obesity standard, the overweight and obesity rate of children and adolescents aged 6-18 years in the United States of America increased from 15.4% during 1971-1974 to 25.6% during 1988-1994 (30). The overweight rate of Canadian children also increased from 11% for boys and 13% for girls in 1981 to 33% (boys) and 27% (girls), respectively, in 1996. Xiao et al. (13) stated that in China, the mean values of BMI z-scores decreased from 2006 to 2014 among Chinese children aged 3-6 years due to the significant increase in height z-scores. The prevalence of obesity increased from 2006 to 2010 and then remained stable until 2014 among children aged 5-6 years. On the other hand, Sagbo et al. (29) suggested that the relative fatness of children with morbid obesity, as measured by the BMI z-score, has remained stable. The proportion of obese and morbidly obese children also plateaued between 2007 and 2014.

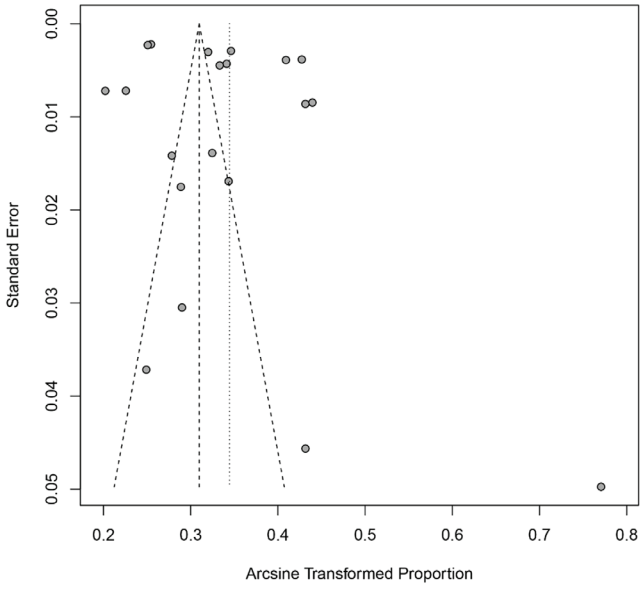

Figure 5. Funnel plot for publication bias

To assess the presence of publication bias in our study, we employed Egger's regression test and funnel plot. Egger's test, a statistical method designed to detect funnel plot asymmetry, yielded p values greater than 0.05 across all analyses, which indicated that there was no significant evidence of publication bias within our dataset. The funnel plots demonstrated a symmetric distribution of studies around the combined effect size. This symmetry in the plots further supports the conclusion drawn from Egger's test, suggesting an absence of noticeable publication bias in our meta-analysis. Results of this meta-analysis showed overall prevalence in both genders was 0.10, the pooled prevalence of obesity in boys was greater than that in girls, nevertheless, there was no statistically significant difference of prevalence in the two groups. There were non-significant differences between earlier period and recent period findings. In China, Xiao et al. (13) showed that the prevalence of obesity was higher in boys than in girls. The reason for the inconsistent results compared to previous study may be the varying definitions of obesity, time periods, ethnicity of children and study designs in studies included. The current study only included studies with children under 12 years old. However, targeted preventive measures should be implemented, such as more exercise and proper food intake for children.

Study Limitations

This study had some limitations that must be considered when analyzing the implications of the results. Firstly, only ten articles could be included as per the pre-defined eligibility criteria, which may limit the generalizability of the results and introduce potential biases. Secondly, the subgroup comparison in different countries was not considered because too few studies were available from different countries or even continents, which needs further

evaluation. Thirdly, the details about different races were not included, and the comparison about different races should be included in future studies. Indeed, the difference in the childhood obesity rate among races has been reported. The data from 1999 to 2000 showed that the obesity rate of black and Hispanic children was almost twice that of white and non-Hispanic children. Especially for black people, the obesity rate of children has increased rapidly in recent decades (31). Again, the small number of studies precluded such an analysis. Fourthly, since the included articles were published from 2017 to 2019, they did not cover the relevant articles in history, which could be conducted in the next step. Finally, there is a lack of PROSPERO registration, no meta-regression could be performed, and the heterogeneity was significant, and more well-designed studies including meta-analysis are needed in the future.

Conclusion

In conclusion, there was no gender and time period difference for obesity rate in children under 12 years old identified in this meta analysis. Comprehensive measures are required to control childhood obesity regardless of the nonsignificant results.

Ethics

Ethics Committee Approval-**Informed Consent:** This article is a meta-analysis. The data comes from published articles and does not require ethical approval and written informed consent.

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Footnotes

Authorship Contributions

Concept: Jinna Yuan, Xuelian Zhou, Ke Huang, Data Collection or Processing: Xuefeng Chen, Wei Wu, Junfen Fu, Analysis or Interpretation: Jinna Yuan, Xuelian Zhou, Ke Huang, Yangli Dai, Guanping Dong, Writing: Xuefeng Chen, Wei Wu, Yangli Dai, Guanping Dong, Junfen Fu.

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Inequalities in Access to Diabetes Technologies in Children with Type 1 Diabetes: A Multicenter, Cross-sectional Study from Türkiye

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What is already known on this topic?

Technology use improves type 1 diabetes management in children, regardless of socioeconomic status. However, access to this technology has socioeconomic barriers.

What this study adds?

It was shown that socioeconomic characteristics affect access to diabetes technologies and glycemic management in a large group of families from Türkiye.

Abstract

Objective: To determine inequalities in access to diabetes technologies and the effect of socioeconomic factors on families with children with type 1 diabetes.

Methods: In this multicenter, cross-sectional study, parents of children with type 1 diabetes completed a questionnaire about household sociodemographic characteristics, latest hemoglobin A1c (HbA1c) values, continuous glucose monitoring (CGM) and insulin pump use of children, the education and working status of parents. These characteristics were compared between technology use (only-CGM, only-pump, CGM + pump, no technology use).

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Results: Among 882 families, only-CGM users, only-pump users, and CGM + pump users were compared with no technology users, adjusting for age, sex, region, education levels, number of working parents, and household income. Children living in the least developed region had lower odds of having only-CGM [odds ratio (OR) = 0.20, 95% confidence interval (CI): 0.12-0.34, $p < 0.001$] and having CGM + pump (OR = 0.07, 95% CI: 0.03-0.22, $p < 0.001$) compared with those living in the most developed region. Children with parents who had not finished high school had lower odds of having only-CGM (mothers: OR = 0.36, 95% CI: 0.19-0.66, $p = 0.001$; fathers: OR = 0.32, 95% CI: 0.18-0.60, $p < 0.001$) or both CGM + pump (mothers: OR = 0.27, 95% CI: 0.11-0.64, $p = 0.003$; fathers: OR = 0.34, 95% CI: 0.15-0.79, $p = 0.012$) rather than no-technology compared to children whose parents have a university degree. Every $840 increase in the household income increased the odds by 5% for having only-CGM (OR = 1.05, 95% CI: 1.02-1.09, $p < 0.001$) or CGM + pump (OR = 1.05, 95% CI: 1.01-1.08, $p < 0.001$).

Conclusion: Socioeconomic factors, such as parental education, region of residence, and income were associated with inequality in access to technologies. The inequalities are more prominent in access to CGM.

Keywords: Continuous glucose monitoring, inequality, technology, type 1 diabetes

Introduction

In the last decade, diabetes technologies, especially continuous glucose monitoring (CGM), have played an increasingly fundamental role in treating children with type 1 diabetes (T1D). In parallel, ensuring equal access to these technologies and evaluating inequalities in a multidimensional manner has become a matter of debate (1,2,3). Inequalities are directly related to the historical and current unequal distribution of social, political, economic, and environmental resources, and one of the groups most affected by inequalities is children (4). Inequalities in access to diabetes technologies should be addressed within the scope of "social determinants of health", screened routinely by healthcare providers during visits, and made the subject of advocacy for the social rights of children (5,6,7).

Equal access to CGM from diagnosis can lower hemoglobin A1c (HbA1c) in children with T1D despite other inequalities and, thus, can be a "leverage" to reduce the impact of inequalities on children's diabetes (8). The relationship between families and CGM goes beyond the numbers, CGM eases families' burden and reinforces their motivation to be "their children's pancreas" (9,10). Therefore, equal access to diabetes technologies contributes to making diabetes treatment more humane, in addition to its glycemic control benefits, such as improving HbA1c and reducing the frequency and fear of hypoglycemia (9,11).

In Türkiye, a medium-income country, 70% of children with T1D have HbA1c over 7.5%, and 35% have HbA1c over 9% (75 mmol/mol) (12). Moreover, in the Southeastern Anatolia region, one of the least developed regions of Türkiye, the prevalence of diabetic ketoacidosis (DKA) at presentation is 65.9%, 63% of these cases being severe DKA, and the frequency of DKA increases up to 87.5% between the ages of 0-4 (13). Despite the evidence and intense advocacy efforts over the last 5 years, Türkiye temporarily reimburses CGM for a limited number of children with T1D who meet strict criteria. Türkiye also provides partial reimbursement (approximately 20%) for insulin pumps. However, the prevalence of diabetes technology use and the characteristics of the population who have access are unknown. The aims of this study were to examine the use of diabetes technology in terms of socioeconomic groups and regions, to investigate the determinants and inequalities of access to diabetes technologies, and the socioeconomic determinants of better glycemic management among technology users. The results may provide useful information to health care decision-makers in addressing inequalities in access to diabetes technology.

Methods

Participants

The study protocol was shared previously (14). In brief, parents of children and adolescents with T1D were recruited from nine pediatric endocrinology centers and the Children Diabetes Foundation Network in Türkiye. The online survey was distributed to the parents during routine visits to the clinics and through the Children Diabetes Foundation's social media groups. Only mothers or fathers whose children were diagnosed with T1D before the age of 18 years were included, caregivers other than the mother or father were excluded. Participants with a diabetes duration of < 3 months were excluded to ensure the families had sufficient experience with T1D. Of note, at the time of the study there was no reimbursement for CGM.

Questionnaire

Briefly, the questionnaire covered the child's clinical and household sociodemographic characteristics, CGM and pump use, the latest HbA1c value, the education level of both parents, the working statuses of both parents, and the financial burden of diabetes (14). Only one parent completed the questionnaire for the entire family. The Ethical Committee at Koç University approved the study

(decision no: 2022.378.IRB3.176, date: 03.11.2022) in accordance with the Declaration of Helsinki.

Statistical Analysis

The primary outcome of the study is the socioeconomic determinants of technology use. Technology use is divided into four categories: CGM use only; pump use only; both CGM and pump use; and no use of technology.

For independent variables the 81 provinces of Türkiye were ranked in six groups based on socioeconomic development, according to the "socioeconomic development ranking of provinces research" of the Turkish Ministry of Industry and Technology (15). According to this ranking, we divided the provinces where the families were located into the six groups but rationalized these into three categories for analysis, which were the most developed region, the least developed region, and the remaining four groups as intermediate developed regions. The highest education levels of parents were analyzed in three categories, finished school before the end of high school, completed high school, and university degree or above.

Household income was collected as Turkish lira, converted to United States (US) dollars based on March 2022 US Dollar/Turkish lira exchange rates.

Descriptive statistics are presented as means with standard deviation for normally distributed continuous variables, median (interquartile range) for non-normally distributed continuous variables or absolute numbers with percentages for categorical variables. For univariate analysis, one-way ANOVA or Kruskal-Wallis tests were used for continuous variables, and the chi-square test was used for categorical comparisons. A multinominal logistic regression model was used to assess technology use (only CGM users, only pump users, and both CGM and pump users compared with no technology use), adjusting for age, sex, region where the family lived (least developed/intermediate/most developed), education levels of mothers, education levels of fathers, number of working parents, and household income.

Another analysis was conducted to evaluate which technology and which factors were associated with better glycemic outcomes among technology users. In this analysis, the factors associated with better glycemic control, indicated by lower HbA1c, in technology users (CGM and/or pump users). For this, a linear regression model used the HbA1c as the dependent variable and age, sex, diabetes duration, CGM use (only pump use vs. CGM with or without pump use), the number of working parents, education levels of mothers, education levels of fathers, the region where the family lives, and household income as predictors. After forward stepwise variable selection, diabetes duration, the region where the family lives, education levels of mothers, CGM use, and household income were included in the model as predictors. Statistical Package for the Social Sciences, version 28.0, was used for the analysis (IBM Inc., Armonk, NY, USA). A p value < 0.05 was considered significant.

Results

Study Population

Of the 1254 responses, 372 were excluded due to missing information or duplicate responses. Among the final 882 responses (77.6% completed by mothers), 692 were from nine pediatric endocrinology clinics and 190 were from the online network of Children Diabetes Foundation. Participants were from 65 out of 81 provinces of Türkiye.

Participant families' characteristics are summarized in Table 1. Of 882 children with T1D (52.5% female, mean age 10.75 ± 4.6 years, diabetes duration 7 ± 3.8 years), 829 children (94%) were living with both parents while 53 children (6%) were with a single parent. In addition, 666 children (75.5%) had at least one sibling, and 86 (13%) of them had a sibling with a chronic medical condition. Twenty-five families (2.8%) had more than one child with T1D.

According to self-reported HbA1c values (n = 738), the mean last HbA1c was 7.5 ± 1.4% (58 mmol/mol). Reported current pump and CGM use were 19.4% and 49.7%, respectively. Of all children, 15% were using both pump and CGM, 4.4% using only pump, 34.7% using only CGM, and 45.9% were not using any diabetes technology.

Technology use (CGM and/or pump use) was 16.1% in the least developed region, 67.8% in the intermediate developed region, and 66.1% in the most developed region. CGM use was 14.4%, 60.1%, and 62.5% in these regions, respectively while pump use was 3.6%, 27.8%, and 22.5%, respectively.

Characteristics of Technology Users and Determinants of Technology Use

Family characteristics of only CGM users, only pump users, both pump and CGM users, and no technology users are shown in Table 2. Technology use did not differ by the sex and living arrangements of children (living with a single parent or both parents).

The number of siblings was higher in families in whom the child with T1D did not use technology. No technology users had similar household incomes to pump only users but lower household incomes than CGM users. Moreover,

Table 1. Characteristics of participants

	All participant families (n = 882)
Children	
Age, years, mean ± SD	10.75 ± 4.6
Sex, female, n (%)	463 (52.5)
Diabetes duration, years, mean ± SD	3.7 ± 3.5
Child's living arrangement, n (%)	
With both parents	829 (94.0)
With a single mother	51 (5.0)
With a single father	2 (1.0)
Children with sibling(s), n (%)	666 (75.5)
Sibling(s) with chronic condition, n (%)[a]	86 (13.0)
Sibling with T1D, n (%)[a]	25 (2.8)
Most recent HbA1c, mean ± SD[b]	7.5 ± 1.4
Current use of CGM, n (%)	438 (49.7)
Current use of pump, n (%)	171 (19.4)
No technology users, n (%)	405 (45.9)
Parents, mother/father	
Parents' age, years, mean ± SD	39 ± 6.4 / 42.3 ± 6.8
Highest educational level, n (%)/n (%)	
Less than high school	282 (32.0) / 227 (25.7)
High school graduate	221 (25.1) / 255 (28.9)
University degree or above	379 (43.0) / 400 (45.4)
Household income, Turkish lira/month, median (IQR)	7500 (4500-14000)
Number of working parents, n (%)	
Both parents are not working	71 (8.0)
Only one parent is working	518 (58.7)
Both parents are working	293 (33.2)
The region where family lives, n (%)	
The least developed	222 (25.2)
Intermediate	273 (31.0)
The most developed	387 (43.9)
Financial loss due to diabetes care, n (%)	
None to minimal loss	113 (12.8)
Moderate financial loss	262 (29.8)
High to severe financial loss	506 (57.4)

[a]Percentages were calculated for children with at least one sibling.
[b]HbA1c was self-reported from 738 responders.
SD: standard devation, HbA1c: hemoglobin A1c, T1D: type 1 diabetes, CGM: continuous glucose monitoring, IQR: interquartile range

their mother or father's education level was lower, they had fewer working parents, they reported less financial burden caused by diabetes, and they mostly lived in the least developed region compared to those using CGM and/or pump ($p < 0.001$ for all) (Table 2). Mean HbA1c levels by technology use are shown in Figure 1A. HbA1c was lower in CGM users compared to pump users [both CGM and pump vs. only pump: 7.05% (54 mmol/mol) vs. 8.0% (64 mmol/mol), $p < 0.001$] and insulin pen users [only CGM vs. no technology: 7.07% (54 mmol/mol) vs. 8.07% (65 mmol/mol), $p < 0.001$]. However, HbA1c did not differ by pump use in CGM users [both CGM and pump vs. only CGM: 7.05% (54 mmol/mol) vs. 7.07% (54 mmol/mol), $p = 0.868$] nor blood glucometer users [only pump vs. no technology: 8.00% (64 mmol/mol) vs. 8.07% (65 mmol/mol), $p = 0.810$]. HbA1c was lower in all regions with CGM use (Figure 1B).

To understand the social determinants of technology use, a multinomial logistic regression analysis was used (Table 3). In this analysis, technology use was assessed between CGM only users, pump only users, and combined CGM and pump users compared with no technology use), adjusting for age, sex, region (least/intermediate/most developed), education levels of mothers, education levels of fathers, number of working parents, and household income. The results showed that children living in the least developed region had lower odds of having CGM only [odds ratio (OR) = 0.20, 95% confidence interval (CI): 0.12-0.34] and having combined CGM and pump (OR = 0.07, 95% CI: 0.03-0.22) compared to living in the most developed region. Children with a mother who had not finished high school had lower odds of having CGM only (OR = 0.36, 95% CI: 0.19-0.66) or combined CGM and pump (OR = 0.27, 95% CI: 0.11-0.64) rather than no technology compared to children whose mother had a university degree or above. Fathers' education levels had a similar association for CGM only (OR = 0.32, 95% CI: 0.18-0.60) and combined CGM and pump users (OR = 0.34, 95% CI: 0.15-0.79) rather than no technology users. Every 12,000 Turkish lira (~840 US dollars) increase in the household income increased the odds by 5% for using CGM (OR = 1.05, 95% CI: 1.02-1.09) and cobined CGM and pump (OR = 1.05, 95% CI: 1.01-1.08).

Factors Associated with Better Glycemic Control in Technology Users

After showing better glycemic management by technology use (Figure 1A), we performed a linear regression analysis to examine variables associated with glycemic control among 431 CGM and/or pump-user children, specifically to investigate the effect of single or multiple technology use.

The model was adjusted for diabetes duration, region where the family lives, mother's education level, technology use (CGM only use, pump only use or CGM and pump use), and household income. Living in the least developed region was associated with 0.54% (6 mmol/mol) higher HbA1c [95% CI: 0.11% (1 mmol/mol) - 0.97% (11 mmol/mol), $p = 0.013$] compared with living in the most developed region. Children whose mothers had attended or completed high school had 0.26% (3 mmol/mol) higher HbA1c [95% CI: 0.01% (0.1

Table 2. Technology use by the characteristics of children and families

	Users of no technology (n = 405)	Pump only users (n = 39)	CGM only users (n = 306)	Both pump and CGM users (n = 132)	p
Age, years, mean ± SD	11.5 ± 4.6[a]	13.8 ± 5.4[a]	9.1 ± 4.1[b]	11.3 ± 4.6[b]	< 0.001
Sex, female, n (%)	216 (53.3)	24 (61.5)	149 (48.7)	74 (56.1)	0.279
Diabetes duration, years, mean ± SD	3.9 ± 3.6[a]	6.7 ± 5.1[b]	2.6 ± 2.6[c]	5.0 ± 3.3[d]	< 0.001
HbA1c, %, mean ± SD	8.1 ± 1.6[a]	8.0 ± 1.4[a]	7.1 ± 1.2[b]	7.1 ± 1.0[b]	< 0.001
Number of siblings, median (IQR)	2 (1-3)[a]	1 (1-2)[b]	1 (0-1)[b]	1 (0-1)[b]	< 0.001
Living arrangement, with both parents, n (%)	380 (93.8)	37 (94.9)	289 (94.4)	123 (93.2)	0.953
Household income, Turkish lira/month, median (IQR)	5000 (4000-7500)[a]	7000 (4250-10000)[a]	10000 (6500-18000)[b]	12000 (8000-20000)[b]	< 0.001
Region where family lives, n (%)					< 0.001
The least developed region	186 (45.9)	4 (10.3)	28 (9.2)	4 (3.0)	
Intermediate developed regions	88 (21.7)	21 (53.8)	109 (35.6)	55 (41.7)	
The most developed region	131 (32.3)	14 (35.9)	169 (55.2)	73 (55.3)	
Education level of mothers, n (%)					< 0.001
University degree or above	78 (19.3)	13 (33.3)	200 (65.4)	88 (66.7)	
High school graduates	110 (27.2)	15 (38.5)	64 (20.9)	32 (24.2)	
Less than high school	217 (53.6)	11 (28.2)	42 (13.7)	12 (9.1)	
Education level of fathers, n (%)					< 0.001
University degree or above	99 (24.4)	10 (25.6)	206 (67.3)	85 (64.4)	
High school graduates	138 (34.1)	18 (46.2)	64 (20.9)	35 (26.5)	
Less than high school	168 (41.5)	11 (28.2)	36 (11.8)	12 (9.1)	
Number of working parent, n (%)					< 0.001
Both parents working	76 (18.8)	9 (23.1)	135 (44.1)	73 (55.3)	
Only one parent working	272 (67.2)	27 (69.2)	162 (52.9)	57 (43.2)	
Both parents not working	57 (14.1)	3 (7.7)	9 (2.9)	2 (1.5)	
Financial loss due to diabetes care, n (%)					< 0.001
None to minimal loss	80 (19.8)	2 (5.1)	24 (7.8)	7 (5.3)	
Moderate financial loss	126 (31.1)	13 (33.3)	97 (31.7)	27 (20.5)	
High to severe financial loss	199 (49.1)	24 (61.5)	185 (60.5)	98 (74.2)	

HbA1c was self-reported from 738 responders. Other data were from 882 responses.
One-way ANOVA, Kruskal-Wallis, or chi-square tests were used as appropriate.
[a,b]Subgroup comparison of continuous variables after Bonferroni correction were shown with superscript letters, while same superscript letters are not significantly different and different letters significantly differ.
SD: standard devation, HbA1c: hemoglobin A1c, CGM: continuous glucose monitoring, IQR: interquartile range

mmol/mol) - 0.51% (6 mmol/mol), p = 0.04] than children whose mothers had a university degree or above. Using only pump was associated with 0.57% (6 mmol/mol) higher HbA1c [95% CI: 0.15% (2 mmol/mol) - 1.00% (11 mmol/mol), p = 0.007] compared to CGM use, with or without a pump. A 12,000 Turkish lira (~840 US dollars) increase in household income was associated with a 0.02% (0.1 mmol/mol) decrease in HbA1c [95% CI: 0.003% (0.1 mmol/mol) - 0.03% (0.1 mmol/mol), p = 0.017]. A one year increase in diabetes duration was associated with a 0.05% (0.1 mmol/mol) increase in HbA1c [95% CI: 0.015 (0.1 mmol/mol) - 0.087% (1 mmol/mol), p = 0.006].

Discussion

This study showed inequality in access to diabetes technologies in Türkiye and the associated socioeconomic

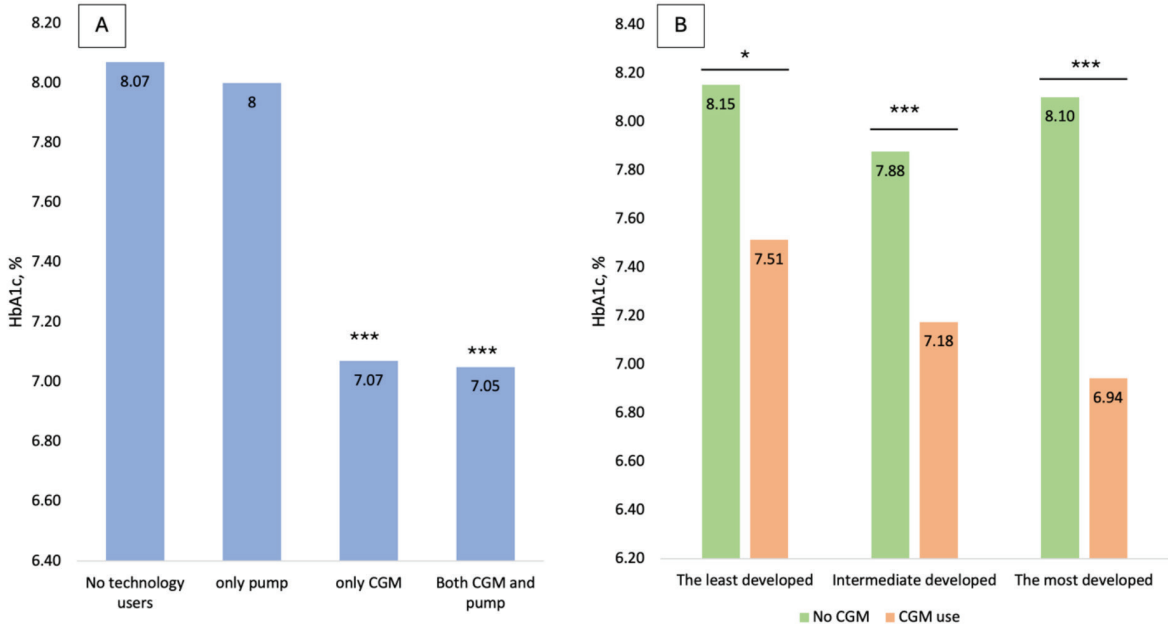

Figure 1. HbA1c levels by technology use and regions. A) HbA1c levels by CGM and/or pump use, three technology categories were compared with no technology users. B) HbA1c levels by CGM use in the least, intermediate and most developed regions

*p < 0.05, **p < 0.01, ***p < 0.001.

HbA1c: hemoglobin A1c, CGM: continuous glucose monitoring

determinants, such as education level of parents, socioeconomic development level of the region of residence, and household income. Moreover, these factors were also associated with HbA1c levels among technology users. Inequality in access to diabetes technologies has emerged as an important problem for children with diabetes, regardless of the socioeconomic development level of the countries, as shown by studies from the United States and New Zealand (2,16). The present study, the first data published from Türkiye, shows significantly lower technology use in underdeveloped regions than in intermediate and most developed regions, and the difference between regions is more prominent for pump use. This latter may be due to several factors, such as CGM use being relatively easy and individual preferences and skills, whereas pump use requires more skills and the presence of healthcare providers familiar with the use of technology.

It has been stated that access to health care results from the interface between the supply-side characteristics of health systems and organizations and the demand-side characteristics of populations (17). Similarly, in the present study it was shown that inequalities in access to diabetes technologies are multi-layered and they are not just related to affordability or coverage by reimbursement. Among those layers, population factors, such as parental education level, household income, and the working status of parents affected the inequality in access to diabetes technologies. However, there are also health system related factors at play, such as the supply and reimbursement of diabetes technologies, and the number and availability of healthcare providers that are experienced with diabetes technologies (18). These factors contributing to inequality affect all individuals in a region, regardless of individual factors. Thus, equitable access to technologies, including CGM and/or automatic insulin delivery systems, requires programs that prioritize the most disadvantaged areas and consider the social determinants of health (5). The present study found a close parallel between household income, parental education, and the number of working parents and, moreover, these were collectively associated with access to technology.

Studies from New Zealand and Germany show that inequalities regarding T1D care and metabolic control are not only socioeconomic but there are also barriers arising from ethnicity, language, and cultural differences (16,19). Another point, that may be equally as important as these obstacles, is whether families who do not use this technology are even aware of the existence and benefits of these technologies. Lack of awareness may be another factor associated with access to technology. However, we

Table 3. Variables related to only pump use, only CGM use, and both CGM and pump use by multinomial logistic regression analysis

	Only pump					Only CGM					Both pump and CGM				
			95% CI					95% CI					95% CI		
	Beta	OR	Lower	Upper	p values	Beta	OR	Lower	Upper	p values	Beta	OR	Lower	Upper	p values
Age	0.1	1.11	1.03	1.19	**0.006**	-0.12	0.89	0.85	0.93	**< 0.001**	-0.01	0.99	0.95	1.05	0.82
Male vs. female	-0.34	0.71	0.35	1.43	0.339	-0.04	0.96	0.66	1.39	0.827	-0.27	0.76	0.48	1.2	0.245
Household income, Turkish lira[a]	0.03	1.04	0.99	1.08	0.128	0.05	1.05	1.02	1.09	**0.001**	0.05	1.05	1.01	1.08	**0.005**
Education level of mothers															
Less than high school vs. university degree or above	-1.11	0.33	0.1	1.04	0.059	-1.04	0.36	0.19	0.66	**0.001**	-1.31	0.27	0.11	0.64	**0.003**
High school vs. university degree or above	-0.35	0.71	0.27	1.88	0.489	-0.67	0.51	0.3	0.87	**0.013**	-0.46	0.63	0.33	1.19	0.157
Education level of fathers															
Less than high school vs. university degree or above	0.39	1.48	0.47	4.6	0.501	-1.13	0.32	0.18	0.6	**< 0.001**	-1.07	0.34	0.15	0.79	**0.012**
High school vs. university degree or above	0.65	1.91	0.74	4.92	0.182	-0.71	0.49	0.3	0.81	**0.005**	-0.39	0.68	0.37	1.23	0.2
Number of working parents															
Both parents working vs. both parents not working	-0.21	0.81	0.18	3.7	0.784	0.13	1.14	0.45	2.89	0.782	1.27	3.57	0.76	16.91	0.108
Only one parent working vs. both parents not working	0.46	1.59	0.43	5.83	0.489	0.37	1.45	0.62	3.38	0.39	1.11	3.03	0.67	13.68	0.15
Region where family lives															
The least developed vs. most developed	-1.11	0.33	0.1	1.08	0.067	-1.61	0.2	0.12	0.34	**< 0.001**	-2.61	0.07	0.03	0.22	**< 0.001**
Intermediate developed vs. most developed	0.96	2.62	1.22	5.62	**0.013**	0	1	0.66	1.53	0.984	0.18	1.2	0.74	1.95	0.463

Reference category was the no technology user group.
[a]A unit increase in household income is 12,000 Turkish lira (approximately 840 US dollars).
OR: odds ratio, CI: confidence interval, CGM: continuous glucose monitoring, US: United States

did not obtain information from parents about whether they were familiar with diabetes technologies. Therefore, there is a need to evaluate the lack of awareness about available technologies, social and cultural barriers related to language, mothers' education, and employment in the least developed regions in a separate study (20). A qualitative study emphasized that inequalities have a complex structure involving people with diabetes, their families, and diabetes teams (21). The choices of people with diabetes are directed by their culture and beliefs, which should be considered, and specific programs should be developed to reduce inequalities instead of giving all responsibility to the people with diabetes concerning their choices for T1D management (21).

An important paradox about diabetes technologies is that diabetes technologies are the most promising developments for the improvement of diabetes treatment, but they may carry the risk of increasing inequalities both worldwide and within countries if the necessary measures are not taken (5). The reason for this is that in today's conditions, it is not the children who need it most, but those who are economically and socially advantaged that benefit most from these technologies. This situation also applies to Türkiye, as shown by our results. We believe that this is unethical, and that socioeconomic inequalities and structural exclusionary processes have a critical detrimental effect on the health of children with diabetes. It seems to us that providing equal access to diabetes technologies from diagnosis may be the first step in reducing the impact of inequalities on glucose management (8). The present study showed that CGM use in all regions resulted in lower HbA1c, regardless of pump use, while the same effect was not found for pump use. However, CGM use was associated with more socioeconomic factors. Therefore, in countries with limited economic opportunities, priority should be given to providing CGM to all children with diabetes (3).

The strengths of this study include the large number of families from all regions and meticulous data collection.

Study Limitations

The limitations of this study included its cross-sectional study design, reliance on self-reported data, and unknown response rates. Another limitation was the failure to inquire about participants' awareness of the existence of diabetes care devices and their benefits, which are determinants of technology access. Furthermore, the lack of information about pump models and the number of users of automated insulin delivery systems hindered glycemic assessment in our study, despite the evident improvement in glycemia associated with automated insulin delivery systems (18).

Conclusion

There were inequalities in access to diabetes technologies, affected by factors such as parental education, regional socioeconomic development, and household income. These disparities are more pronounced in terms of access to CGM, despite its significant contribution to improving glycemic control. Thus, there is a need for specific initiatives to overcome disparities in technology access for children with T1D, especially those from disadvantaged socioeconomic backgrounds.

Ethics

Ethics Committee Approval: The Ethical Committee at Koç University approved the study (decision no: 2022.378. IRB3.176, date: 03.11.2022) in accordance with the Declaration of Helsinki.

Informed Consent: Consent form was filled out by all participants.

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Footnotes

Authorship Contributions

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ORIGINAL ARTICLE

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Hospital Admission for Diabetic Ketoacidosis in Thai Children and Adolescents with Type 1 Diabetes: A National Study During 2015-2019

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What is already known on this topic?

Despite a globally increasing incidence of pediatric type 1 diabetes mellitus (T1D), the incidence of hospitalization for diabetic ketoacidosis (DKA), a life-threatening yet preventable complication of T1D, varies among countries. Understanding the incidence trend in DKA admission rates may strengthen the preventive measure for DKA.

What this study adds?

The incidence of T1D and DKA admissions in Thailand increased progressively during 2015-2019. School-aged children, adolescents, females, and those residing in the Northeast area were at increased risk for DKA admission. This study underscores the importance of diabetic care among Thai youth with T1D, particularly for those with higher risks.

Abstract

Objective: To study the national incidence of admission for diabetic ketoacidosis (DKA) in Thai children and adolescents with type 1 diabetes mellitus (T1D) and characterize risk factors for DKA admission.

Methods: Admission records of children and adolescents with T1D during the years 2015-2019 were retrieved from the Thai health coverage system of all schemes. Hospitalization was categorized according to patients' age groups (< 1, 1-5, 6-12 and 13-17 years), sex and geographical regions (Bangkok, Central, Northeast, North and South). DKA admission incidence and rate were calculated and compared among subgroups.

Results: The annual incidences of T1D and DKA admissions per 100,000 child-years progressively increased over the study period (T1D: 12.0 to 15.0, $p < 0.001$ and DKA: 4.8 to 7.3, $p < 0.001$). About half of DKA admissions (52%) were recurrent episodes. DKA admission rate was 1.49 admissions/patient. The incidence of DKA admission was greatest in individuals aged 13-17 years (13-17 years: 10.3; 6-12 years: 6.3; 1-5 years: 1.7; and < 1 year: 0.6 per 100,000 child-years, $p < 0.001$). DKA admission incidence was greater in females than males (7.6 vs. 4.3 per 100,000 child-years, $p < 0.001$). Across the geographical regions, the greatest percentage of recurrent DKA (57%), rate of increased annual incidence of DKA admission (3.8 to 7.8 per 100,000 child-years), and DKA admission rate (1.64 admissions/patient) were found in the Northeast region.

Conclusion: During the years 2015-2019, rising annual incidences of T1D and DKA admissions among Thai youth were observed. Individuals older than 6 years, being female, and resided in the Northeast region conveyed a higher risk for DKA hospitalization.

Keywords: Diabetic ketoacidosis, type 1 diabetes, hospitalization, children, adolescent

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Introduction

Diabetic ketoacidosis (DKA) is a life-threatening complication that occurs mainly in patients with type 1 diabetes mellitus (T1D). DKA is a common manifestation at the initial diagnosis of T1D in children and adolescents, with incidence varying widely from 13% to 80% (1). The worldwide incidence of T1D in children and adolescents has dramatically increased during the last 20 years, and currently, over 100,000 children develop T1D annually (2). Unlike the generally rising trend in T1D, hospitalization for DKA differs between countries. The incidence of DKA admission increased in the United States of America (USA) and Canada (3,4) but decreased in the Netherlands, Italy and Korea (5,6,7), whereas it remained stable in Germany and China (8,9). This diversity might be due to international variations in the recurrent DKA rate of individuals with established T1D, accessibility to the healthcare system, and early recognition of hyperglycemia and DKA (1,5,6).

In parallel with the global trend, the incidence of T1D in Thai children and adolescents rose from 0.14 in 1984 to 0.6 per 100,000 person-years in 2014 (10,11). A previous nationwide, population-based study demonstrated a decreasing trend of DKA incidence in Thailand during the years 2015-2020. However, the DKA incidence was calculated per the number of youth with T1D, not the total population (12). That study also excluded recurrent DKA admissions. In addition, only data from the Universal Health Coverage Scheme was analyzed (12), despite the fact that the Thai health coverage system consists of three public insurance programs, including the Universal Health Coverage Scheme, the Social Health Insurance, and the Civil Servant Medical Benefit Scheme (13). As a result, national data focusing specifically on the incidence of DKA admission and its secular trends in Thai children and adolescents with T1D remain unavailable. Recent studies revealed increased risk factors for DKA development in patients who had limited access to medical services and delayed recognition of hyperglycemia (1,14). Despite having national coverage, access to health care services is limited, particularly in rural areas where public transportation is unavailable and travel expenses are unaffordable for local low-socioeconomic status populations (15,16). Moreover, the cost of the testing strip for self-monitoring blood glucose (SMBG) is currently not covered by most insurances (17). These factors might cause a delay in detection of hyperglycemia and thus DKA.

DKA is primarily preventable, while the cost of DKA treatment is high (18). Understanding the trend in DKA admission, identifying individuals who are vulnerable to DKA development, and the consequences of DKA are essential for implementing the national preventive strategy for DKA. Therefore, the aim of this study was to describe a robust national trend in pediatric DKA hospitalization and identify characteristics of T1D youth who had higher risks for DKA admission.

Methods

Study Population and Data Collection

The admission data of T1D and DKA were retrospectively retrieved between the years 2015 and 2019 inclusive from databases of the Universal Health Coverage Scheme, the Social Health Insurance, and the Civil Servant Medical Benefit Scheme. Inclusion criteria were hospital admissions of children and adolescents aged under 18 years who had T1D and DKA diagnosis using the International Code of Diseases (ICD)-10 of E10 and E10.1, respectively. Exclusion criteria were admissions of diabetic patients with ICD-10s other than E10. Duplicated DKA admissions of the same patients over the study period were labelled as recurrent DKA episodes. However, for the first record of DKA admission, particularly in the earlier years of the study period, we could not distinguish DKA in individuals with newly-diagnosed diabetes from those with known diabetes who had DKA before the study period began due to the lack of a specific ICD-10 code. Characteristics, including patient age and sex, hospital level and region, season, comorbidity, and discharge status were collected. Age ranges of the patients were <1, 1-5, 6-12, and 13-17 years. The hospital level included primary, secondary, tertiary, and private hospitals. The hospital region was determined based on five geographical areas of Thailand, which are Bangkok, Central, Northeast, North, and South. According to the data of the Thai meteorological department, the climate consists of three seasons, including summer, rains, and winter (19). We reported the groups of comorbidities according to ICD-10 classification, and infectious diseases were further categorized into organ systems.

The study was approved by the Ethics Committee on Human Research of the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand (decision no: MURA2024/136, date: 16.02.2024) and conformed to the provisions of the Declaration of Helsinki. Informed consent was not obtained from the patients because the data were anonymous and were extracted from the health care schemes with permission.

Hospitalization Parameters

The parameters of hospitalization are as follows.

- Incidence of hospital admission for T1D or DKA (per 100,000 child-years) =

The number of T1D or DKA admissions x 100,000 / Total population

- The percentage of DKA admission among patients with T1D (%) =

The number of DKA admissions x 100 / The number of T1D admissions

- The admission rate of DKA (admissions/patient) =

The number of DKA admissions / The number of patients admitted for DKA

- The mortality rate of DKA (%) =

The number of deceased patients admitted for DKA x 100 / The number of DKA admissions

Statistical Analysis

IBM Statistical Package for the Social Sciences statistics for Windows, version 24.0 (IBM Corp. Armonk, NY, USA), and RStudio (R version 4.2.3; RStudio Inc, Vienna, Austria) were used for statistical analysis. Datasets were compared using the chi-squared test. A p value of less than 0.05 was considered to imply statistical significance.

Results

Overall Pediatric Hospitalization for T1D and DKA

Of 64,677,608 child-years, 8,708 admissions from individuals with T1D (13.5 per 100,000 child-years) and 3,846 admissions from individuals with DKA (5.9 per 100,000 child-years) were recorded (Tables 1, 2). DKA was the main indication (44%) for hospital admission among patients with T1D. The admission rates of T1D and DKA were 1.66 and 1.49 admissions/patient, respectively. The majority of the hospitalized patients with T1D were individuals aged 13-17 years (50%), female (60%), and those residing in the Northeast region (35%) (Table 1). Among DKA hospitalizations, 2007 (52%) were recurrent episodes, and 226 (5.9%) were identified referrals. The majority of patients with DKA were admitted to tertiary (48%) and secondary hospitals (43%), whereas the remaining patients were admitted to primary (6%) and private hospitals (3%). In addition, most DKA admissions occurred in rainy season (mid-May to mid-October, 40%) and winter (mid-October to mid-February, 34%), while 26% were observed in summer (mid-February to mid-May).

The Trend in DKA Admission During the Study Period

The annual incidence of T1D admission rose from 12.0 in 2015 to 15.0 per 100,000 child-years in 2019 ($p < 0.001$) (Figure 1). Likewise, the respective annual incidences of DKA admission increased from 4.8 to 7.3 per 100,000 child-years ($p < 0.001$). Following these findings, the respective percentages of DKA admission among T1D hospitalization increased progressively from 40% to 49% ($p < 0.001$) (Figure 2). Despite overall increases in the incidence and percentage of DKA admission, the mortality rate of DKA admission reduced from 2.4% to 1.2% ($p = 0.06$). The median (interquartile range) length of hospital stay for DKA was 5 (3, 9) days, without significant change throughout the study.

DKA Admissions According to Age Group, Sex and Region

The majority of pediatric DKA admissions in this study were patients in the age groups 13-17 years (50%) and 6-12 years (42%) (Table 2). Following from this, the highest incidence of DKA admission was identified in individuals aged 13-17

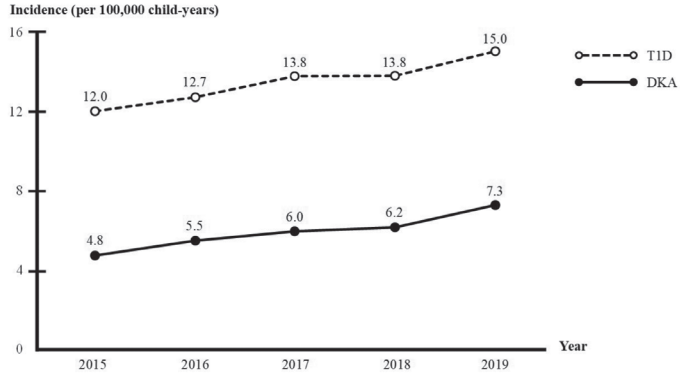

Figure 1. Incidence trend of hospital admission for type 1 diabetes mellitus and diabetic ketoacidosis

T1D: type 1 diabetes mellitus, DKA: diabetic ketoacidosis

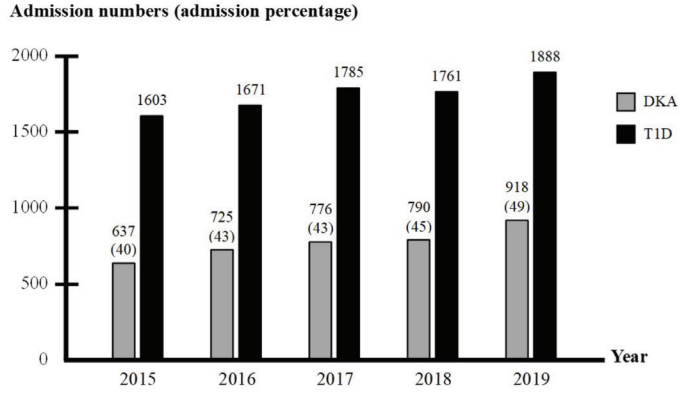

Figure 2. Hospital admission for type 1 diabetes mellitus and diabetic ketoacidosis of individuals aged under 18 years

T1D: type 1 diabetes mellitus, DKA: diabetic ketoacidosis

Table 1. Total admission, population and incidence of hospital admissions in patients with type 1 diabetes during the year 2015-2019

Year	Admission numbers						Total population						Incidence per 100,000 child-years					
	All	2015	2016	2017	2018	2019	All	2015	2016	2017	2018	2019	All	2015	2016	2017	2018	2019
Total	8,708	1,603	1,671	1,785	1,761	1,888	64,677,608	13,321,565	13,121,876	12,939,015	12,747,946	12,547,206	13.5	12.0	12.7	13.8	13.8	15.0
Age groups (years)																		
<1	50	12	19	3	9	7	3,009,424	637,546	623,309	607,525	584,548	556,496	1.7	1.9	3.0	0.5	1.5	1.3
1-5	742	150	155	172	153	112	17,194,614	3,628,116	3,566,589	3,469,736	3,328,164	3,202,009	4.3	4.1	4.3	5.0	4.6	3.5
6-12	3,545	588	705	766	715	771	25,758,480	5,189,861	5,179,687	5,140,056	5,139,904	5,108,972	13.8	11.3	13.6	14.9	13.9	15.1
13-17	4,371	853	792	844	884	998	18,715,090	3,866,042	3,752,291	3,721,698	3,695,330	3,679,729	23.4	22.1	21.1	22.7	23.9	27.1
Gender																		
Male	3,497	655	674	701	749	718	33,230,782	6,842,917	6,740,708	6,648,212	6,551,186	6,447,759	10.5	9.6	10.0	10.5	11.4	11.1
Female	5,211	948	997	1,084	1,012	1,170	31,446,826	6,478,648	6,381,168	6,290,803	6,196,760	6,099,447	16.6	14.6	15.6	17.2	16.3	19.2
Region																		
Bangkok	1,010	203	234	193	216	164	4,811,349	999,836	984,944	970,192	942,542	913,835	21.0	20.3	23.8	19.9	22.9	17.9
Central	2,286	432	438	496	438	482	16,429,931	3,341,918	3,316,553	3,286,895	3,257,522	3,227,043	13.9	12.9	13.2	15.1	13.4	14.9
Northeast	3,084	525	556	631	650	722	21,725,957	4,524,269	4,425,863	4,340,872	4,259,661	4,175,292	14.2	11.6	12.6	14.5	15.3	17.3
North	1,114	214	220	214	222	244	10,444,785	2,155,930	2,118,232	2,087,249	2,057,531	2,025,843	10.7	9.9	10.4	10.3	10.8	12.0
South	1,214	229	223	251	235	276	11,265,586	2,299,612	2,276,284	2,253,807	2,230,690	2,205,193	10.8	10.0	9.8	11.1	10.5	12.5

years (10.3 per 100,000 child-years), and it was followed by that of children aged 6-12 years (6.3 per 100,000 child-years). Recurrent DKA episodes frequently occurred in patients aged 13-17 years (57%) and those aged 6-12 years (51%). The annual incidences of DKA admission in these two groups increased from the year 2015 to 2019 [13-17 years: 9.0 to 12.7 (↑41%); 6-12 years: 4.6 to 7.8 (↑68%) per 100,000 child-years]. The percentages of DKA admission were 46% in individuals aged 6-12 years and 44% in those aged 13-17 years. Individuals aged 6-12 years also had the highest mean DKA admission rate (1.55 admissions/patient).

Regarding sex predominance, the majority of individuals admitted for DKA were female (63%). Compared to males, females had higher incidence of DKA admission (7.6 vs. 4.3 per 100,000 child-years), percentage of DKA admission (46% vs. 41%), percentage of recurrent DKA events (55% vs. 47%), and DKA admission rate (1.56 vs. 1.39 admissions/patient) (Table 2). Furthermore, the annual incidences of DKA admission from 2015 to 2019 increased more pronouncedly in females than males [5.9 to 9.5 (↑63%) vs. 3.8 to 5.2 (↑39%) per 100,000 child-years].

Across the five geographical regions, Bangkok had the highest incidence of DKA admission (9.1 per 100,000 child-years) but the lowest DKA admission rate (1.31 admissions/patient) (Table 2). The peak percentage of DKA admission was in the Central region (52%). Recurrent DKA events most frequently occurred in the Northeast region (57%). Compared with other regions, the Northeast area also had the maximum increase in annual incidence of DKA admission from 2015 to 2019 [Northeast: 3.8 to 7.8 (↑108%), North: 3.1 to 5.0 (↑62%), South: 4.3 to 6.2 (↑44%), Central: 6.5 to 8.2 (↑25%), Bangkok: 8.3 to 9.6 (↑16%) per 100,000 child-years]. In addition, the highest DKA admission rate was observed in the Northeast region (1.64 admissions/patient), which remained far above other areas for most of the study period (Figure 3).

Comorbidities of T1D and DKA Admissions

T1D patients hospitalized for DKA shared similar comorbidities to those admitted for non-DKA conditions (Table 3). Respiratory tract infections were the most common comorbidities in hospitalized T1D patients, irrespective of the presence of DKA. Mental and behavioral disorders were among the most frequent comorbidities in both groups, while adjustment disorder and major depressive disorder were common diagnoses.

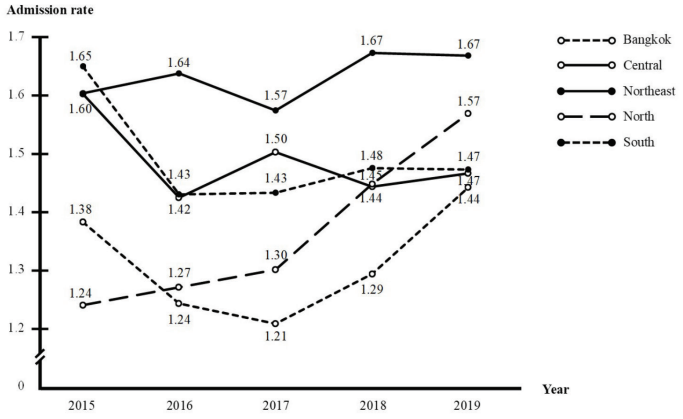

Figure 3. Trend in hospital admission rate for diabetic ketoacidosis (the number of admissions/the number of patients) according to regions

Discussion

From 2015 to 2019, the annual incidence of DKA admission increased progressively, highlighting the importance of DKA prevention. Recurrent DKA events accounted for more than half of DKA admissions. Indeed, such a proportion could be underestimated because first-recorded hospitalizations might be repeated DKA episodes in individuals with known T1D. The findings were consistent with data observed in both the USA and Canada, where incidences of DKA admission increased with the increase in recurrent DKA events (3,4). Accordingly, the increased percentage of recurrent DKA episodes might explain the increased annual incidence of DKA admission in our study. Risk factors for DKA admission could be multifactorial, and some of them affect most T1D individuals while others impact a specific group of individuals (1,3,4). Data from the

Table 2. Diabetic ketoacidosis hospitalization parameters of patients with type 1 diabetes during the year 2015-2019

Year	Total admissions (incidence per 100,000 child-years)						Percentage of DKA admission	Recurrent DKA events (%)	DKA admission rate
	2015-2019	2015	2016	2017	2018	2019	2015-2019	2015-2019	2015-2019
Total	3,846 (5.9)	637 (4.8)	725 (5.5)	776 (6.0)	790 (6.2)	918 (7.3)	44	2,007 (52)	1.49
Age groups (years)									
< 1	18 (0.6)	6 (0.9)	7 (1.1)	0 (0)	4 (0.7)	1 (0.2)	36	5 (28)	1.38
1-5	288 (1.7)	42 (1.2)	67 (1.9)	70 (2.0)	56 (1.7)	53 (1.7)	39	71 (25)	1.20
6-12	1,616 (6.3)	241 (4.6)	314 (6.1)	326 (6.3)	337 (6.6)	398 (7.8)	46	831 (51)	1.55
13-17	1,924 (10.3)	348 (9.0)	337 (9.0)	380 (10.2)	393 (10.6)	466 (12.7)	44	1,100 (57)	1.50
Gender									
Male	1,442 (4.3)	257 (3.8)	266 (3.9)	255 (3.8)	328 (5.0)	336 (5.2)	41	673 (47)	1.39
Female	2,404 (7.6)	380 (5.9)	459 (7.2)	521 (8.3)	462 (7.5)	582 (9.5)	46	1,334 (55)	1.56
Region									
Bangkok	437 (9.1)	83 (8.3)	97 (9.8)	81 (8.3)	88 (9.3)	88 (9.6)	43	208 (48)	1.31
Central	1,192 (7.3)	218 (6.5)	228 (6.9)	251 (7.6)	231 (7.1)	264 (8.2)	52	612 (51)	1.48
Northeast	1,204 (5.5)	170 (3.8)	208 (4.7)	233 (5.4)	266 (6.2)	327 (7.8)	39	685 (57)	1.64
North	424 (4.1)	67 (3.1)	89 (4.2)	82 (3.9)	84 (4.1)	102 (5.0)	38	189 (45)	1.37
South	589 (5.2)	99 (4.3)	103 (4.5)	129 (5.7)	121 (5.4)	137 (6.2)	49	313 (53)	1.48

DKA: diabetic ketoacidosis

Table 3. Five most common comorbidities of hospital admission for type 1 diabetes mellitus and diabetic ketoacidosis in children and adolescents

Rank	T1D			DKA		
	ICD-10	Diagnosis	N (%)	ICD-10	Diagnosis	N (%)
1	J00-J22	Respiratory tract infections	832 (9.6)	J00-J22	Respiratory tract infections	376 (9.8)
2	A00-A09	Intestinal infections	406 (4.7)	A30-A49	Other bacterial diseases	205 (5.3)
3	N30-N39	Other diseases of urinary system	375 (4.3)	N30-N39	Other diseases of urinary system	201 (5.2)
4	F00-F99	Mental and behavioral disorders	345 (4.0)	A00-A09	Intestinal infections	142 (3.7)
5	K20-K31	Diseases of esophagus, stomach and duodenum	309 (3.5)	F00-F99	Mental and behavioral disorders	141 (3.7)

ICD-10: International Code of Diseases-10, T1D: type 1 diabetes mellitus, DKA: diabetic ketoacidosis

Thai T1D registry showed that only 28% of youth performed SMBG at least four times/day, which is below the standard of care (17,20). On top of that, low frequency of SMBG was significantly associated with poor diabetes control, a known risk factor for developing recurrent DKA (14,17). Lack of coverage for glucose strip tests is likely a major problem for a number of Thai children and adolescents with T1D. Together with the increased incidence of DKA admission found in our study, the health insurance coverage for blood glucose strip tests in Thai T1D youth should be endorsed.

Compared to their younger counterparts, T1D patients over 6 years of age had a significantly higher incidence of DKA admission. Previous reports also showed the maximum rates of DKA admission in adolescents followed by school-aged children (18,21). Non-adherence to treatment is a common risk factor for developing DKA in adolescents (22). According to the data of T1D patients in the Thai registry, school-aged children had the lowest proportion of those achieving hemoglobin A1c targets (17). High admission rates in school-aged children might reflect a lack of comprehensive diabetic education for school personnel, which is indispensable for these vulnerable individuals (23). We believe that diabetic education for school nurses in Thailand is still lacking.

Consistent with other studies, we found that females had a higher incidence of DKA admission than males (3,4,6,7,9,24). Possible explanations might include insulin omission and intentional insulin restriction, which were more common in females and were risk factors for DKA in patients with known diabetes (14,25). Females were also more likely to receive the diagnoses of impaired psychosocial adjustment and psychiatric disorders, which were associated with poor glycemic control and diabetes-related complications (26). Interestingly, we found that mental and behavioral disorders were not uncommon in hospitalized T1D patients (Table 3). These findings reflect that female youth with T1D require more intensive and holistic care in which diabetes self-education and routine psychosocial support is essential.

Bangkok had the highest incidence of DKA admission but the second lowest DKA admission number and the lowest DKA admission rate. The total number of individuals in Bangkok was undeniably the smallest of all regions (Table 1). In addition, Bangkok has the most referral tertiary medical centers. These factors might lead to a falsely high incidence of DKA admission. In contrast, the Northeast region had the highest hospitalization parameters, including recurrent DKA admission, increased incidence of DKA admission, and DKA admission rate. Residents of the Northeast area have the lowest socioeconomic status according to poverty indices, such as the lowest average monthly profits and the highest numbers of poor people (27). Traveling in the area is problematic while the transportation costs are high and the health care availability is the least of all regions in Thailand (16). As a result, the Northeast region had the maximum rate of unmet healthcare needs for both in-patient and out-patient departments (16). Limited access to medical services, a potential risk factor for developing DKA, would plausibly contribute to the highest DKA admission in the Northeast area (1,14).

Infections were among the common precipitating causes of DKA, with varied frequency from 14% to 58% in different countries (28). Korbel et al. (29) demonstrated that respiratory infections were the most common infectious disease in hospitalized children with T1D in the USA. Similarly, we found that respiratory tract infections were prevalent in patients with T1D hospitalized for DKA and non-DKA. Our findings thus emphasized the importance of preventive measures for respiratory infections and sick day management among youth with T1D.

Over four decades, several studies among Thai children and adolescents have focused on the regional or national incidence of T1D using either questionnaires or medical records, but none specifically reported the incidence of DKA admission (10,11,30,31,32,33,34,35). To the best of our knowledge, this is the first study using the databases of all Thai health coverage systems that show national hospitalization data of patients with T1D and DKA.

Study Limitations

Limitations of this study included a relatively short study period (5 years) and the diagnosis of T1D and DKA, which were based solely on ICD-10 codes. Hence, newly diagnosed and known T1D were indistinguishable.

Conclusion

Increased incidences of T1D and DKA admissions among Thai youth during 2015-2019 were observed. Individuals who had a higher risk of being admitted for DKA were those over 6 years of age, being female, and residing in the Northeast region.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee on Human Research of the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand (decision no: MURA2024/136, date: 16.02.2024) and conformed to the provisions of the Declaration of Helsinki.

Informed Consent: Informed consent was not obtained from the patients because the data were anonymous and were extracted from the health care schemes with permission.

Footnotes

Authorship Contributions

Concept: Taninee Sahakitrungruang, Pat Mahachoklertwattana, Design: Taninee Sahakitrungruang, Pat Mahachoklertwattana, Data Collection or Processing: Somboon Wankanit, Kaewjai Thepsuthammarat, Pat Mahachoklertwattana, Analysis or Interpretation: Somboon Wankanit, Kaewjai Thepsuthammarat, Preamrudee Poomthavorn, Pat Mahachoklertwattana, Literature Search: Somboon Wankanit, Preamrudee Poomthavorn, Pat Mahachoklertwattana, Writing: Somboon Wankanit, Kaewjai Thepsuthammarat, Preamrudee Poomthavorn, Taninee Sahakitrungruang, Pat Mahachoklertwattana.

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Endocrine Disorders in Children with Primary Mitochondrial Diseases: Single Center Experience

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What is already known on this topic?

Primary mitochondrial diseases (MD) can manifest with endocrine abnormalities characterized by problems in hormone production and secretion. The initial clinical manifestation of primary MD may be a hormonal deficit.

What this study adds?

This study examined the genetics, phenotype, auxological data, and hormonal profiles of children and adolescent patients with MD. To the best of our understanding, this study represents the most extensive investigation conducted on this specific patient population in Türkiye.

Abstract

Objective: Endocrine abnormalities may be the only clinical manifestation of primary mitochondrial disorders. The aim of this study was to evaluate the endocrinological characteristics of mitochondrial disease (MD) in a cohort from a single center.

Methods: Pediatric patients diagnosed with MD were categorized on the basis of their specific genetic abnormalities. The auxologic data, pubertal development, and, based on their clinical symptoms, hormonal profiles were obtained.

Results: Twelve of the cohort of 26 patients (46%) were female. In 15 (57.6%), the MD was caused by nuclear DNA mutations (nDNA group). Four patients had Leigh syndrome, two patients had Leber's Hereditary Optic Neuropathy syndrome, two patients had Mitochondrial Encephalopathy Lactic Acidosis and Stroke Like episodes, and one patient had Kearns-Sayre syndrome clinical phenotype. The median age at diagnosis was 2.91 (0.59-16.8) years, and the median age at first endocrine evaluation was 4.62 (1.26-18) years. The mean height standard deviation score (SDS) was -1.34 ± 2.12, and the mean body mass index SDS was -0.82 ± 1.96 for all patients. Of the 26 patients, 6 (23%) had a range of hormonal deficits. Ovarian insufficiency, central adrenal insufficiency, central hypothyroidism, diabetes mellitus, and critical illness-related adrenal insufficiency were all observed. Three of the patients were initially monitored in the endocrine clinic for hormone deficiencies but it was later determined that the hormonal abnormalities were caused by underlying MD.

Conclusion: Individuals diagnosed with MD, particularly those with specific genetic abnormalities, are considered a high-risk group for developing hormonal deficits. Endocrine abnormalities may be one of the primary early warning symptoms for MD.

Keywords: Primary mitochondrial disease, genotype-phenotype, endocrine disorders, endocrin abnormalities

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Introduction

Primary mitochondrial diseases (PMDs) are multisystemic diseases that encompass a broad spectrum of conditions. The incidence of mitochondrial diseases (MDs) is estimated at 1/4,500-5,000 (1). These disorders are caused by point mutations or by large deletions in either the mitochondrial (mtDNA) or nuclear DNA (nDNA), which both alter the structure and function of the mitochondria. In addition to the well-known pattern of maternal inheritance of MD, mutations in two genes can result in autosomal dominant, autosomal recessive, and rare X-linked disorders. Occasionally, sporadic cases may occur (2). Clinical manifestations are extremely variable, and early symptoms of these disorders may manifest at any age. Mitochondrial inheritance patterns differ, in addition to being complex. A single cell may contain hundreds or thousands of mtDNA copies. Homoplasmy occurs when all cells' mtDNA copies are identical (mutant or wild type). Heteroplasmy refers to the presence of mutant or normal mtDNA in a cell. However, the ratio of mutant mtDNA heteroplasmy may not correlate with the patient's clinical symptoms. The precise reason for this is unknown (3). MDs are in the subgroup of inherited metabolic diseases that affected patients develop energy deficiencies. In the classification of MDs, specific clinical, radiological, biochemical findings and physiological analyzes are taken into consideration. However, since MDs has a wide spectrum of phenotypes and genotypes, it is the most difficult group of metabolic diseases to classify. Defects in respiratory chain function and oxidative phosphorylation affect mitochondrial energy metabolism, leading to multisystemic organ failure. PMDs are multisystemic diseases that primarily affect metabolically active tissues, such as the brain, kidney, heart, skeletal muscles, and endocrine organs. MDs constitute a large genetic group and are considered rare diseases or even, despite the prevalence of some types, a very rare disease. Therefore, due to the nature of the disease, it is almost impossible to create a single homogeneous genetic study group unless it is a multicenter, multinational study (4). Mitochondrial cocktails, which consist of antioxidant combinations, exhibit synergistic effects in enhancing final energy production and mitigating oxidative stress. This, in turn, contributes to slowing disease progression by decreasing both the frequency and severity of metabolic attacks (5). Endocrine abnormalities may be one of the early warning symptoms of PMDs (6). Although diabetes mellitus (DM) is a well-known illness resulting from mitochondrial dysfunction, PMDs can exhibit hormonal deficiencies, such as ovarian insufficiency, adrenal insufficiency, hypoparathyroidism, growth hormone deficiency, and hypopituitarism. In MDs such as Kearns-Sayre syndrome (KSS), which is characterized by extensive mtDNA rearrangements, endocrine abnormalities are prevalent (7). All steroid hormones are synthesized using energy supplied by the mitochondria, and poor oxidative phosphorylation results in mitochondrial dysfunction, which impairs the production of intracellular hormones and the secretion of extracellular hormones (8). When patients with multisystemic diseases have endocrine abnormalities, it should be kept in mind that this population may have PMD. Although endocrinological involvement in MDs has been recognized for a long time, publications describing genetic and phenotypic characteristics are quite limited.

In the present study pediatric patients with PMDs were assessed for any endocrinological abnormalities. All included patients had diagnoses confirmed genetically and phenotypically. Although three patients in the cohort had first been monitored in the endocrine clinic due to hormone deficiencies, it was later discovered that the cause of the hormone deficiencies was underlying MD.

Methods

Patients

Patients with confirmed PMD were evaluated in this cross-sectional, descriptive study. The auxological indices, clinical records, and hormonal profiles of patients when they were first admitted to the Bakırköy Dr. Sadi Konuk Training and Research Hospital Outpatient Pediatric Endocrinology and Metabolism Clinics were collected. All patients' clinical characteristics were reported, and they were categorized as having either nDNA or mtDNA mutations. These genetic changes were further categorized according to the areas affected, as previously described (9). The databases Mitocarta and Mitomap were used to improve the classification of the patients' genetic results. The study protocol was approved by Bakırköy Dr. Sadi Konuk Training and Research Hospital Clinical Trials Ethical Committee (decision number: 2023-08-07, date: 17.04.2023).

Parameters for the Study

The metabolic features of the patients were documented from July 2016 to September 2023 and subsequently used to classify the identified mitochondrial disorders. The auxologic data, including height, weight, body mass index (BMI), and head circumference of patients were evaluated using the child metrics program and Turkish children's references (10). In addition, child metrics were used to assess the birth auxologic data for the patients, using Turkish neonatal reference data (11). Hormonal profiles were obtained based on individual patients' clinical symptoms. Anthropometric

parameters, nutritional status, vitamin supplements, and thyroid functions were monitored in the metabolism outpatient clinic, and in consultation with the endocrinology outpatient clinic when there were abnormalities in growth parameters and hormonal profiles. Bone metabolism and other enzyme and hormone profiles, including calcium, phosphorus, magnesium, alkaline phosphatase, parathyroid hormone (PTH) and 25-hydroxyvitamin D levels, were assessed using serum electrolytes. Vitamin D status was assessed by testing serum 25-hydroxyvitamin D. Vitamin D levels were classified as sufficient (20-100 ng/mL), insufficient (15-20 ng/mL), or deficient (< 15 ng/mL) (12). Glycated hemoglobin (HbA1c) was used to assess glucose metabolism. The blood glucose levels of hypoglycemic patients undergoing serial glucose monitoring were also assessed. Gonadotropin levels were acquired from patients whose secondary sex characteristics were considered clinically unusual. Girls older than 13 years and boys older than 14 years who had no pubertal signs were considered to have delayed puberty. The levels of thyroxine (fT4) and thyroid-stimulating hormone (TSH) were measured in every patient. Thyroid autoantibodies of patients whose thyroid function tests were abnormal were collected. Of note, none of the patients had goiters on physical examination. Serum triiodothyronine (fT3) levels were tested in 19 of the patients. In 19 patients with abnormal fT4 and TSH values in the initial evaluation, fT3 levels were also examined.

All patients' basal adrenocorticotropic hormone (ACTH) and cortisol levels were evaluated. Patients with low baseline cortisol levels and hypoglycemic symptoms were tested for adrenocortical insufficiency. A low-dose (1 µg) synthetic ACTH (Cosyntropin) (Alfasigma S.p.a. Via Ragazzi del '99 n.5 40133 Bologna, Italy) test was conducted on patients with basal cortisol levels below 15 µ/dL and with serum cortisol levels below 20 µ/dL during hypoglycemia. Serum cortisol levels were assessed at 10, 20, and 30 minutes. Adrenal insufficiency was indicated when cortisol levels fell below 20 µ/dL following an ACTH injection.

In patients whose growth continued, serum insulin like growth factor-1 (IGF-1) and IGF binding protein-3 (IGFBP-3) levels were evaluated. The standard deviation scores for IGF-1 and IGFBP-3 levels were calculated against the child metrics program (10).

Metabolic Medical Treatment

Cases were supported with medical treatment at the indicated doses: L-arginine 200 mg/kg/day, divided into three doses; coenzyme Q10 15 mg/kg/day, divided into two doses; vitamin B1 10 mg/kg/day, divided into two doses; vitamin B2 10 mg/kg/day, divided into two doses; vitamin B6 30 mg/kg/day, once a day; L-carnitine 50 mg/kg/day, divided into two doses; lipoic acid 10 mg/kg/day, once a day; dichloroacetate 25 mg/kg/day, divided into three doses; and vitamin C 100 mg/kg/day, divided into two doses.

Nutritional Assessment of the Patients

The parents of the patients were trained to keep food records to evaluate the medical nutrition treatments, and three days of food records (two weekdays and a weekend day) were kept. A photographic food catalog was used to determine the amounts and portion sizes of the foods consumed (13). Based on these food records, daily mean energy and macronutrient intake were calculated using a nutrient database program (BeBis 8.2. software), based on the United States Department of Agriculture's FoodData Central and TurKomp National Food Composition Database (14,15,16,17). The energy requirements of patients were calculated according to age and gender according to Food and Agriculture Organization of the United Nations/World Health Organization/UNU equations (18,19).

Biochemical Analysis

Venous blood samples were obtained from the antecubital vein into vacutainer tubes, following an overnight fast by the participants. The plain tubes were centrifuged at 2000 g (10 min) to obtain serum for routine biochemical analyses. Blood samples were immediately centrifuged in EDTA tubes at 1000xg, at 4 °C (10 min) for the ACTH analyses. Another EDTA tube was used to measure HbA1c. Routine biochemical parameters were determined on a Roche Cobas C8000 modular auto-analyzer using commercial kits (Roche Diagnostics, CA, USA). Plasma ACTH was measured using a solid phase, two-site enzyme chemiluminescent system (Immulite 2000 XPi, Siemens Healthcare Diagnostics, USA). HbA1c levels were measured by an Arkray Adams HA-8160 analyzer, using reversed-phase cation exchange high performance liquid chromatography (Arkray KDK, Kyoto, Japan).

Genetic Analysis

Genomic DNA and mtDNA were isolated from peripheral blood lymphocytes. The initial test was for mtDNA sequencing using an in-house developed fragmentation-based methodology (20). The fragmentation process was performed using the Ion Xpress™ Plus Fragment Library Kit. Patients with unidentified genetic variation (no heteroplasmic or homoplasmic causative variant associations in mtDNA) were investigated with exome sequencing. They were examined by clinical exome sequencing (CES) using the Illumina Clinical-Exome Sequencing TruSight One Gene Panel. In the CES, the libraries generated were sequenced

using Illumina Nextseq500 next-generation sequencing platforms. The detected variants were then confirmed by conventional Sanger sequencing.

Statistical Analysis

All data were statistically analyzed using the GraphPad InStat program (v3.05; GraphPad Software Inc, San Diego, CA, USA). Parametrically distributed data were analyzed using descriptive statistics, including mean ± standard deviation, while non-parametric data were analyzed using median, minimum, and maximum. Categorical variables are given as count and percentage. The Kolmogorov-Smirnov test was used to test the normality of variable distribution and the homogeneity of the variance.

Results

The study included twenty-six pediatric patients. Twenty-three (88.5%) with PMD were monitored in a tertiary center pediatric metabolism unit. The remaining three patients were initially diagnosed in a tertiary center pediatric endocrinology unit with primary ovarian insufficiency, DM, and ACTH deficiency. Of the whole group, 12 patients (46%) were female and the MD of 15 patients (57.6%) was caused by nuclear DNA mutations (Tables 1, 2).

Patients' metabolic phenotype data were used to categorize recognized mitochondrial syndromes when the study was conducted. When the 26 patients in our study were evaluated in terms of the clinical findings, four (15.4%) patients had Leigh syndrome, two (7.7%) had Leber's Hereditary Optic Neuropathy (LHON) syndrome, two (7.7%) had Mitochondrial Encephalopathy Lactic Acidosis and Stroke-like episodes (MELAS), and one (3.85%) had a KSS clinical phenotype. Tables 1 and 2 summarize protein and complex deficiencies caused by mutations.

The median age of patients at MD diagnosis was 2.91 (0.59-16.8) years, and the median age at their first endocrinologic evaluation was 4.62 (1.26-18) years. The mean height SDS was -1.34 ± 2.12, with 38.4% (10/26) of all patients having a height SDS < -2 SDS. The mean BMI SDS was -0.82 ± 1.96 for all patients. Three individuals in our cohort had BMIs greater than 1.5 SDS, while eight had BMIs less than -1.5 SDS (Table 3).

The birth data included 13 (52%) with weight information, 12 (48%) with length measurements and 8 (38%) with head circumference measurements. The demographic data for the study group are presented in Table 3.

Twenty-two (84.6%) were prepubertal, and four were at pubertal stage 5. Three of the pubertal stage 5 patients were female (patients 5, 19, and 26) and one was male (patient 18). These female patients all had regular menstrual cycles. Patient 5 had ovarian insufficiency, but she had been given pubertal induction using hormone replacement therapy and had regular menstrual cycles (Table 3).

Serum calcium, phosphorus, and magnesium concentrations were in the normal range for all patients. The mean PTH level was 38.63 ± 23.59 pg/mL (Table 4). Of the cohort, 17 (65.4%) had 25-hydroxyvitamin D levels greater than 20 ng/mL but the median 25-hydroxyvitamin D level was 20 (4.71-94.2) ng/mL. Vitamin D deficiency and vitamin D insufficiency rates in the cohort were 19.2% and 15.4%, respectively.

Of the patients, 16.7% currently followed a ketogenic diet (mean fat ratio of energy: 63.4%); 41.7% of the patients followed a diet rich in fat (mean fat ratio of energy: 44.1%), and 41.6% of the patients did not follow any specific diet. Furthermore, 16.6% were breastfed, and 38.5% of the patients used enteral nutrition products. The rate at which the energy requirement of the patients was met was 89.16 ± 20.20% (minimum-maximum: 58.9-123.6%). All patients met the recommended daily allowance values for protein. On average, 43.33 ± 10.69% of daily energy intake was provided from carbohydrates, 15.17 ± 7.22% from protein, and 43.33 ± 10.48% (minimum-maximum: 32.0-66.7%) from fat. A mean of 14.58 ± 4.48% of daily energy was obtained from saturated fats.

Six (23%) had a range of hormonal deficiencies. Four of these six patients were in the nDNA group. Half of individuals with proven PMD had received a diagnosis of hormonal deficiency and underwent their initial assessment in the endocrinology unit.

Patients with Hormonal Deficiencies in the nDNA Group

Critical Illness-related Adrenal Insufficiency

Patient 4 was a one-year-old female with neuromotor retardation, epilepsy, hypertrophic cardiomyopathy, cystic encephalomalasia, and growth retardation phenotype with autosomal recessive homozygous mutation in the *NDUFV1* gene. She had developed cathecolamine refractory shock and had persistent low blood glucose levels (< 50 mg/dL). She was in the pediatric intensive care unit during hospitalization with septic and metabolic shock. After initiation of 200 mg/m²/day hydrocortisone treatment, her blood pressure levels normalized, and normoglycemia was maintained. This patient was diagnosed with critical illness-related adrenal insufficiency. During periods of hypoglycemia and hypotension, her ACTH and cortisol levels were 96 (7.2-63.3) pg/mL and 68 (6.2-22.6), µg/dL (6.2-22.6), respectively.

Table 1. Characteristics of detected variants in 15/26 patients with proven mitochondrial disease

	Family number	Patient numbers	Gene; phenotype MIM number; inheritance	Variant in nucleotide	Variant in peptide	Associated region	Zygosity	Phenotypic features	Endocrine system findings
1. Mutations in genes encoding structural subunits of respiratory chain proteins	1	**1,2**	*FOXRED1;* # 618241; AR	c.473G > T	p.(G158V)	C I	Hom.	Epilepsy, NMR, strabismus, GR, autism, NMR Leigh syndrome	Vitamin D insufficiency (1), vitamin D deficiency (2)
	2	**3**	*NDUFS7;* # 618224; AR	c.511G > A	p.(D171N)	C I	Hom.	Myopathy, elevated CK	Normal
	3	**4**	*NDUFV1;* # 618225; AR	c.1018G > A	p.(D340N)	C I	Hom.	NMR, epilepsy, HCM, cystic encephalomalasia, GR Leigh syndrome	Criticall ilness related adrenal insufficiency Short stature
2. Mutations in genes encoding ancillary or assembly factors for the respiratory chain function	4	5	*COX15;* # 615119; AR	c.[1011dup]; [1030T > C]	p.([T338fs];[S344P])	C IV	CH	Myopathy, NMR	Ovarian insufficiency Short stature
	5	6	*MICU1;* # 615673; AR	c.330 + 1G > T	p.(?)	MCC	Hom.	Mild autism, mild NMR	Vitamin D deficiency
	6	7,8	*NNT* # 614736, AR	c.1225C > T	p.(Q409fs*)	IMM	Hom.	Dystonia, walking difficulty	Vitamin D insufficiency (8)
	7	9	*RMND1,* # 614922, AR	deletion in 6q25.1	p.(?)	COXPD	Hom.	SNHL, CRF, GR	Ovarian insufficiency Short stature Vitamin D deficiency
3. Mutations in genes encoding mtDNA translation factors	8	10	*ELAC2;* # 615440; AR	c.[85C > T]; [86G > T]	p.([R29C];.[R29L])	COXPD	CH	Epilepsy, hypotonia, NMR, GR	Slightly elevated anti-TPO antibodies Short stature
4. Mutations in genes encoding mitochondrial enzymes	9	11	*ECHS1D;* # 616277; AR	c.476A > G	p.(Q159R)	Mt Mrx	Hom.	Hypotonia, severe NMR, GR	Short stature
5. Defects of intergenomic signaling	10	12	*RRM2B;* # 612075; AR	c.462A > G	p.(Lys154 =)	MM	Hom.	SNHL, DM	DM
6. Other miscellaneous	11	13	*SERAC1;* # 614739; AR	c.1404-2A > G	p.(?)	MM	Hom.	MEGDEL, NMR, hypotonia, GR	Normal
	12	14		c.1396dupA	p.(M446Nfs*15)		Hom.		Short stature (14)
	13	15	*SLC19A3* # 607483; AR	c.597dupT	(p.H200Sfs*r25)	MM	Hom.	Epilepsy, NMR, blindness	Sightly elevated anti-TPO antibodies

VUS: variant of unknown significance, Hom.: homozygous, AR: autosomal recessive, C-I: complex-1, C-IV: complex-4, CK: creatinine kinase, CH: compound heterozygous, COXPD: combined oxidative phosphorylation deficiency, CRF: chronic renal failure, DM: diabetes mellitus, GR: growth retardation, HCM: hypertrophic cardiomyopathy, IMM: inner mitochondrial membrane, MM: mitochondrial membrane, MCC: mitochondrial calcium channel, MMrx: mitochondrial matrix, MEGDEL: 3-methylglutaconic aciduria with deafness-encephalopathy Leigh-like syndrome, NMR: neuromotor retardation, SNHL: sensorineural hearing loss, TPO: thyroid peroxidase

Table 2. Genotype and phenotype characteristics and endocrine dysfunctions in patients with mitochondrial DNA mutations

	Patient no	Gene; locus MIM number; inheritance	Nucleotide change	Amino acid change	Associated region	Zygosity	Phenotypic features	Endocrine system findings
1. Large-scale rearrengements								
2. Single-nucleotide variants (point mutation) in genes encoding structural proteins	16	*MT-ATP6;* * 516060; Mt-in	m.8993T > C	p.L156P	C-V	Heteroplasmy (89%)	Dystonia, hypotonia, contractures, walking difficulty, GR	Normal
	17	MT-ND4 * 516003 Mt-in	m.11467A > G	p.L236L	C-1	Homoplasmy (99%)	Hypoglycemia, encephalopaty, liver failure	Vitamin D insufficiency
	18	*MT-ND5;* * 516005; Mt-in	m.12372G > A	p.L12L	C-I	Homoplasmy (99%)	LHON, epilepsy, NMR	Vitamin D insufficiency
	19		m.12706T > C	p.F124L	C-I	Homoplasmy (97%)	MELAS, cortical blindness, epilepsy	Vitamin D deficiency
	20	*MT-ND1;* * 516000; Mt-in	m.4216T > C	p.Y304H	C-I	Homoplasmy (99%)	LA, nystagmus, NMR, GR Leigh syndrome	Central adrenal insufficiency Vitamin D insufficiency Short stature
	21	*MT-ND3;* * 516002; Mt-in	m.10398A > G	p.T114A	C-I	Homoplasmy (99%)	Microcephaly, contractures, LHON, NMR, GR	Central hypothyroidism Vitamin D deficiency Short stature
3. Mutation in genes encoding tRNA	22	*MT-TA;* * 590000; Mt-in	m.5631G > A	tRNA Ala	Mitochondrial-nuclear crosstalk	Homoplasmy (100%)	HCM, SNHL, GR, myopathy, lactate and CK elevation	Normal
	23	*MT-TN;* * 590010; Mt-in	m.5667G > A	tRNA Asn	Mitochondrial-nuclear crosstalk	Heteroplasmy (88%)	Strabismus, epilepsy, NMR	Vitamin D insufficiency
	24	*MT-TL1;* * 590050; Mt-in	m.3243A > G	tRNA Leu	Mitochondrial-nuclear crosstalk	Heteroplasmy (87%)	MELAS, ptosis, LA, myopathy	Normal
	25	*MT-TL2;* * 590055; Mt-in	m.12308A > G	tRNA Leu	Mitochondrial-nuclear crosstalk	Homoplasmy (97%)	NMR, autism	Normal
4. Mutation in genes encoding rRNA								
5. Other miscellaneous	26	MT-CR	16519T > C	(non-coding)	Entire Control Region	Homoplasmy (100%)	KSS, hypotonia, NMR, GR	Vitamin D deficiency Short stature

C-I: complex-1, C-V: complex-5, CK: creatinine kinase, GR: growth retardation, HCM: hypertrophic cardiomyopathy, MCR: mitochondrial control region, Mt-in: mitochondrial inheritance, KSS: Kearns-Sayre syndrome, LA: lactic acidemia, LHON: Leber hereditary optic neuropathy, MELAS: mitochondrial encephalopathy with lactic acidosis and stroke like episodes, NMR: neuromotor retardation, SNHL: sensorineural hearing loss

Table 3. Demographic and clinical characteristics of the whole cohort

	Number of patients (%)	Mean ± SDS or Median (min-max)
Age (years) at mitochondrial diagnosis* (median)	26 (100)	2.91 (0.59-16.8)
Age (years) at endocrine system evaluation* (median)	26 (100)	4.62 (1.26-18)
Sex		
Female	12 (46.2)	
Male	14 (53.8)	
Gestational age	13 (52)	38.77 ± 1.54
Birth weight SDS	13 (52)	-0.43 ± 2.22
Birth height SDS	12 (48)	-0.20 ± 1.64
Birth head circumference SDS	8 (32)	0.42 ± 1.89
Height SDS	26 (100)	-1.34 ± 2.12
Weight SDS	26 (100)	-1.36 ± 2.26 [(-7.04)-2.33]
BMI SDS	26 (100)	-0.82 ± 1.96
Head circumference SDS	11 (42.3)	-3.51 ± 2.35
Pubertal stage		
1	22 (84.6)	
2		
3		
4		
5	4 (15.4)	

*Non-parametric distribution according to Kolmogorov-Smirnov test.
BMI: body mass index, SDS: standard deviation score, min-max: minimum-maximum

Table 4. Biochemical and hormonal profiles of study population

	Number of patients (%)	Mean ± SDS or Median (min-max)
TSH (mIU/mL)	26 (100)	2.49 ± 1.27
Free T4 (ng/dL)*	26 (100)	1.25 (0.85-4.09)
Free T3 (pg/mL)	19 (73)	3.97 ± 0.95
ACTH (pg/mL)*	26 (100)	35 (4-365)
Cortisol (µg/dL)*	26 (100)	14.95 (5-68)
Calcium (mg/dL)	26 (100)	9.79 ± 0.56
Phosphorus (mg/dL)	26 (100)	4.57 ± 0.91
Magnesium (mg/dL)	26 (100)	2.1 ± 0.18
ALP (U/L)	26 (100)	203.5 ± 71.52
PTH (pg/mL)	26 (100)	38.63 ± 23.59
25 OH vitamin D (ng/mL)*	26 (100)	20 (4.71-94.2)
HbA1c %*	26 (100)	5.2 (4.7-7.25)
FSH (mIU/mL)*	6 (23)	9.5 (3.05-280)
LH (mIU/mL)*	7 (26.9)	8.3 (0.85-66)
IGF-1 (ng/mL) SDS*	23 (88.5)	0.6 (-2.1-9.03)
IGFBP-3 (mg/L) SDS*	22 (84.6)	-0.25 (-2.38-7.07)

*Non-parametric distribution according to Kolmogorov-Smirnov test.
TSH: thyroid stimulation hormone, T4: thyroxine, T3: tri-iodothyronine, ACTH: adrenocorticotrophic hormone, ALP: alkaline phosphatase, PTH: parathyroid hormone, 25 OH vitamin D: 25-hydroxyvitamin D, HbA1c: glycolysated haemoglobin A1c, FSH: follicle stimulating hormone, LH: luteinising hormone, IGF-1: insulin like growth factor-1, IGFBP-3: IGF binding protein-3, min-max: minimum-maximum

Ovarian Insufficiency

Two patients (patients 5 and 9) had ovarian insufficiency. Both were 46,XX karyotypes and had elevated gonodotropin levels after 13 years of age. Patient 5 was initially followed in the metabolism unit with mild myopathy and neuromotor retardation. Her baseline follicle stimulating hormone (FSH), luteinising hormone (LH), and estradiol levels were 61.99 mIU/mL, LH 19.76 mU/mL, and estradiol 5 pg/mL, respectively.

Patient 9 had sensorineural hearing loss (SNHL), chronic renal failure (CRF), and growth retardation. This patient was admitted to the pediatric endocrinology unit due to the absence of breast development. Her baseline FSH, LH, and estradiol levels were 280 mIU/mL, 66.34 mU/mL, and < 20 pg/mL, respectively. Investigation for MD was initiated because of her multisystemic involvement.

Diabetes Mellitus

Patient 12 was a nine-year-old female diagnosed with insulin-dependent DM when she was 4.8 years old. At the time of diagnosis, her glucose level was 299 mg/dL, c-peptide was 0.415 µg/L (0.9-7.1), HbA1c was 9.1%, islet cell antibodies (ICA) were positive at 1/10 (< 1/4), insulin antibodies were positive at 13% (NR 4-10%), glutamic acid decarboxylase antibodies were very high at 1803 IU/L (NR 0-5). This patient had SNHL. Her hearing loss was diagnosed when she was 13 months old. Her parents were first-degree cousins. Four years after the diagnosis of diabetes, her growth was normal, her mean HBA1c was 7% during the follow-up period, her mean daily insulin dose was 0.5 unit/kg/day, and her c-peptide level was low at 0.296 µg/L (0.9-7.1). This patient was evaluated for unusual causes of DM and a mutation was identified in the *RRM2B* gene.

Patients with Hormonal Deficiencies in the mtDNA Group

Central Adrenal Insufficiency

Patient 20 was an eighteen-month-old hypotonic boy admitted to the pediatric endocrinology outpatient clinic for hypoglycemic attacks. During hypoglycemia, when his blood glucose level reached 28 mg/dL, his ACTH and cortisol levels were 12 pg/mL and 5 µg/dL, respectively. A 1 µg ACTH stimulation test was performed, and his peak cortisol was 11.85 µg/dL. The patient was diagnosed with central adrenal insufficiency; no additional pituitary hormone deficiencies were present. The patient's neurological development was retarded. He was unable to walk or sit without support. Magnetic resonance imaging of the pituitary showed that it was normal. The patient also had lactic acidemia, nystagmus, and neuromotor and growth retardation phenotypes, and a mutation was identified in the *MT-ND1* gene.

Central Hypothyroidism

Patient 21 had a mutation in the *MT-ND3* gene, was phenotypically LHON, and had microcephaly, neuromotor and growth retardation, and contractures. This patient was diagnosed with MD at the age of 1.16 years and with central hypothyroidism at the age of 2.34 years. TSH was 1.3 mIU/mL, fT4 was 0.87 ng/dL, and fT3 was 3.7 pg/mL (2.41-5.5) in her first assessment with no other acute illnesses. Her thyroid function tests were TSH 0.8 mIU/mL, fT4 0.81 ng/dL, and fT3 2.2 pg/mL (2.41-5.5) at the baseline time of the ACTH stimulation test. Her basal ACTH level was 22 pg/mL, and her cortisol level was 8.26 mg/dL. 1 mcg ACTH stimulation test was performed, and her peak cortisol level was found to be 22 mcg/dL, and L-thyroxine treatment started at 10 mcg/kg/daily. There were no other pituitary hormone deficiencies. Magnetic resonance imaging of the pituitary showed it to be normal.

In addition to the six MD patients who had endocrinological hormone secretion deficiency, two patients in the nDNA group had anti-thyroid peroxidase antibody (ATPO) positivity, despite their normal thyroid function tests and normal thyroid gland ultrasonography. The ATPO levels for patient 10 and patient 15 were 16 IU/mL and 22.6 IU/mL, respectively. Anti-TPO levels below 13 IU/mL are considered normal in our laboratory references.

The classification of the genotype-phenotype and endocrinological characteristics of patients with nDNA and mtDNA mutations are presented in Tables 1 and 2, respectively. The study population characteristics, and hormonal profiles can be seen in Tables 3 and 4, respectively.

Discussion

Hormone synthesis and secretion are both energy-dependent processes, dependent on the energy generating systems of the cellular mitochondria. This dependency makes the endocrine glands sensitive to mitochondrial dysfunction. PMDs may result in one or more hormone deficiencies, depending on the severity of the mitochondrial disorder. In addition, due to the random distribution of mitochondria during embryogenesis, patients with affected endocrine glands may present with unpredictable clinical characteristics (9). It is known that findings may be very different, depending on whether the pathogenic variation is inherited in the nDNA or the mtDNA, together with its type. Even within the same family, clinical findings of varying severity may develop depending on penetrance and on the inherent variability of these conditions (1). In this paper, the pattern of endocrinological involvement in patients with MDs, as diagnosed in our single tertiary center, are presented.

MD prevalence is estimated as 1/5000 in the adult age group (2/3 of whom are mtDNA), and as 5-10/100,000 in the pediatric age group (80% of whom are nDNA) (3). In the North American Mitochondrial Disease Consortium (NAMDC) Patient Registry study, 60% of pediatric patients had mtDNA mutations (9), which was similar in our cohort. The explanation for this is that patients with nDNA mutations are diagnosed earlier as more severe clinical findings develop at an earlier stage of life.

The Mitochondrial Society 2017 guideline recommends annual or biannual endocrinological evaluation for these patients, even if they have no hormonal dysfunction at the time of diagnosis (21). An Australian cohort with a mean age of 5.09 years at diagnosis was considerably older than our cohort at diagnosis (22). In contrast to our study, in a large cohort that consisted of patients of any age, female dominance was observed (9). However, in our cohort, there were more male than female patients (Table 3). This may be related to the fact that our group included a relatively limited number of patients.

Twenty-three percent of our patients (two with mtDNA and four with nDNA mutations) had already recognized endocrinological abnormalities. It is difficult to estimate the prevalence of endocrinological disorders in all MDs. Theoretically, in all cases of MD, the endocrine glands are sensitive to energy deficiency and oxidative stress. However, it has been shown that some well-known mutations can cause distinct endocrine findings (23,24). While endocrinological follow-up is recommended for all patients with MDs, patients with these identified mutations should be evaluated more closely. MD patients are known to show poor growth, quite apart from any growth hormone insufficiency. There are many factors affecting growth in this group of patients. Most patients show prenatal and postnatal growth failure. Von Kleist-Retzow et al. (25) observed that 22.7% of 300 mitochondrial respiratory chain deficiency patients had intrauterine developmental retardation. Feeding difficulties, restriction of energy production, frequent infections, and multiple organ system failure all have negative effects on normal growth patterns. Skeletal changes, such as joint contractures, scoliosis, or kyphosis, also have negative effects on MD patients. Growth hormone deficiency may be the first symptom of MD (26,27). The majority of our patients were close to normal height but, within our group, there were ten (38.5%) with SDS values below -2 at the time of first endocrine assesment. None was growth hormone deficient. In this respect, supportive treatment is found to be valuable in MD patients, even without growth hormone deficiency. Despite the fact that not all of our patients had completed their growth, the mean height was slightly greater than that of an adult MD cohort from the United Kingdom published in 2009 (27).

Hypo- or hyper-gonadotropic hypogonadism are both well-known entities in MD patients. Ovarian insufficiency alone, with or without SNHL, is frequently seen in MDs (28). Recently, some studies have reported that the *RMND-1* gene is a candidate for premature ovarian insufficiency. Our patients had phenotypic features similar to those of these studies, including SNHL and CRF (29,30,31,32,33,34).

Primary adrenal insufficiency is the main cause of adrenal insufficiency in MDs. To the best of our knowledge, secondary adrenal insufficiency has been reported only in a Japanese woman at fifty years of age (35). In another report including 18 patients with single, large-scale mitochondrial DNA deletions, 39% of patients had impaired basal adrenocortical function (36). In our cohort there were two patients with adrenal insufficiency, and only one of them had a critical illness related adrenal insufficiency. This patient had elevated cortisol levels, despite having clinical adrenal insufficiency. Under conditions of critical stress, the elevation of the cortisol level is explained by the adrenal gland's subacute response to the insult, decreasing cortisol clearance, and shifting to increased cortisol receptor activation (37). None of our patients had primary adrenal insufficiency, including two siblings with *NNT* mutations. Both of these had dystonia and difficulty walking, although neither had hypoglycemia, fatigue, or hyperpigmentation, and their reactions to the 1 µg ACTH test were normal (peak cortisol responses were 19 and 22 µg/dL, respectively). This mutation is a well-known cause of familial glucocorticoid deficiency (38).

DM is a common occurrence in MDs. Decreased sensitivity and insulin production are both seen in some pathophysiologies. The *MT-TL-1* gene m.3243A > G mutation is the most prevalent mutation in diabetes-associated MD (39). Patient 23 had this genetic abnormality, but no clinical or biochemical signs of diabetes. This genotype is associated with the MELAS phenotype, as was the case in our patient. In the general population, the prevalence of this genotype is 1/400 and the mean age for diagnosis of diabetes is thirty-eight years. This mutation is suggested to be responsible for ~0.5-2.9% of all diabetes (28,39,40). In our cohort there was only one female patient with DM who had a mutation in *RRM2B* gene. Mutations in *RRM2B* have been reported as a cause of MD (28). In a Japanese diabetic cohort with m.3243A > G mutations, one patient had high positivity for anti-GAD and ICA antibodies, and the authors suggested the coexistence of MD with autoimmunity in this patient. In this Japanese study, another 12 patients had slightly elevated ICA antibodies. It is suggested that this may be due to an autoimmune response to mitochondrial cell injury (39).

In the NAMDC study mentioned earlier, the prevalence of hypothyroidism in their MD cohort was 4.3%. This percentage was considered close to that of the general population (6.3%) in the USA (9). It has also been reported that hypothyroidism is more prevalent with nDNA deletions than with mtDNA variants (28). In our relatively small cohort there was only one patient with central hypothyroidism, and no one in our cohort had primary hypothyroidism. However, we did have two patients with slightly elevated anti-TPO antibodies. Some studies report that MD patients frequently have autoimmune disorders, but we have no proof that autoimmunity is more prevalent in MD patients than in the general population (41). One study reported KSS in a patient with autoimmune thyroid disease (42). There hasn't been an objective evaluation of mitochondrial cocktails' effectiveness in terms of the endocrine and other involved systems. Unfortunately, it is not easy to evaluate its effectiveness.

Study Limitations

This study was a cross-sectional observational study in which we analyzed 26 patients' auxological data and endocrinological parameters at the time of the study. We were unable to access some of these patients' birth auxologic data. Furthermore, we evaluated the auxologic data of the patients at a specific time only, and were unable to document their growth velocity. In future, prospective follow-up studies conducted with patients with PMD should provide more comprehensive data on growth patterns and the development of other endocrine disorders in this specific patient population. Furthermore, PMDs are rare diseases, and our study focused on data from a single center only, so the study group consisted of a limited number of cases. However, this work does present an extensive preliminary endocrinological assessment of children with mitochondrial disorders.

Conclusion

Individuals diagnosed with MD, particularly those with specific genetic abnormalities, are considered a high-risk group for developing hormonal deficits. Endocrine abnormalities may serve as an early warning sign for PMDs. Timely identification and treatment of hormonal insufficiency can significantly influence the course of clinical development. Conducting further research in this area will provide additional positive outcomes for evidence-based guidelines regarding the endocrinological assessment of patients with PMD.

Ethics

Ethics Committee Approval: The study protocol was approved by Bakırköy Dr. Sadi Konuk Training and Research Hospital Clinical Trials Ethical Committee (decision number: 2023-08-07, date: 17.04.2023).

Informed Consent: Consent form was filled out by all participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Esra Deniz Papatya Çakır, Melike Ersoy, Nihan Çakır Biçer, Concept: Esra Deniz Papatya Çakır, Melike Ersoy, Asuman Gedikbaşı, Design: Esra Deniz Papatya Çakır, Melike Ersoy, Asuman Gedikbaşı, Data Collection or Processing: Esra Deniz Papatya Çakır, Melike Ersoy, Nihan Çakır Biçer, Analysis or Interpretation: Esra Deniz Papatya Çakır, Melike Ersoy, Nihan Çakır Biçer, Asuman Gedikbaşı, Literature Search: Esra Deniz Papatya Çakır, Melike Ersoy, Nihan Çakır Biçer, Writing: Esra Deniz Papatya Çakır, Melike Ersoy, Nihan Çakır Biçer.

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ORIGINAL ARTICLE

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Experience in a PTEN Hamartoma Tumor Syndrome Expertise Centre: Yield of Thyroid Ultrasound Surveillance in Children with PTEN Hamartoma Tumor Syndrome

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What is already known on this topic?

Literature describes PTEN hamartoma tumor syndrome (PHTS) patients who developed thyroid abnormalities, including differentiated thyroid carcinoma (DTC), before the age of 18 years. However, the exact risk for DTC under age 18 is unknown and no consensus has been reached thus far about the age to initiate thyroid ultrasound surveillance in children with PHTS.

What this study adds?

This study provides unique data about thyroid surveillance in children with PHTS. An expertise center undertook thyroid ultrasound surveillance in children, at least from age 12 years, in order to early detect DTC, and identified 5% with DTC and 84% with thyroid abnormalities on ultrasound. To minimize unnecessary invasive fine needle aspirations and surgeries, surveillance should preferably be assigned to experienced clinicians.

Abstract

Objective: Children with PTEN hamartoma tumor syndrome (PHTS) are at increased risk for developing thyroid abnormalities, including differentiated thyroid carcinoma (DTC). The Dutch PHTS guideline recommends ultrasound surveillance starting from age 18 years. Since the literature describes PHTS patients who developed DTC before this age, the Dutch PHTS expertise center has initiated annual ultrasound surveillance starting from age 12 years. The purpose of this study was to identify the yield of thyroid ultrasound surveillance using this cut-off.

Methods: A retrospective, single center, cohort study was conducted. Pediatric PHTS patients who received thyroid ultrasound surveillance before age 18 years between 2016-2023 were included. Medical records were reviewed. Primary outcomes included prevalence and time to develop thyroid nodules ≥10 mm, nodular growth, goiter, thyroiditis and DTC. Descriptive statistics and Kaplan-Meier analyses were performed.

Results: Forty-three patients were included. Two (5%) were diagnosed with DTC at ages 12 and 17 years. Both DTCs were identified as minimally invasive follicular carcinoma at stages pT3NxMx and pT1NxMx respectively. A total of 84% were diagnosed with thyroid

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abnormalities at a median age of 12 (9-18) years. Most common findings were benign, including nodular disease (74%), goiter (30%) and autoimmune thyroiditis (12%). Nodular growth was observed in 14 patients (33%) resulting in (hemi)thyroidectomy in 7 (16%).
Conclusion: Thyroid ultrasound surveillance resulted in the detection of DTC in 2/43 (4.65%) PHTS patients before age 18. These findings support the recommendation to initiate thyroid ultrasound surveillance in children with PHTS from at least age 12, preferably within an expertise center.
Keywords: PTEN hamartoma tumor syndrome, thyroid ultrasound surveillance, differentiated thyroid carcinoma, thyroid nodules, goiter, children, Cowden syndrome

Introduction

The PTEN hamartoma tumor syndrome (PHTS) is a rare autosomal dominant hereditary syndrome caused by pathogenic variants in the phosphatase and tensin homolog gene (*PTEN*). *PTEN* is a tumor suppressor gene, that regulates cellular growth, migration and apoptosis (1). Currently, PHTS encompasses Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome and PTEN-related Proteus syndrome, previously thought to be distinct disorders. Clinical characteristics of PHTS in children include macrocephaly, developmental delay, autism or intellectual disability, hamartomas together with cutaneous manifestations, such as papillomatosis, lipomas, trichilemmomas and vascular malformations (2).

Patients with a *PTEN* variant are at increased risk for developing both benign and malignant tumors in a variety of organs, most frequently in thyroid, breasts and endometrium. For thyroid cancer, the current Dutch PHTS guideline recommends annual palpation of the thyroid starting from PHTS diagnosis and ultrasound surveillance starting from the age of 18 years (3). The European PHTS guideline also recommends ultrasound surveillance starting from the age of 18 years (4). However, differentiated thyroid carcinoma (DTC) can develop in childhood (2,4,5). Previous studies have estimated the incidence of DTC in pediatric patients at 4-12% (2,6,7) and the median (range) age of DTC diagnosis in children is estimated at 12 (4-17) years (6). PHTS patients evaluated in the Radboud University Medical Centre (Radboudumc), the Dutch PHTS expertise center, have been offered annual thyroid ultrasound surveillance starting from the age of 12 years since 2016. Yet, the evidence for this surveillance is limited, as few studies have been performed to evaluate thyroid surveillance outcomes in pediatric cohorts. Current surveillance guidelines are mostly based on expert opinion. Recommendations regarding the age to initiate thyroid surveillance range from age 7 to 18 years, or even from the time of PHTS diagnosis and are not incorporated by current guidelines (5,6,8,9). Moreover, pathology rates are likely overestimated due to ascertainment bias.

This study analyzed the value and clinical outcomes of thyroid ultrasound surveillance in the pediatric PHTS cohort of the Radboudumc, starting from the age of 12 years. The aim was to provide evidence and recommendations regarding thyroid ultrasound surveillance in pediatric PHTS patients between the ages of 12 and 18 years.

Methods

Setting and Participants

A retrospective, single center, cohort study was conducted including pediatric PHTS patients evaluated in the Radboudumc from 2016 until 2023. All patients who were diagnosed with PHTS (clinically or genetically confirmed) and underwent thyroid ultrasound surveillance before the age of 18 years were included. A clinical diagnosis was based on a positive family history of PHTS in combination with clinical features. Genetic diagnosis was confirmed using either Sanger sequencing, in case of positive family history or high clinical suspicion, or whole exome sequencing, in case of a broader differential diagnosis. No minimal follow-up time was required. Ultrasound examinations were performed by specialized radiologists, who used standardized protocols to assess thyroid surveillance ultrasound imaging. The multidisciplinary expertise team included pediatric endocrinologists, a pediatric neurologist, clinical geneticists, nuclear radiologists and pathologists experienced in pediatric thyroid cancer, and conformed with the Dutch recommendation guideline for the management of pediatric DTC (10). All patients gave informed consent to participate in this study. The Research Ethics Committee of the Radboudumc (CMO Arnhem-Nijmegen) declared that ethical approval was not required for this retrospective study.

Data Collection

Patient data were obtained from the electronic medical records of the Radboudumc. For each patient, information regarding diagnosis, genetics, clinical characteristics, physical examination, laboratory values, ultrasound results and pathology reports were collected. Data up to and including age 18 years were collected. Primary outcomes included the prevalence and time to develop thyroid nodules with size ≥10 mm, nodular growth, goiter, thyroiditis and

DTC. Nodular disease was defined as presence of any nodule, regardless of its size. Nodular growth was defined as growth that was clinically relevant according to the radiologist. This was considered by the radiologist in case of a 20% increase in ≥2 nodule dimensions and an enlargement of ≥2 mm or a ≥50% increase in volume (11). The World Health Organization (WHO) normative values for thyroid volume in children were used by the radiologists to define goiter (12). Thyroiditis was defined as signs of thyroiditis visible on ultrasound according to the radiologist. Autoimmune thyroiditis was referred to as presence of thyroid autoantibodies in combination with signs of thyroiditis on ultrasound. The definition DTC was used when cancer of the thyroid was histologically proven. Secondary outcomes included physical examination findings, laboratory values, presence of nodules < 10 mm, presence of adenomas, fine needle aspiration (FNA) results, and treatment information. Treatment encompassed potassium iodide, hemithyroidectomy, cervical lymph node dissection and adjuvant iodine-131. The Bethesda classification was used to describe FNA results (13).

Statistical Analysis

Descriptive analyses were performed. Continuous data are presented as medians (range) or means [standard deviation (SD)]. Categorical data are presented as absolute numbers and percentages. Kaplan-Meier analyses were performed to estimate the probability of developing thyroid ultrasound abnormalities over time. Right censoring was applied at last performed follow-up ultrasound or at the age of 18 years, whichever came first. Log-rank tests were performed to evaluate associations between patient characteristics and time-to-event of ultrasound abnormalities. P values of < 0.05 were considered statistically significant. To correct for ascertainment bias, Kaplan-Meier analyses were performed without the index patients. Index patients were defined as patients who developed thyroid abnormalities prior to PHTS diagnosis. Statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics, version 27.0 (IBM Inc., Armonk, NY, USA).

Results

Study Population and Clinical Characteristics

The medical files of all 142 pediatric PHTS patients known at the Radboudumc were screened for the presence of thyroid ultrasound reports. A total of 99 patients were excluded, mainly since they had not begun to receive thyroid ultrasound surveillance (Figure 1). The final study cohort consisted of 43 patients: 27 males (63%) and 16 females (37%), including three sibling pairs (Table 1). The median age at PHTS diagnosis was 5 (1-14) years. In the majority of patients (79%), macrocephaly contributed to the PHTS diagnosis. Both mild and severe developmental delay were common clinical presenting features (65%). In 4 (9%) thyroid abnormalities led to PHTS diagnosis. During follow-up, macrocephaly was present in almost all patients (98%), with an median head circumference SD score (SDS) +3.9 (2.9-4.9). Subcutaneous lipomas were already present in 13 patients (30%). In a total of 14 patients (33%) a palpable mass was identified by physical examination of the thyroid gland and all 14 had thyroid abnormalities (nodules ≥10 mm, goiter or thyroiditis) on their first ultrasound, although in one patient swelling was probably caused by an enlarged lymph node. A total of 20 patients (47%) had a family history of thyroid abnormalities (in both PHTS patients and healthy individuals), whereas 16 patients (36%) had no family history documented.

Table 1. Characteristics of the pediatric PHTS study cohort (n = 43)

Demographic	n (%) or median (range)
Sex	
Female	16 (37%)
Male	27 (63%)
Age PHTS diagnosis	5 (1-14)
Presenting clinical features	
Macrocephaly	34 (79%)
Developmental delay	28 (65%)
Autism and intellectual disability	5 (12%)
Family history of PHTS (at presentation)	9 (21%)
Thyroid pathology	4 (9%)
Skin lesions	8 (19%)
PHTS diagnosis	
Clinically confirmed	3 (7%)
Genetically confirmed	40 (93%)
Sanger sequencing	28 (70%)
Whole exome sequencing	9 (23%)
Unknown	3 (8%)
DNA tested on	
Blood	28 (70%)
Additional testing skin lesion	2 (5%)
Unknown	12 (30%)
PTEN variant	
Pathogenic	34 (85%)
Likely pathogenic	3 (8%)
Variant of uncertain significance	1 (3%)
Unknown	2 (5%)
PHTS inheritance	
De novo	20 (50%)
Maternal	15 (38%)
Paternal	3 (8%)
Unknown	2 (5%)
Family history of thyroid pathology	
Yes	20 (47%)
No	7 (16%)
Unknown	16 (37%)

PHTS: PTEN hamartoma tumor syndrome

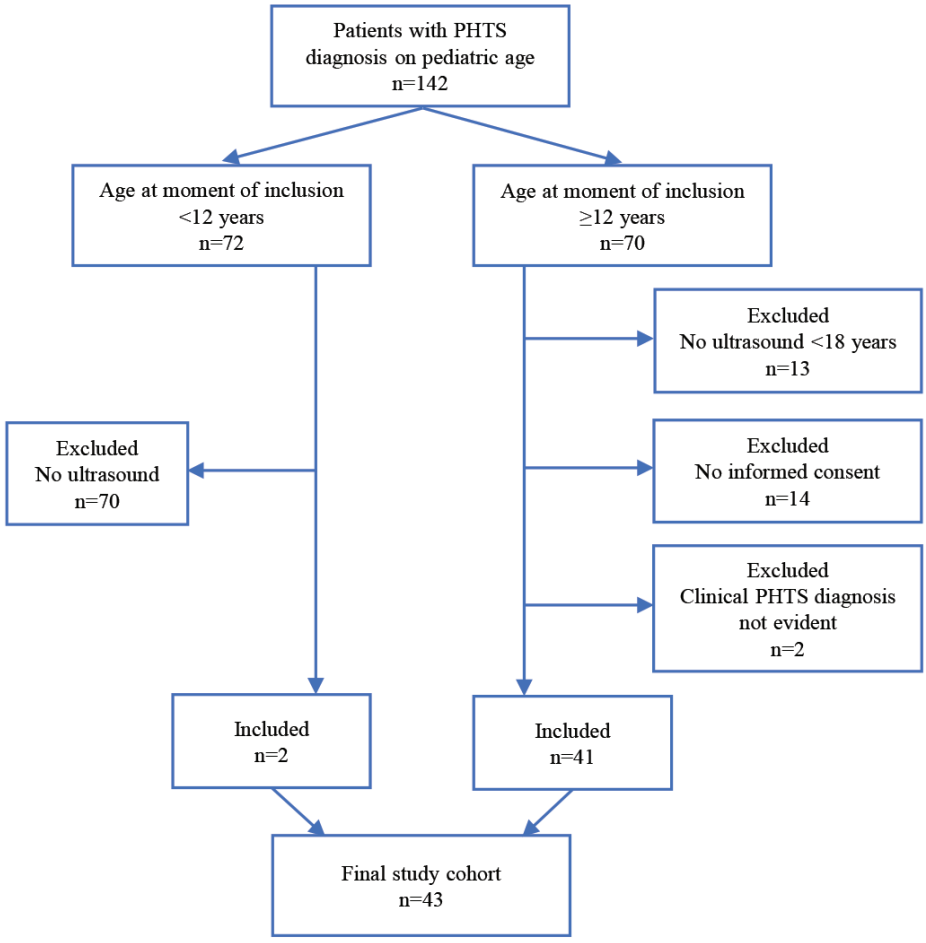


Figure 1. Flowchart of the selection for inclusion in the study cohort

PHTS: PTEN hamartoma tumor syndrome

A *PTEN* variant was confirmed in 93% (40/43), while 7% (3/43) had a clinically confirmed PHTS diagnosis and a first-degree relative with PHTS. Details of the *PTEN* variant were available for 95% (38/40). Most variants (97%, 37/38) were categorized as (likely) pathogenic and a single variant (3%, 1/38) was categorized as variant of uncertain significance (VUS). In half of the patients PHTS inheritance was *de novo*.

Thyroid Ultrasound Findings

All patients received one or more ultrasound(s) during follow-up, with a median follow-up time between the first and last ultrasound of 2 (0-7) years. Age at initial ultrasound varied between 2 and 16 years, with a median age of 12 years. The majority of patients received their initial ultrasound because of regular surveillance (79%, n = 34), others due to a palpable thyroid mass (14%, n = 6), because of parents' request (n = 1), unexplained decline in development (n = 1) or due to an early case of DTC in family history (n = 1). Thyroid abnormalities were present on initial ultrasound in 72% (31/43), whereas 12% (5/43) developed abnormalities during follow-up. Ultrasound findings are presented in Table 2.

In total, 166 thyroid ultrasounds were performed in the pediatric cohort, with a median number of 3 (1-9) ultrasounds per individual. Eight cases (19%) received only 1 ultrasound, showing abnormalities (nodules < 10 mm) in 4 of them.

Nodular disease was present in 74% (32/43). In 30 out of 32 patients (94%), this was multinodular disease. Nearly half of the patients (49%, n = 21) developed a nodule ≥10 mm. Figure 2 shows the time to diagnosis of nodular disease and nodular growth. Nodular growth was observed in 33% (14/43), leading to close monitoring in 8 patients (indicating that surveillance was repeated within 3, 6 or 9 months instead of annually). In 7 patients, nodular growth led to FNA performance.

Five patients (12%) had features of thyroiditis on ultrasound. All of them had presence of serum anti-thyroid peroxidase autoantibodies (anti-TPO), confirming diagnosis

Table 2. Thyroid pathology findings in 43 pediatric PHTS patients

Thyroid pathology	n	%	Median age at diagnosis (range)
Physical examination			
Palpable mass thyroid	14	33	13 (9-16)
Ultrasound features			
Normal	7	16	Not applicable
All types of thyroid pathology	36	84	12 (9-18)
Nodule < 5 mm*	4	9	13 (9-14)
Nodule ≥5 mm < 10 mm**	7	16	17 (13-18)
Nodule ≥10 mm	21	74	12 (10-16)
Nodular growth	14	33	14 (11-18)
Thyroiditis	5	12	12 (11-14)
Goiter	13	30	12 (10-16)
Laboratory results			
Pathology in laboratory***	8	19	13 (11-17)
Benign pathology results			
Hyperplastic nodules	1	2	13
Follicular adenoma	3	7	14 (11-15)
Multinodular goiter	1	2	13
Lymphocytic thyroiditis	2	5	12 (11-13)
Malignant pathology results			
Minimal invasive follicular carcinoma	2	5	15 (12-17)

*Patients had nodule(s) of < 5 mm only.
**Patients had nodule(s) of ≥5 mm < 10 mm only.
***Pathology in laboratory was defined as presence of thyroid autoantibodies or presence of thyroid dysfunction based on serum thyroid function tests.
PHTS: PTEN hamartoma tumor syndrome

of autoimmune thyroiditis. In 3 of them, additional anti-thyroglobulin autoantibodies (anti-TG) were found. Goiter was diagnosed on ultrasound in 13 patients (30%). Figure 3 shows the cumulative risk for thyroiditis and goiter over time.

Seven patients (16%) had a normal thyroid ultrasound. They all had a proven pathogenic *PTEN* variant. Five out of them were aged 12 years (or younger) at the end of the study follow-up time.

Thyroid Abnormalities and Associations

Figure 2 shows that females in our cohort tended to develop nodules ≥10 mm slightly earlier than males, although this was not significant ($p = 0.237$). Female sex led to significant earlier development of nodular growth ($p = 0.008$), goiter ($p = 0.005$) and thyroiditis ($p = 0.027$), shown in Figures 2 and 3. Moreover, patients with head circumference SDS > 4.0 were significantly more likely to develop a nodule ≥10 mm ($p = 0.006$) compared to patients with head circumference SDS ≤4.0. The cumulative risk of thyroid abnormalities was not significantly associated with the presence of lipomas, family history of thyroid abnormalities or developmental delay as a presenting clinical feature, as shown in Table 3. Results for Kaplan-Meier analyses, excluding index patients, were similar (data shown in Supplementary Figure 1).

Laboratory Results

Serum thyroid function was measured in 67% (29/43) of the patients. The majority (90%, 26/29) was euthyroid. Levothyroxine treatment was needed in one patient with hypothyroidism after total thyroidectomy. Anti-TPO was measured in 15 patients, whereas anti-TG was measured in 10 patients. Five patients had serum thyroid autoantibodies present.

Benign Thyroid Abnormalities Before 12 Years of Age

Before the age of 12 years, 9 patients received ultrasound surveillance. Eight of them already showed nodular thyroid abnormalities and 4 such cases underwent FNA and treatment (n = 2) or close monitoring (n = 2) before this age. The treatment performed before the age of 12 years included a hemithyroidectomy in one case (histologically reported as follicular adenoma) and the start of potassium iodide in another case. All of the abnormalities found before the age of 12 years, were identified by a palpable thyroid mass and

Table 3. Log-rank tests for associations with thyroid abnormalities

Nodule ≥10 mm	**p**	**Goiter**	**p**
Characteristics		Characteristics	
Sex	0.237	Sex	**0.005**
Head circumference ≤4.0 / > 4.0	**0.006**	Head circumference ≤4.0 / > 4.0	0.140
Presence of lipomas	0.336	Presence of lipomas	0.242
Developmental delay	0.276	Developmental delay	0.651
Family history thyroid disease	0.116	Family history thyroid disease	0.277
Nodular growth	**p**	**Thyroiditis**	**p**
Characteristics		Characteristics	
Sex	**0.008**	Sex	**0.027**
Head circumference ≤4.0 / > 4.0	0.182	Head circumference ≤4.0 / > 4.0	0.506
Presence of lipomas	0.573	Presence of lipomas	0.718
Developmental delay	0.753	Developmental delay	0.196
Family history thyroid disease	0.723	Family history thyroid disease	0.613

For each outcome (nodule ≥10 mm, nodular growth, goiter and thyroiditis), different clinical characteristics have been analyzed to test if there was an association. Every clinical characteristic was divided in 2 subgroups before performing a log-rank test, namely:
1. Female sex/male sex
2. Head circumference ≤4.0 / > 4.0
3. Presence of lipomas yes/no
4. Developmental delay as presenting feature yes/no
5. Having a family member with thyroid disease yes/no

eventually determined to be benign. The cumulative risk of abnormalities at age 12 was 30% for developing nodules ≥10 mm, 8% for nodular growth, 23% for goiter, and 8% for thyroiditis (Figures 2, 3)

Cytology and Treatment

A total of 11 patients (26%) underwent one or more FNA(s), with 10 years being the youngest age of FNA performance. Six patients received multiple FNAs (up to a maximum of 4), mainly since their first cytology resulted in Bethesda 1 (13). Ultimately, in 2 patients cytology was classified as Bethesda 1, 4 patients had Bethesda category 2, 3 patients had Bethesda category 3 and 2 patients had Bethesda category 4 (13). A total of 9 patients (21%) needed treatment, at a median age of 13 (11-17) years. Treatment included potassium iodide in 4 patients (9%). Six patients (14%) needed a hemithyroidectomy and 1 patient (2%) needed a total thyroidectomy. Prior to surgery, 6 out of 7 (86%) had a palpable thyroid mass.

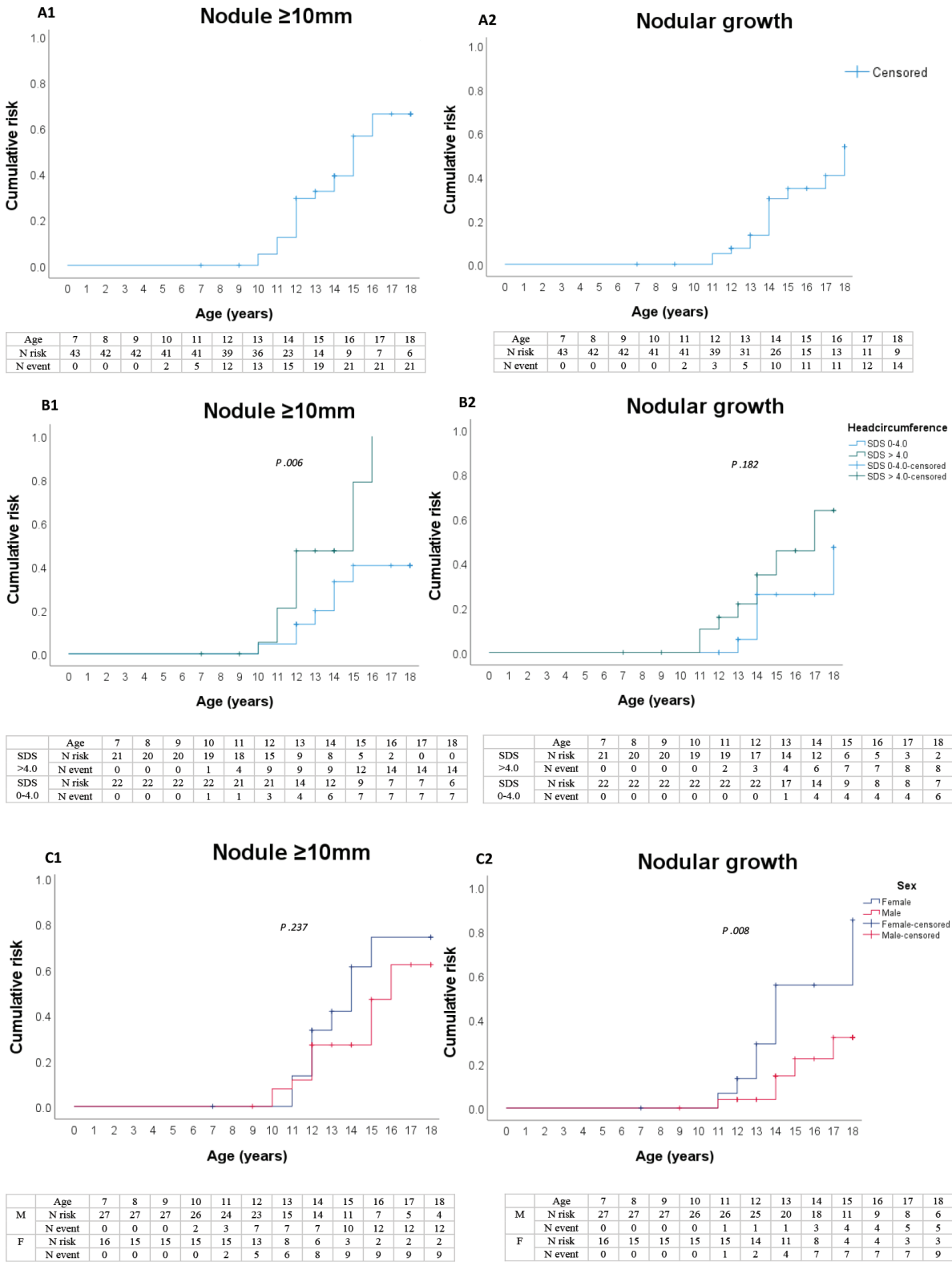
Pathology Results

DTC was diagnosed in 2 patients (5%). One female patient was diagnosed with a minimally invasive follicular carcinoma of 5.6 cm (pT3NxMx) at the age of 12 years. She had a pathogenic *PTEN* variant and had already been diagnosed with multinodular goiter. After noticing a growing mass in her neck, clinicians identified this as a remarkably solid, fast growing, thyroid mass. Ultrasound imaging confirmed nodular growth with a volume that doubled (from 6.1 mL to 12.8 mL) within 8 months. After 2 FNAs (Bethesda category 1 and 3), she underwent a hemithyroidectomy. Histopathology confirmed DTC whereafter a rest total thyroidectomy was performed with cervical lymph node dissection and adjunctive iodine-131. Another male patient was diagnosed with a minimally invasive follicular carcinoma of 1.5 cm (pT1NxMx) at age 17 years. PHTS diagnosis was clinically confirmed and he had a first-degree relative with a *PTEN* variant classified as VUS. No thyroid mass was found by physical examination. At age 15 years, ultrasound showed nodular growth and FNA resulted in Bethesda 2. After 2 years of close monitoring, the nodule volume almost doubled within 6 months (from 1.5 mL to 2.9 mL) and ultrasound imaging showed an irregular nodular margin. These ultrasound features, together with a low Technetium uptake on scintigraphy led to performance of a second FNA (Bethesda 4) and hemithyroidectomy thereafter. Given the small and low invasive character of this DTC, a favourable prognosis was predicted. Therefore, a total thyroidectomy and adjunctive iodine-131 were not performed, in shared decision with the patient.

In 2 out of 7 patients that underwent surgery, histopathology was reported as malignant, while 5 had benign outcomes (Table 2). All 5 patients with benign outcome had a palpable mass of the thyroid and multinodular disease.

Discussion

Our study provides important and unique data about thyroid abnormalities in children diagnosed with PHTS, which may be used to establish surveillance guidelines for pediatric PHTS patients. Two patients (5%) in our pediatric PHTS cohort developed minimally invasive DTC at ages 12 and 17



Age	7	8	9	10	11	12	13	14	15	16	17	18
N risk	43	42	42	41	41	39	36	23	14	9	7	6
N event	0	0	0	2	5	12	13	15	19	21	21	21

Age	7	8	9	10	11	12	13	14	15	16	17	18
N risk	43	42	42	41	41	39	31	26	15	13	11	9
N event	0	0	0	0	2	3	5	10	11	11	12	14

	Age	7	8	9	10	11	12	13	14	15	16	17	18
SDS >4.0	N risk	21	20	20	19	18	15	9	8	5	2	0	0
	N event	0	0	0	1	4	9	9	9	12	14	14	14
SDS 0-4.0	N risk	22	22	22	22	21	21	14	12	9	7	7	6
	N event	0	0	0	1	1	3	4	6	7	7	7	7

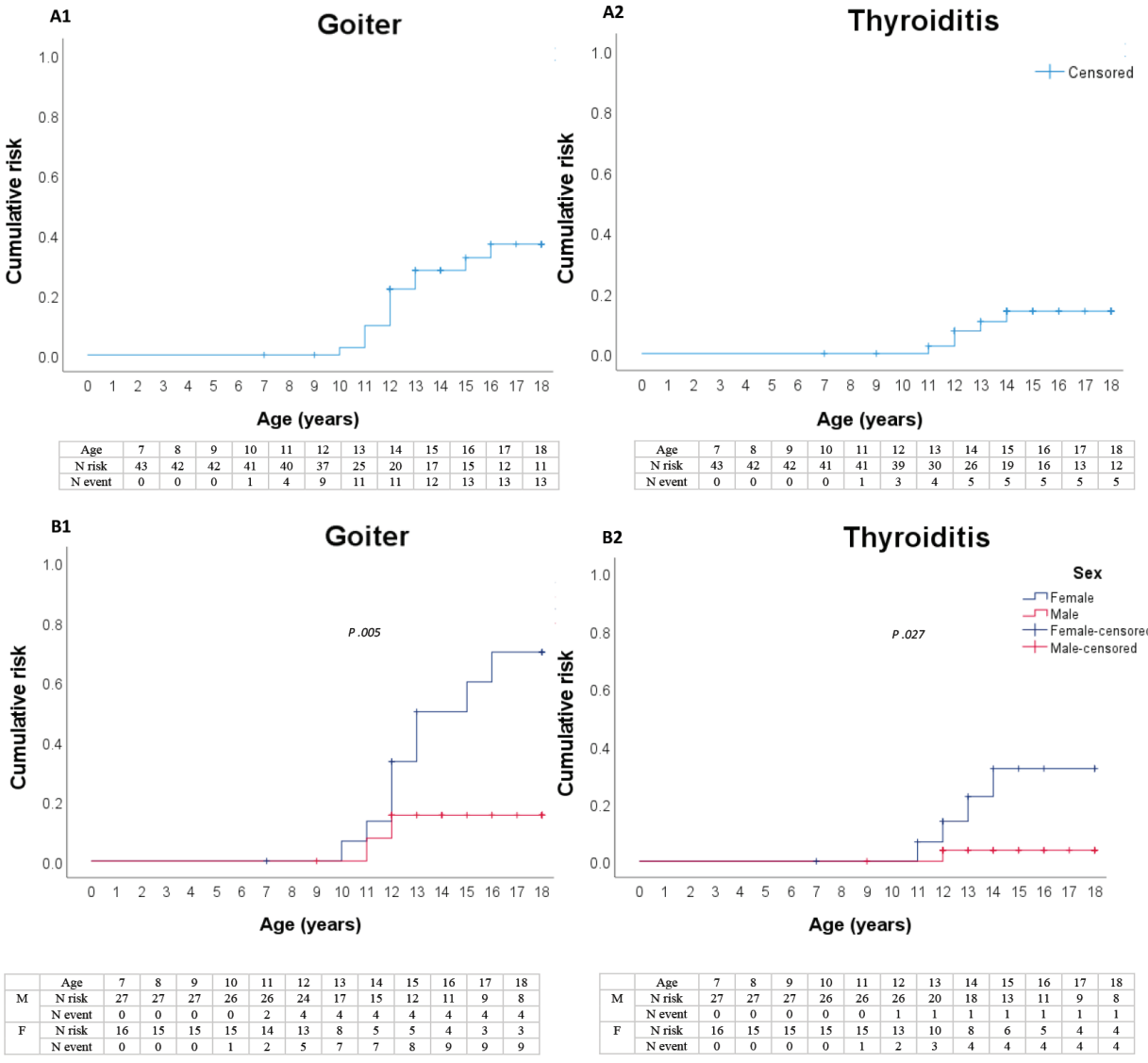
	Age	7	8	9	10	11	12	13	14	15	16	17	18
SDS >4.0	N risk	21	20	20	19	19	17	14	12	6	5	3	2
	N event	0	0	0	0	2	3	4	6	7	7	8	8
SDS 0-4.0	N risk	22	22	22	22	22	22	17	14	9	8	8	7
	N event	0	0	0	0	0	0	1	4	4	4	4	6

	Age	7	8	9	10	11	12	13	14	15	16	17	18
M	N risk	27	27	27	26	24	23	15	14	11	7	5	4
	N event	0	0	0	2	3	7	7	7	10	12	12	12
F	N risk	16	15	15	15	15	13	8	6	3	2	2	2
	N event	0	0	0	0	2	5	6	8	9	9	9	9

	Age	7	8	9	10	11	12	13	14	15	16	17	18
M	N risk	27	27	27	26	26	25	20	18	11	9	8	6
	N event	0	0	0	0	1	1	1	3	4	4	5	5
F	N risk	16	15	15	15	15	14	11	8	4	4	3	3
	N event	0	0	0	0	1	2	4	7	7	7	7	9

Figure 2. Cumulative risk of thyroid nodular disease. Time-to-event is presented for a nodule ≥10 mm (1) and for nodular growth (2), for all patients (A), by head circumference SDS (B) and by sex (C) (M = male, F = female). The number of patients at risk (N risk) and the cumulative number of events (N event) are presented for each age category from age 7 years onwards. Log-rank tests p values are provided

SDS: standard deviation score



	Age	7	8	9	10	11	12	13	14	15	16	17	18
	N risk	43	42	42	41	40	37	25	20	17	15	12	11
	N event	0	0	0	1	4	9	11	11	12	13	13	13

	Age	7	8	9	10	11	12	13	14	15	16	17	18
	N risk	43	42	42	41	41	39	30	26	19	16	13	12
	N event	0	0	0	0	1	3	4	5	5	5	5	5

	Age	7	8	9	10	11	12	13	14	15	16	17	18
M	N risk	27	27	27	26	26	24	17	15	12	11	9	8
	N event	0	0	0	0	2	4	4	4	4	4	4	4
F	N risk	16	15	15	15	14	13	8	5	5	4	3	3
	N event	0	0	0	1	2	5	7	7	8	9	9	9

	Age	7	8	9	10	11	12	13	14	15	16	17	18
M	N risk	27	27	27	26	26	26	20	18	13	11	9	8
	N event	0	0	0	0	0	1	1	1	1	1	1	1
F	N risk	16	15	15	15	15	13	10	8	6	5	4	4
	N event	0	0	0	0	1	2	3	4	4	4	4	4

Figure 3. Cumulative risk of goiter and thyroiditis. Cumulative risk of goiter (1) and thyroiditis (2) on ultrasound. Time-to-event is presented for all patients (A) and by sex (B) (M = male, F = female). The number of patients at risk (N risk) and the cumulative number of events (N event) are presented for each age category from age 7 years onwards. Log-rank tests p values are provided

years, which is a high and relevant rate when considering the short median follow-up period (2 years). The number of DTCs in our cohort is similar to the previously reported incidence rate of 4% to 12% for DTC in pediatric PHTS cohorts and these results are comparable to the yield of surveillance in adults from the same PHTS expertise center (2,6,7,15). It is known that the DTC rate in PHTS children (older than age 10 years) is significantly higher than the general population (16). The young age at DTC diagnosis in this study is in line with previous childhood diagnoses/reports, with the youngest reported case of DTC at 4 years of age (6,17). Current available studies have recommended surveillance starting from the age of 10 years (2,4,6), from the age of 7 years (5,18) or immediately after PHTS diagnosis (19,20). The Pediatric Cancer Working Group of the American Association for Cancer Research recommends to start surveillance when cancer incidence reaches ≥5% (21). Taken together, clinicians in our expertise center would recommend thyroid surveillance from at least the age of 12 years to detect DTC at an early stage and that even surveillance from age 10 could be considered.

The majority of thyroid abnormalities (94%) identified by ultrasound were benign, including nodular disease, goiter, thyroiditis and follicular adenomas. These findings are in line with previously published studies in both adults and children (2,4,15,19,22,23). It is remarkable that 5 out of 7 patients who underwent (hemi)thyroidectomy

had benign histopathology results, despite prior close ultrasound monitoring and/or FNA performance. These findings demonstrate that it is challenging to predict the presence of cancer based on ultrasound and/or FNA in PHTS. The high prevalence of (growing) benign lesions in children with PHTS poses a challenge for the interpretation of the ultrasound and FNA results, even for experienced clinicians. As a result, surgery with pathology examination is still required sometimes. Therefore, surveillance should preferably be performed within a center experienced in PHTS or pediatric thyroid abnormalities in order to minimize the burden of (unnecessary) FNAs and proceed to surgery only in cases of high suspicion of a malignancy. Characteristics, such as nodular growth, a palpable solid and growing mass or suspicious ultrasound findings (including irregular margins, hypoechogenicity, solid pattern, microcalcifications, intranodular vascularity and taller-than-wide shape) should lead to performance of FNA and further diagnostics (10).

Treatment included the use of potassium iodide in a few patients (9%) with nodular growth. Potassium iodide is effective in reducing the volume of benign solitary thyroid nodules, however the effect on nodular growth in PHTS patients, is not known (14).

Clinical factors that might influence DTC diagnosis were studied. Females tended to develop nodules ≥10 mm, nodular growth, goiter and thyroiditis at an earlier age than males. These results are consistent with previous observations in both the general population (24,25) and pediatric PHTS cohorts (5). A possible explanation for this association is the growth-promoting effect of estrogen on nodular growth (26). Another association was found between head circumference SDS >4 and nodular disease, consistent with findings by Tuli et al. (4). Although further research is needed to confirm these associations in larger PHTS cohorts, these findings may lead to improvement of thyroid cancer surveillance programs (e.g. initiating surveillance earlier in females, patients with head circumference SDS >4 and possibly also in patients with other clinical features). It should be noted that the clinical PHTS phenotypes in children have not reached full development and continue to develop as the children grow older.

It is worth noting that 6 out of 7 patients (86%) that underwent (hemi)thyroidectomy had a palpable mass of the thyroid prior to surgery. A palpable thyroid mass was also found in all patients (n=4) that required treatment or close monitoring before the age of 12 years. This suggests that they could have been identified by physical examination only. In one DTC case, thyroid palpation led to suspicion of a malignancy, while the other cancer patient had no palpable thyroid mass. It is known that palpation is not sensitive and therefore ultrasound surveillance cannot be replaced by palpation only (27). However, since palpation is a non-invasive and low-cost method which helped to diagnose DTC in one case, we agree with the recommendation of the Dutch PHTS guideline (2015) to palpate the thyroid annually starting from PHTS diagnosis (3).

Although eight patients had laboratory abnormalities, only one needed levothyroxine therapy after undergoing a total thyroidectomy and the majority was euthyroid. Five out of 8 had thyroid autoantibodies in addition to signs of thyroiditis on ultrasound. This data suggests that measuring serum thyroid function or thyroid autoantibodies might only be useful post-operative or after discovering signs of thyroiditis on ultrasound. This is comparable to the findings of Smith et al. (5), although, this needs to be confirmed in larger cohorts.

The most important advantage of surveillance in pediatric patients is the early detection of DTC in order to prevent advanced disease and decrease morbidity (28). In our cohort we detected DTC at an early stage rather than advanced disease. Another advantage is the noninvasive and low-cost character of ultrasound surveillance. In our experience, it is parents' preference to start surveillance immediately after PHTS diagnosis. However, the most important disadvantage of ultrasound surveillance is the high risk of false-positive results and parents need to be counseled for that (27). Implementing thyroid ultrasound surveillance as standard care from age 10-12 years onwards, will consequently lead to more (possibly unnecessary) invasive FNAs and surgeries, especially when performed by a less experienced clinician. Moreover, minimally invasive follicular thyroid carcinomas, as found in our cohort, are associated with low recurrence and/or metastases rates (29). Thus, although our surveillance led to diagnosis at an early stage, more research is needed to investigate the clinical benefit of earlier cancer detection. With our current knowledge, surveillance from age 10-12 years onwards is justified and shared decision making is important to make considerations based on individual preferences.

Besides discussion about the time to initiate ultrasound surveillance, consensus about surveillance intervals has not yet been reached. While some studies recommend annual surveillance (2,7,30), others suggest prolonged surveillance intervals in specific cases (5,6,9,15,31). Our study could not provide recommendations about stratifying ultrasound surveillance intervals, since the collected data was not appropriate to address this issue.

Study Limitations

The major strength of this study is the relatively large cohort of pediatric PHTS patients who received regular follow-up within one hospital. As a worldwide recognized expertise center in the care for PHTS patients, our institute has an experienced multidisciplinary team of clinicians. Due to the small size of our team, there is little chance of inter-observer variation. Given the fact that PHTS is a rare disease, a retrospective study performed in an academic expertise center, seemed to be most appropriate. Despite the retrospective design, surveillance outcomes were well-documented with limited missing data. In all PHTS studies, the reported numbers of thyroid pathology are probably overestimated, since (asymptomatic) unrecognized PHTS individuals are not evaluated in hospitals and therefore not included in studies. To minimize the effect of overestimation due to ascertainment bias, Kaplan-Meier analyses were performed without the four index patients (9%) that had thyroid pathology prior to PHTS diagnosis (Supplementary Figure 1). All graphs were comparable to the ones including the index patients, suggesting that the effect of ascertainment bias might be moderate. One limitation is the effect of selection bias, since only 43 out of 142 patients had available ultrasound reports and were included in the study. It should be noted that not every participant reached the age of 18 years at the end of this study. Furthermore, benign thyroid abnormalities were already present at initial ultrasound in the majority of patients (72%). As a result, our findings do not represent accurate incidence rates, as abnormalities might have developed before the first performance of surveillance. As the aim of surveillance is to identify DTC at an early stage, providing exact DTC numbers is more relevant. Left-censoring should be applied in larger cohorts to improve current incidence rates. In future research it would be useful to assess the risk of malignancy for each nodule, based on ultrasound images, according to the TI-RADS classification as performed by Drissen et al. (2023) (11,15). To provide more robust conclusions about the yield of ultrasound surveillance, studying a larger cohort of pediatric patients would be ideal, preferably in a prospective design and during a longer follow-up period.

Conclusion

According to the results of our study and the previous literature, thyroid ultrasound surveillance is useful from age 10-12 years onwards. Children with PHTS often have benign thyroid lesions, as part of their clinical phenotype. However, distinguishing these benign abnormalities from potential malignancies is challenging. A fast growing nodule and an atypical aspect of the nodule warrants suspicion and should lead to close monitoring or further invasive diagnostics, such as FNA. If diagnosis remains uncertain, hemithyroidectomy is required, which can still reveal a high percentage of benign histopathology results. We conclude that thyroid surveillance is useful, however, given its complexity, it should preferably be performed in a center with excellence in pediatric thyroid abnormalities or PHTS.

Ethics

Ethics Committee Approval: The Research Ethics Committee of the Radboudumc (CMO Arnhem-Nijmegen) declared that ethical approval was not required for this retrospective design.

Informed Consent: Retrospective study.

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Footnotes

Authorship Contributions

Concept: Esther M.G. Bormans, Linda A.J. Hendricks, Meggie M.C.M. Drissen, Martin Gotthardt, Hedi L. Claahsen-Van der Grinten, Nicoline Hoogerbrugge, Jolanda H. Schieving, Design: Esther M.G. Bormans, Janneke H.M. Schuurs-Hoeijmakers, Petra van Setten, Linda A.J. Hendricks, Meggie M.C.M. Drissen, Data Collection or Processing: Esther M.G. Bormans, Linda A.J. Hendricks, Meggie M.C.M. Drissen, Jolanda H. Schieving, Analysis or Interpretation: Esther M.G. Bormans, Janneke H.M. Schuurs-Hoeijmakers, Linda A.J. Hendricks, Meggie M.C.M. Drissen, Hedi L. Claahsen-Van der Grinten, Nicoline Hoogerbrugge, Literature Search: Esther M.G. Bormans, Linda A.J. Hendricks, Meggie M.C.M. Drissen, Writing: Esther M.G. Bormans, Janneke H.M. Schuurs-Hoeijmakers, Petra van Setten, Linda A.J. Hendricks, Meggie M.C.M. Drissen, Martin Gotthardt, Hedi L. Claahsen-Van der Grinten, Nicoline Hoogerbrugge, Jolanda H. Schieving.

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The Effect of Dietary Acid Load on Cardiometabolic Risk, Psychological Resilience and Sleep Quality in Adolescents with Obesity

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What is already known on this topic?

Diet composition affects acid-base balance by providing acid or base precursors. High dietary acid load (DAL) may have detrimental effects on cardiovascular health, mental health and sleep quality.

What this study adds?

High DAL was associated with high cardiometabolic risk, insulin resistance, and low psychological resilience and poor sleep quality in adolescent with obesity.

Abstract

Objective: Mild metabolic acidosis may adversely affect cardiovascular risk factors, and diet-dependent acid-base load may impair mental health and sleep quality. The aim of this study was to investigate the effects of dietary acid load (DAL) on cardiometabolic risk factors, psychological resilience, and sleep quality in adolescents with obesity.

Methods: Obese adolescents participated in the study. Biochemical parameters, anthropometric measurements and blood pressures were measured. Three-day retrospective food intake records were collected from the adolescents, and potential renal acid load (PRAL), net endogenous acid production (NEAP), and DAL were derived from food intake records. Psychological resilience was assessed by the Child and Youth Resilience Measure (CYRM-12) and sleep quality was assessed by the Pittsburgh Sleep Quality Index (PSQI).

Results: A total of 205 adolescents with obesity (105 males, 100 females) aged 13-18 years participated. Body mass index, fat mass, fat percentage, fasting insulin, triglyceride, systolic blood pressure, homeostasis model assessment for insulin resistance (HOMA-IR) and PSQI scores were significantly higher and psychological resilience levels were significantly lower in high tertiles of DAL ($p < 0.05$). Adolescents in the lowest tertile of DAL scores had higher consumption of whole grains, vegetables, dairy, legumes, and higher intakes of potassium and calcium than adolescents in the highest tertile of the DAL scores ($p < 0.05$). Red meat, and white meat consumption and sodium intake were higher in adolescents in the high tertiles ($p < 0.05$). Energy intakes were found to be significantly lower in the first tertile of PRAL and DAL scores compared to the other tertiles ($p < 0.05$). A linear regression model ahowed an increase in NEAP, PRAL and DAL scores led to a decrease in psychological resilience score and an increase in PSQI and HOMA-IR scores ($p < 0.05$).

Conclusion: High DAL was associated with high cardiometabolic risk, insulin resistance, and low psychological resilience and poor sleep quality.

Keywords: Dietary acid load, cardiometabolic risk, psychological resilience, sleep quality

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Introduction

The dramatic increase in the prevalence of overweight and obesity in children and adolescents has become an important global public health problem (1). Adolescent obesity is a strong predictor of adult obesity and is associated with higher cardiometabolic risk, mortality and morbidity (2). Studies have shown that, in addition to physical diseases caused by obesity such as insulin resistance, cardiovascular diseases, metabolic syndrome, non-alcoholic liver disease, polycystic ovary syndrome, and respiratory abnormalities during sleep, children and adolescents with high body mass index (BMI) tend to have psychosocial problems, including depression, anxiety and social withdrawal, poor quality of life and sleep and behavioral problems (2,3,4).

There is a close relationship between healthy lifestyle and well-being of individuals. Therefore, teaching activities that increase lifelong well-being is important for healthy lifestyle behavior (5). In recent years, increasing interest in the relationship between healthy life and well-being has provided an opportunity to investigate lifestyle factors related to psychological resilience (6). Psychological resilience is the ability to cope with and adapt to challenging conditions and stress (7). Despite the lack of longitudinal studies specifically designed to associate psychological resilience with health outcomes, evidence suggests that psychological resilience has a positive association with the most common health risk factors (8). Psychological resilience is associated with decreased risk of obesity, cardiovascular disease, type 2 diabetes, cancer and increased life expectancy (8). Similarly, it has been reported that improving the sleep quality of children may be an effective strategy in the prevention and treatment of pediatric obesity (9).

Diet therapy is an important and modifiable environmental factor that may affect adverse health problems, psychological disorders and sleep disorders caused by obesity in adolescents (10). Psychological resilience and sleep quality may be affected by dietary factors and may be a determinant of diet quality (7,10). Although the relationship between dietary habits and some psychological or psychosocial factors has been partially investigated (11), research on diet and psychological resilience is limited (6,7,12) and there are no studies on this subject in adolescents with obesity. It was reported that a Mediterranean-type diet model or vegetable-based diet models, increase in dietary polyphenol or antioxidant intake and diversity in fruit and vegetable consumption were positively associated with psychological resilience and sleep quality (6,9). In another study, adults with high psychological resilience had better diet quality and consumed more seafood, whole grain foods and fruits, and less processed and starchy foods, and sugary and fatty foods (13).

Diet composition may significantly affect the acid-base balance of the body (14). Studies have shown that metabolic acidosis may be associated with obesity (15,16). It has been suggested that acidic diets may increase the risk of some chronic diseases, including obesity, whereas alkaline diets may provide protection from these chronic diseases (17). It has been reported that an acidogenic diet leads to the accumulation of hydrogen ions related with weight gain (18). Excessive consumption of animal products, meat and Western-style dietary patterns leads to higher organic acid production and fatty acid oxidation, especially in individuals with obesity. Plant-based diets reduce the dietary acid load (DAL) (19). In another study, it was also found that high DAL was associated with the risk of depression and anxiety in individuals (20). Furthermore, it has been reported that children with high DAL at baseline had more emotional problems and increased hyperactivity ten years later (21). Low DAL and plant-based diets may have beneficial effects on mental and sleep disorders by suppressing inflammation and reducing oxidative stress because they contain high amounts of antioxidants, phytochemicals, flavonoids, vitamins and minerals (10). However, no study has examined the effect of DAL on psychological resilience and sleep quality in adolescents with obesity. To that end, the aim of this study was to investigate the relationship between DAL and cardiometabolic risk factors, psychological resilience, and sleep quality in adolescents with obesity.

Methods

Study Design, Setting, and Participants

This study was carried out on adolescents with obesity who attended the Pediatric Endocrinology Outpatient Clinic at Gazi University Faculty of Medicine Children's Hospital between February 2022 and May 2022. The inclusion criterion was obesity (BMI ≥95th percentile) in adolescents who did not have any chronic disease diagnosed by a doctor, did not take hormone therapy, and did not use medication. Exclusion criteria were being overweight and having any concomitant chronic medical disease of any type (syndromic, metabolic, neurological) except for metabolic syndrome secondary to obesity, not having clinically normal mental development, having diagnoses of autism spectrum disorder or schizophrenia and related psychotic disorders, and having mental problems that would prevent them from participating in the survey interviews or completing the scales.

Approval was obtained from the Gazi University Faculty of Medicine Ethics Committee (approval number: 230, date: 20.03.2023). Clear explanations were provided with regard to the purpose of the study, after which written informed consent was obtained from the adolescents in accordance with the Declaration of Helsinki.

Data Collection and Evaluation

Data was collected in face-to-face interviews through a questionnaire that included adolescent socio-demographics and dietary habits, the Child and Youth Resilience Measure (CYRM-12), Pittsburgh Sleep Quality Index (PSQI), anthropometric measurements, biochemical findings, and three-day food consumption records (see below).

Anthropometric Measurements and Body Composition Analysis

Body weight and body composition analysis were performed using a bioelectrical impedance analysis device (Javon BC 360 Jawon Medical Co, Ltd. Korea) between 8.00-10.00 in the morning. Height was measured (cm) with feet close together and the head in Frankfurt plane with a portable stadiometer with sensitivity of 0.1 cm. BMI was calculated as weight in kg/height in m^2. BMI standard deviation score (SDS) was calculated according to the standards established for Turkish children (22). Waist circumference (cm) was measured from the midpoint between the lowest rib and the iliac crest. Hip circumference (cm) was measured horizontally at the largest circumference of the hip. Neck circumference (cm) was measured at the midpoint of the neck, between mid-cervical spine and mid-anterior neck, to within 1 mm, using non-stretchable plastic tape with the subjects standing upright. Waist circumference-to-height ratio was computed as the ratio of waist circumference to height.

Biochemical Parameters and Blood Pressure

The fasting blood glucose, fasting insulin, total cholesterol, low-density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), and triglyceride levels of the children, which were routinely analyzed by Gazi University, Faculty of Medicine, Department of Pediatric Endocrinology, were recorded. Venous blood samples were obtained from all of the patients from the antecubital region between 08:00 and 08:30 am after an 8-12 hour overnight fast. Fasting glucose was measured with the enzymatic UV (hexokinase method) test method using a Beckman AU5800 (Beckman Coulter Inc, Brea CA, USA). HDL-C, LDL-C, total cholesterol, and triglyceride levels were measured with the enzymatic colorimetric method using the same autoanalyzer. Insulin levels were measured with the one step principle enzymatic immunoassay method using a Beckman UniCel DxI 800 (Beckman Coulter Inc, Brea CA, USA). The BP of each adolescent was measured twice following a 5-minute rest period from the right arm with a 30-second interval. Systolic and diastolic blood pressure Z scores were calculated for adolescents. The adolescent's gender, age, height and blood pressure were used for this calculation, as previously reported (23).

Assessment of Insulin Resistance

The homeostasis model assessment for insulin resistance (HOMA-IR) value was calculated using the "fasting blood glucose (mmol/L) x fasting insulin (μU/mL)/22.5" formula (24). A value of HOMA-IR above 3.16 was used as a cut-off value for both genders for insulin resistance.

Assessment of Psychological Resilience

CYRM-12 was used to determine the psychological resilience levels of adolescents participating in the study. The 28-item original form of the scale, which was developed in the light of data collected from eleven different countries, consists of three subscales and eight sub-dimensions. The short form study of the scale was conducted by Liebenberg et al. (25) in 2013 and a 12-item structure was obtained as a result of two different studies. The factor loading values of the scale ranged between 0.39 and 0.88 and the internal consistency coefficient was found to be 0.84. The scale, which has a five-point Likert scale, is graded between "strongly agree" (5) and "strongly disagree" (1). A high score indicates a high level of resilience. There are no reverse items in the scale. The total score that can be obtained is 60. The scale was adapted to Turkish and reliability and validity study was conducted by Arslan (26) (2015).

Assessment of Sleep Quality

PSQI was used to determine sleep quality. PSQI is a questionnaire consisting of 19 questions evaluating the sleep of individuals during the previous month. PSQI has seven components including sleep duration, sleep disturbance, sleep latency, daytime dysfunction due to sleepiness, sleep efficiency, overall sleep quality, and sleep medication use. The total PSQI score is the sum of the seven components. Each item is evaluated on a 0-3 point scale and the total score varies between 0-21. High scores indicate poor sleep quality. A total PSQI score of ≤5 indicates "good" and >5 indicates "poor" sleep quality. PSQI was developed by Buysse et al. (27) (1989) and adapted to Turkish by Ağargün et al. (28) (1996).

Dietary Intake

Three-day food consumption records were obtained from the participants. Adolescents were trained by the researcher

on how to keep food consumption records. The dietary energy and nutrients were analyzed using the Nutrition Information Systems Package Program (BeBiS, Ebispro for Windows, Germany; Turkish version/BeBiS 7.0). The Food and Nutrient Photo Catalogue was used to ensure that patients correctly specified the amount of food they consumed. The daily energy, protein, fat, fiber, whole grain, refined grain, fruit, vegetable, dairy, red meat, white meat, legumes, phosphorus, calcium, potassium, sodium and magnesium intakes of adolescents were calculated from food consumption records.

Calculation of Dietary Acid Load

The potential renal acid load (PRAL), net endogenous acid production (NEAP) and DAL scores were derived from nutrient intake equations. The PRAL score was calculated using an algorithm described by Remer et al. (29) where PRAL (mEq/d) = 0.49x protein intake (g/day) + 0.037x phosphorus (mg/day) - 0.021 x potassium (mg/day) - 0.013 x calcium (mg/day) - 0.026 x magnesium (mg/day).

In addition, another measure of DAL score was estimated using the algorithm described by Frassetto et al. (30) where NEAP (mEq/d) = [54.5 x protein intake (g/day) + potassium intake (mEq/day)] -10.2.

Higher values of PRAL and NEAP reflect more acidic dietary intake, whereas lower values indicate more alkaline dietary intake.

DAL (mEq/day) = [body surface area (m^2) × 41 (mEq/day) / 1.73 m^2 + PRAL] (31). The DuBois (32) formula: [0.007184 × $height^{0.725}$ × $weight^{0.425}$] was used to calculate body surface area.

Statistical Analysis

The assumptions required for the suitability of the data for parametric tests were examined. Compliance with normal distribution was analyzed by the Shapiro-Wilk test. One-way analysis of variance (ANOVA) was used for variables that met the normal distribution and parametric test specifications, and the Kruskal-Wallis test was used for variables that did not meet the parametric specifications. In cases where there were differences between groups, pairwise comparisons were made using the independent Student's t-test for parametric measurements and the Mann-Whitney U test for non-parametric measurements. In addition to this descriptive information, Linear regression models were established between the independent variables and the dependent variables in order to examine the effect, direction and explanation rates of any obtained relationships. The "crude" model was used when analyzing the linear regression model with only dependent and independent variables, and were calculated separately as an adjusted model with personal characteristics and other variables from the measurements that may affect the relevant variables, respectively. Comments were made on the slope coefficient (β) obtained in all relevant models. Statistical analysis was performed using Statistical Package for the Social Sciences statistics, version 22.0 (IBM Inc., Armonk, NY, USA) and p values < 0.05 were considered statistically significant for all data.

Results

The study included 205 adolescents (105 male, 100 female) aged 13-18 years. The mean DAL indices were 5.11 ± 7.1 mEq/d, 46.11 ± 11.9 mEq/d and 51.07 ± 16.8 mEq/d for PRAL, NEAP and DAL, respectively. The mean PSQI and psychological resilience scale scores were 7.94 ± 2.7 and 37.06 ± 9.6, respectively.

Table 1 displays the means and standard deviations for age, anthropometric measures, and biochemical parameters and blood pressure values according to quartile categories of DAL indices. The cutoffs for DAL indices were constructed as follows: tertiles for the PRAL index were < 1.81, 1.81 to 8.91, and > 8.91 mEq/d, for the NEAP index < 38.61, 38.61 to 53.41, and > 53.41 mEq/d, and for the DAL index < 40.1, 40.1 to 59.61, and > 59.61 mEq/d. Table 1 shows that BMI SDS, fat mass, fat percentage, fasting insulin, triglyceride, systolic blood pressure SDS, HOMA-IR values, and PSQI scores were higher and psychological resilience scores were lower in high tertiles of PRAL, NEAP and DAL compared to the low tertiles ($p < 0.05$). There was no difference observed between tertiles in other parameters.

Table 2 shows the effects of dietary intakes on DAL. Energy intakes were found to be significantly lower in the 1st tertile of PRAL and DAL scores compared to the other tertiles. Adolescents in the lowest tertile of the three DAL scores had higher intakes of whole grains, vegetables, dairy, legumes, potassium and calcium than adolescents in the highest tertile of the DAL scores ($p < 0.05$). Red meat, white meat and sodium consumption were found to be higher in adolescents in the high tertiles ($p < 0.05$). There was no difference observed between tertiles in other parameters.

Simple linear regression models of HOMA-IR, psychological resilience and PSQI variables were established with NEAP, PRAL and DAL variables, respectively. Afterwards, these models were organized as the crude model, Model 1, by adding the specified variables respectively. In the next step, Model 2 was obtained by adding the specified variables to Model 1. A one-unit increase in NEAP score led to an average increase of 0.09 units in HOMA-IR levels, an

Table 1. Anthropometric measurements, biochemical findings, psychological resilience levels and sleep quality of adolescents according to PRAL, NEAP and DAL tertiles

Variables	Total	PRAL			p	NEAP			p	DAL			p
		1st tertile < 1.81 n = 66	2nd tertile 1.81-8.91 n = 69	3rd tertile > 8.91 n = 70		1st tertile < 38.61 n = 68	2nd tertile 38.61-53.41 n = 69	3rd tertile > 53.41 n = 68		1st tertile < 40.1 n = 69	2nd tertile 40.1-59.61 n = 67	3rd tertile > 59.61 n = 69	
Age (years)	14.76 ± 1.94	14.74 ± 1.81	14.71 ± 1.99	14.83 ± 2.06	0.933*	14.61 ± 1.79	14.83 ± 1.74	14.83 ± 2.28	0.788*	14.67 ± 1.75	14.69 ± 1.82	14.91 ± 2.25	0.775*
Sleep duration (hours)	8.18 ± 1.31	8.18 ± 1.09	7.94 ± 1.28	8.42 ± 1.53	0.267*	8.12 ± 1.16	8.14 ± 1.19	8.27 ± 1.58	0.823*	8.17 ± 1.16	8.06 ± 1.17	8.3 ± 1.6	0.763*
BMI SDS	2.08 ± 0.2	1.97 ± 0.3[a]	1.96 ± 0.2[a]	2.23 ± 0.5[b]	**0.002**	1.99 ± 0.1[a]	2.11 ± 0.3[a,b]	2.18 ± 0.5[b]	**0.029***	1.99 ± 0.7[a]	2.06 ± 0.2[a,b]	2.14 ± 0.3[b]	**0.021**
Hip circumference (cm)	108.42 ± 13.53	108.91 ± 10.38	105.98 ± 10.09	110.47 ± 18.46	0.073*	107.41 ± 10.18	108.77 ± 10.25	109.05 ± 18.73	0.475*	108.43 ± 10.34	107.44 ± 10.02	109.42 ± 18.76	0.385*
Waist circumference (cm)	99.42 ± 11.27	97.35 ± 11	98.48 ± 9.75	102.48 ± 12.53	0.071	97.27 ± 11.15	99.98 ± 11.09	100.99 ± 11.48	0.263	97.55 ± 11.22	99.02 ± 11.12	101.71 ± 11.33	0.201
WHtR	0.63 ± 0.05	0.62 ± 0.06	0.62 ± 0.10	0.64 ± 0.06	0.125	0.64.0.08	0.62 ± 0.09	0.64 ± 0.07	0.291	0.63 ± 0.10	0.65 ± 0.08	0.63 ± 0.05	0.213
Fat mass (kg)	27.08 ± 9.1	25,82 ± 7.9[a]	25.11 ± 8.1[a]	30.38 ± 10.4[b]	**0.024***	24.72 ± 8.3[a]	26.66 ± 8.28[a]	29.86 ± 10.09[b]	**0.023**	25.27 ± 8.23	26.42 ± 8.49	29.56 ± 10.14	0.148*
Fat percentage (%)	34.96 ± 7.1	34.45 ± 6.23	33.82 ± 7.34	36.65 ± 7.49	0.13	33 ± 6.74[a]	35.11 ± 7.47[a,b]	36.76 ± 6.7[b]	**0.044***	33.58 ± 6.72	35.22 ± 7.2	36.06 ± 7.3	0.236
Fat free mass (kg)	43.5 ± 8.27	45.23 ± 10.43[a]	44.07 ± 9.22[a]	44.82 ± 7.34[b]	0.231	43.66 ± 5.13[a]	44.53 ± 6.11[a,b]	44.31 ± 9.19[b]	0.340	44.65 ± 5.14[a]	44.91 ± 2.28[a]	43.20 ± 4.69[b]	0.322
Fasting glucose (mg/dL)	90.82 ± 11.98	88.62 ± 7.83	92.01 ± 11.37	91.77 ± 15.46	0.408*	90.67 ± 9.33	89.31 ± 7.88	92.53 ± 16.9	0.796*	89.45 ± 8.61	90.09 ± 9.11	92.94 ± 16.59	0.581*
Fasting insulin (IU/mL)	24.25 ± 16.45	19.94 ± 11.58[a]	24.67 ± 13.93[a,b]	28.11 ± 21.55[b]	**0.02***	19.18 ± 11.79[a]	22.49 ± 11,14[a]	31.15 ± 22.11[b]	**< 0.001***	18.31 ± 11[a]	23.39 ± 11.83[a]	31.07 ± 22[b]	**< 0.001***
LDL-C (mg/dL)	92.75 ± 22.15	90.24 ± 22.1	92.58 ± 23.53	95.45 ± 20.85	0.299*	91.82 ± 20.51	93.49 ± 22.32	92.92 ± 23.94	0.934	91.18 ± 20.78	93.48 ± 21.75	93.57 ± 24.2	0.843
HDL-C (mg/dL)	45.64 ± 9.85	46.32 ± 9.43	45.8 ± 9.23	44.8 ± 10.99	0.468*	47 ± 10.79	45.24 ± 9,1	44.7 ± 9.69	0.489*	47.31 ± 9.28	45.09 ± 10.4	44.55 ± 9.81	0.182*
Total-C (mg/dL)	162.08 ± 32.59	159.13 ± 26.91	160.87 ± 36.71	166.28 ± 33.48	0.551	157.94 ± 27.17	161.35 ± 34.59	166.97 ± 35.37	0.409	158.14 ± 24.38	160.81 ± 36.19	167.33 ± 35.69	0.383
Triglyceride (mg/dL)	124.6 ± 77.53	104.98 ± 47.83[a]	129 ± 103.73[a,b]	139.64 ± 66.32[b]	0.026*	102.17 ± 57.3[a]	128.56 ± 92.1[a,b]	142.92 ± 74.5[b]	**0.012***	99.36 ± 55.47[a]	131.96 ± 95.12[a,b]	142.17 ± 70.84[b]	**0.004***
Systolic blood pressure SDS	1.34 ± 0.86	1.33 ± 0.87	1.36 ± 0.83	1.35 ± 0.78	0.194	1.35 ± 0.77[a]	1.37 ± 0.89[a]	1.74 ± 0.43[b]	**0.011***	1.32 ± 0.79[a]	1.34 ± 0.91[a]	1.66 ± 0.71[b]	**0.018***
Diastolic blood pressure SDS	0.89 ± 0.71	0.89 ± 0.44	0.93 ± 0.46	0.96 ± 0.80	0.245	0.93 ± 0.74	0.97 ± 0.90	1.03 ± 0.63	**0.147***	0.95 ± 0.62	0.98 ± 0.75	1.01 ± 0.89	**0.109***
HOMA-IR	5.46 ± 3.74	4.39 ± 2.63[a]	5.6 ± 3.31[a,b]	6.38 ± 4.78[b]	**0.019***	4.32 ± 2.71[a]	4.99 ± 2.66[a]	7.08 ± 4.93[b]	**< 0.001***	4.05 ± 2.48[a]	5.24 ± 2.83[a,b]	7.09 ± 4.9[b]	**< 0.001***
Psychological resilience	37.06 ± 9.59	42.52 ± 1.04[a]	37.87 ± 1.32[a]	30.76 ± 1.28[b]	**< 0.001***	41.8 ± 1.16[a]	38.29 ± 1.38[a]	31.04 ± 1.19[b]	**< 0.001**	43 ± 0.87[a]	38 ± 1.43[b]	30.09 ± 1.13[c]	**< 0.001**
PSQI scores	7.94 ± 2.78	6.11 ± 0.29[a]	8.27 ± 0.40[b]	9.43 ± 3.60[b]	**< 0.001***	6.35 ± 0.35[a]	7.85 ± 0.36[b]	9.63 ± 0.36[c]	**< 0.001**	6.06 ± 0.31[a]	8.02 ± 0.36[b]	9.74 ± 0.36[c]	**< 0.001**

*Kruskal-Wallis test statistic.

**There is no difference between groups containing the same letter.

BMI SDS: body mass index standard deviation score, WHtR: waist circumference-to-height ratio, LDL-C: low-density lipoprotein-cholesterol, HDL-C: high-density lipoprotein-cholesterol, HOMA-IR: homeostasis model assessment for insulin resistance, PSQI: Pittsburgh Sleep Quality Index, PRAL: potential renal acid load, NEAP: net endogenous acid production, DAL: dietary acid load

Table 2. Daily dietary intakes of adolescents according to PRAL, NEAP and DAL tertiles

Variables	Total	PRAL				NEAP				DAL			
		1st tertile < 1.81 n = 66	2nd tertile 1.81-8.91 n = 69	3rd tertile > 8.91 n = 70	p	1st tertile < 38.61 n = 68	2nd tertile 38.61-53.41 n = 69	3rd tertile > 53.41 n = 68	p	1st tertile < 40.1 n = 69	2nd tertile 40.1-59.61 n = 67	3rd tertile > 59.61 n = 69	p
Energy (kal)	2047.55 ± 241.62	1947.39 ± 209.53[a]	2063.81 ± 245.29[b]	2130.74 ± 236.59[b]	**< 0.001**	1996.39 ± 231.72	2077.19 ± 254.85	2067.78 ± 234.04	0.213	1941.61 ± 228.97[a]	2111.44 ± 227.2[b]	2086.83 ± 237.88[b]	**< 0.001**
Carbohydrates (%)	48.96 ± 4.44	48.12 ± 4.4	49.86 ± 4.5	48.87 ± 4.31	0.162	49.42 ± 4.56	49.1 ± 4.16	48.35 ± 4.61	0.497	48.96 ± 4.4	49.06 ± 4.61	48.86 ± 4.38	0.977
Proteins (%)	16.5 ± 4.93	17.09 ± 4.82	15.9 ± 4.42	16.53 ± 5.53	0.503	16.5 ± 4.49	16.54 ± 4.61	16.46 ± 5.71	0.997	16.73 ± 4.79	16.6 ± 4.51	16.16 ± 5.53	0.846
Fats (%)	34.54 ± 3.71	34.79 ± 3.43	34.25 ± 4.03	34.6 ± 3.71	0.772	34.08 ± 3.46	34.36 ± 3.98	35.19 ± 3.65	0.332	34.31 ± 3.41	34.34 ± 3.94	34.98 ± 3.8	0.622
Fiber (g)	18.8 ± 3.56	18.79 ± 3.37	18.87 ± 3.73	18.75 ± 3.64	0.986	18.97 ± 3.76	19.18 ± 3.37	18.24 ± 3.56	0.416	19.16 ± 3.53	18.94 ± 3.76	18.31 ± 3.41	0.497
Whole grains (g)	56.3 ± 18.99	64.02 ± 22.83[a]	54.66 ± 15.85[b]	50.29 ± 15.14[b]	**0.002**	62.38 ± 22.23[a]	57.81 ± 18.08[a]	48.64 ± 13.33[b]	**0.002**	64.21 ± 23.22[a]	55.94 ± 16.05[a,b]	48.77 ± 13.54[b]	**< 0.001**
Refined grains (g)	370.12 ± 82.36	360.25 ± 75.26	370.88 ± 89.02	379.18 ± 82.64	0.547	367.18 ± 79.72	356.74 ± 77.52	387.01 ± 88.49	0.197	368.29 ± 78.54[a,b]	350.18 ± 75.31[b]	392.74 ± 88.94[a]	0.042
Fruits (g)	287.57 ± 153.47	324.46 ± 171.66	282.91 ± 158.55	255.54 ± 120.68	0.094	327.55 ± 174.14	280.29 ± 145.64	255.18 ± 132.33	0.071	319.25 ± 172.35[a]	303.94 ± 153.11[a,b]	238.79 ± 121.73[b]	**0.027**
Vegetable (g)	283.81 ± 102.3	320.05 ± 106.75[b]	282.3 ± 100.37[a,b]	249.15 ± 88.53[b]	**0.003**	312.26 ± 108.85[a]	285.5 ± 96.33[a,b]	253.6 ± 94.99[b]	**0.021**	336.32 ± 101.39[a]	255.06 ± 90.49[b]	261.31 ± 96.31[b]	**< 0.001**
Dairies (g)	412.08 ± 112.82	456.2 ± 79.14[a]	395.27 ± 113.8[b]	385.5 ± 128.52[b]	**0.004**	441.96 ± 97.75[a]	422.46 ± 110.77[a,b]	371.37 ± 119.27[b]	**0.007**	453.26 ± 77.7[a]	416.42 ± 126[a,b]	366.37 ± 113.18[b]	**< 0.001**
Red meat (g)	87.07 ± 31.26	75.33 ± 25.58[a]	86.31 ± 31.43[a]	99.61 ± 32.11[b]	**< 0.001**	72.41 ± 27.35[a]	93.1 ± 32.59[b]	95.43 ± 28.84[b]	**< 0.001**	69.87 ± 22.79[a]	96.04 ± 34.44[b]	94.91 ± 28.46[b]	**< 0.001**
White meat (g)	67.21 ± 31.4	49.76 ± 23.51[a]	67 ± 29.35[b]	84.87 ± 31.01[c]	**< 0.001**	55.65 ± 25.3[a]	60.42 ± 28.97[b]	85.85 ± 31.42[b]	**< 0.001**	51.65 ± 23.31[a]	61.44 ± 29.3[a,b]	88.78 ± 29.08[a]	**< 0.001**
Legumes (g)	23.73 ± 15.89	30.1 ± 15.46[a]	24.43 ± 15.94[a]	16.63 ± 13.5[b]	**< 0.001**	31.49 ± 16.79[a]	22.65 ± 14.52[b]	17.09 ± 13.04[b]	**< 0.001**	32.59 ± 16.17[a]	21.64 ± 13.98[b]	17.05 ± 13.55[b]	**< 0.001**
Phosphorus (mg)	983.36 ± 177.92	976.15 ± 182.85	997.71 ± 174.12	975.6 ± 179.86	0.791	1004.19 ± 203.39	997.75 ± 160.23	947.52 ± 166.2	0.247	1002.54 ± 202.27	1006.86 ± 167.02	939.67 ± 157.54	0.126
Potassium (mg)	3457.9 ± 529.59	3647.3 ± 523.29[a]	3507.27 ± 507.52[a]	3216.98 ± 473.51[b]	**< 0.001**	3655.89 ± 469.63[a]	3542.71 ± 460.28[a]	3171.41 ± 540.55[b]	**< 0.001**	3723.26 ± 456.62[a]	3478.83 ± 482.74[b]	3170.7 ± 506.96[c]	**< 0.001**
Calcium (mg)	977.87 ± 164.8	1044.75 ± 194.39[b]	955.33 ± 140.68[a]	935.38 ± 135.94[a]	**0.003**	1037.72 ± 158.83[b]	972.52 ± 160.23[a,b]	923.6 ± 158.58[a]	**0.003**	1040.05 ± 161.51[b]	951.9 ± 170.36[a]	942.79 ± 146.85[a]	**0.007**
Magnesium (mg)	329.81 ± 67.56	335.59 ± 66.99	334.06 ± 70.66	319.61 ± 65.09	0.458	332.23 ± 69.34	327.33 ± 68	329.78 ± 66.63	0.941	341.67 ± 68.39	332.15 ± 66.22	315.11 ± 66.73	0.157
Sodium (mg)	4.46 ± 0.7	4.22 ± 0.66[a]	4.41 ± 0.7[a]	4.76 ± 0.64[b]	**< 0.001**	4.22 ± 0.67[a]	4.41 ± 0.7[a]	4.77 ± 0.63[b]	**< 0.001**	4.12 ± 0.58[a]	4.51 ± 0.74[b]	4.76 ± 0.61[b]	**< 0.001**

*ANOVA.
**There was no difference between groups marked with the same superscript letter.
PRAL: potential renal acid load, NEAP: net endogenous acid production, DAL: dietary acid load

average increase of 0.116 units in PSQI scores, and an average decrease of 0.412 units in psychological resilience. A one-unit increase in PRAL score resulted in an average increase of 0.150 units in HOMA-IR levels, an average increase of 0.204 units in PSQI scores and an average decrease of 0.692 units in psychological resilience. Finally, a one-unit increase in DAL score led to an average increase of 0.072 units in HOMA-IR levels, an average increase of 0.095 units in PSQI scores, and an average decrease of 0.332 units in psychological resilience. Linear regression model results with adjustment variables are shown in Table 3.

Discussion

In this cross-sectional study of Turkish adolescents with obesity, high DAL was positively associated with anthropometric markers, including BMI, body weight, waist circumference, fat percentage and fat mass and biochemical markers, such as fasting insulin, HOMA-IR score, triglyceride levels, and systolic blood pressure. Similarly, it has been reported that an acidogenic diet was associated with a higher risk of overweight/obesity, and abdominal obesity and adiposity related to body fat percentage in children and adolescents (33). In the present study, adolescents with obesity and high scores of PRAL, NEAP and DAL had significantly higher fat mass, higher fat percentage, and lower fat free mass. In addition, waist circumference, which is a marker of abdominal obesity, increased as the DAL of adolescents increased. Mirzababaei et al. (17) reported that high DAL scores were negatively related with resting metabolic rate and directly related with waist circumference in women who were overweight and who had obesity. In one study, it was found that high PRAL scores were positively correlated with waist circumference and BMI in young Japanese women, while in another study, a positive correlation was observed between high NEAP scores and hip circumference, BMI, fat mass and body adiposity index (34). Sorraya et al. (33) showed a direct correlation between neck circumference, which is an indicator of subcutaneous adipose tissue distribution, and NEAP, whereas no such correlation was observed in other anthropometric markers. In the present study, no relationship was found between waist and hip circumferences and DAL scores, whereas adolescents had significantly higher BMI levels at higher scores of DAL indicators. According to the results of a meta-analysis, an increase in PRAL scores in women and NEAP scores in men was associated with higher BMI (35). Fatahi et al. (36) found a relationship between DAL scores and BMI, whereas no significant relationship was observed between PRAL and NEAP scores and BMI. These differences in the studies may be attributed to race and ethnicity, sample size, age range and health status of the study population, measurement of DAL by different methods, differences in

Table 3. Linear models of dietary acid loads with risk of insulin resistance, psychological resilience and sleep quality

		HOMA-IR				Psychological resilience				PSQI scores			
Variables		**Beta**	**95% CI (LB; UB)**	**p***	**R^2**	**Beta**	**95% CI**	**p***	**R^2**	**Beta**	**95% CI**	**p***	**R^2**
NEAP	Crude model	0.090	(0.04; 0.141)	0.001	0.083	-0.412	(-0.528; -0.296)	0.000	0.262	0.116	(0.082; 0.15)	0.000	0.249
	Model 1	0.091	(0.038; 0.143)	0.001	0.103	-0.376	(-0.494; -0.257)	0.000	0.105	0.099	(0.066; 0.133)	0.000	0.129
	Model 2	0.058	(0.005; 0.111)	0.031	0.146	-0.299	(-0.419; -0.18)	0.000	0.147	0.081	(0.047; 0.115)	0.000	0.156
PRAL	Crude model	0.150	(0.065; 0.235)	0.001	0.081	-0.692	(-0.888; -0.496)	0.000	0.261	0.204	(0.148; 0.261)	0.000	0.271
	Model 1	0.159	(0.067; 0.25)	0.001	0.308	-0.624	(-0.831; -0.416)	0.000	0.291	0.171	(0.113; 0.229)	0.000	0.365
	Model 2	0.100	(0.012; 0.188)	0.027	0.359	-0.545	(-0.743; -0.348)	0.000	0.346	0.143	(0.086; 0.2)	0.000	0.380
DAL	Crude model	0.072	(0.037; 0.108)	0.000	0.106	-0.332	(-0.41; -0.254)	0.000	0.338	0.095	(0.072; 0.118)	0.000	0.330
	Model 1	0.075	(0.038; 0.113)	0.000	0.343	-0.308	(-0.391; -0.226)	0.000	0.342	0.082	(0.059; 0.105)	0.000	0.394
	Model 2	0.049	(0.011; 0.087)	0.012	0.390	-0.272	(-0.354; -0.189)	0.000	0.390	0.070	(0.046; 0.094)	0.000	0.419

*Calculated by linear regression.
- Crude model: Simple linear regression.
- Model 1: All models are adjusted for age, BMI, gender, physical activity, sleep duration.
- Model 2: All models are adjusted for age, BMI, gender, physical activity, sleep duration, energy, carbohydrates, proteins, fats, fiber, phosphorus, potassium, calcium, magnesium, sodium.
BMI: body mass index, PRAL: potential renal acid load, NEAP: net endogenous acid production, DAL: dietary acid load, PSQI: Pittsburgh Sleep Quality Index, HOMA-IR: homeostasis model assessment for insulin resistance, CI: confidence interval

the evaluation of dietary intake, and differences observed in the dietary habits and lifestyles of the participants.

Several possible mechanisms have been proposed to explain the relationship between DAL and obesity. Accumulation of hydrogen ions due to an acidogenic diet results in body weight gain (18). Diet-induced acidosis decreases the production of adipokines such as leptin, adiponectin and resistin, which may prevent appetite suppression and provide energy balance.

DAL is associated with metabolic changes and obesity prevalence in adults (17,18) and tends to induce a chronic low-grade metabolic acidosis in children, which is associated with the formation of systemic acidosis and its metabolic consequences (37). Animal product foods, such as meat, fish, chicken, eggs, cheese and cereals are rich in sulphur-containing amino acids, phosphorus and chloride and are potentially acid forming, whereas fruits and vegetables are rich in malate, citrate and glutamate and are potentially base forming (14,15). Excessive consumption of animal foods, except milk which has a neutral DAL since the amount of phosphorus is compensated by the amount of calcium, and Western-style dietary patterns lead to higher organic acid production and fatty acid oxidation, insulin resistance, increased blood pressure and cortisol levels and decreased insulin sensitivity, especially in individuals with obesity (17,19,35). In the current study, red and white meat and sodium consumption were significantly greater in the adolescents with the highest dietary acid scores, whereas whole grain, legumes, calcium and potassium consumption were significantly higher in those with the lowest dietary acid scores. Similarly, Mirzababaei et al. (17) found that individuals with high DAL scores had higher intakes of eggs, meat, sodium, grain, fat and energy. In this study, fasting insulin levels of obese adolescents were significantly higher in the higher tertiles of PRAL, NEAP and DAL, and fasting insulin, triglyceride, systolic blood pressure and HOMA-IR levels were significantly higher in the higher tertiles of NEAP and DAL. In another study, it was found that children and adolescents with obesity who had insulin resistance had higher PRAL and NEAP scores than those without insulin resistance, and one unit increase in DAL scores increased insulin resistance by approximately 3% (38). Similarly in the present study, a one point increase in NEAP, PRAL and DAL scores increased insulin resistance by 9, 15 and 5% respectively. In a study conducted in young Japanese women, it was found that high PRAL scores were positively associated with systolic and diastolic blood pressure, and total and LDL cholesterol levels (34). A meta-analysis reported that high PRAL scores were associated with high triglyceride levels, whereas no relationship was observed between dietary acid scores and other serum lipid parameters (35). Rezazadegan et al. (15) reported that high PRAL and NEAP scores were associated with high fasting blood glucose and high PRAL scores were associated with high triglyceride levels in children and adolescents with obesity, which

is similar to the findings of the present study. In another study, high DAL scores were found to be associated with insulin resistance but not with fasting blood glucose and hemoglobin A1c levels (39). These differences in findings may again be due to differences in the study population and sample size, differences in the calculation of DAL or various uncontrolled confounders.

There are several possible mechanisms explaining the relationship between DAL and cardiometabolic risk factors. Metabolic acidosis increases glucocorticoid secretion and plasma cortisol concentrations, which may induce insulin resistance (40). High consumption of fruits and vegetables leads to higher intake of carotenoids and antioxidants (hence lower DAL) and has a protective role against inflammation and oxidative stress leading to obesity and metabolic disorders (17). In addition, animal foods with high DAL are proinflammatory and may increase inflammatory cytokines that are associated with lipid disorders and insulin resistance, especially in individuals with obesity (15).

It has been reported that there is a relationship between unhealthy dietary patterns and the mental health of children and adolescents and that healthy foods, such as vegetables, fruits, oil seeds and whole grain products have protective effects on depressive symptoms, mental health and sleep disorders (10,21). It has also been reported that prolonged exposure to high DAL caused unfavorable somatic effects (21). In a study conducted in an adult population, adherence to a Mediterranean diet or vegetable-based dietary pattern were associated with high levels of psychological resilience and consumption of processed meat was associated with low levels of psychological resilience (6), but the effects of DAL on psychological resilience have not been examined in previous studies. In the present study, it was observed that psychological resilience levels of adolescents with obesity were significantly lower in the higher tertiles of DAL scores. Beezhold et al. (41) found that compliance with a vegetarian diet and less consumption of animal-based foods in diets were associated with a better mood and Daneshzad et al. (10) found that high DAL scores in women with diabetes and high consumption of animal-based diets compared to plant-based diets increased mental health problems such as anxiety, depression and stress. In a study involving Australian university students, higher resilience score was significantly associated with higher serves per day of vegetables and fruit, higher frequency of breakfast consumption, and lower frequencies of soft drink, cordial or sports drink and takeaway food (12). In the present study, a one point increase in NEAP, PRAL and DAL scores decreased psychological resilience by 41, 69, 33% respectively.

It has been reported that dietary factors play a role in the etiology and treatment of sleep problems, low consumption of vegetables and high consumption of foods with high fat and energy density are associated with sleep disorders (42). We found that adolescents with high DAL had significantly lower sleep quality and to the best of our knowledge, there is only one study investigating DAL and sleep quality, and in that study, similar to our study, it was observed that women with diabetes who were in the highest group of PRAL and NEAP scores had worse sleep quality (10). In a study conducted on individuals aged 12-18 years, it was reported that unhealthy foods such as sugar-sweetened beverages, prepackaged products, confectionery and fast-foods were associated with poor sleep quality (43). In the present study, DAL increased and sleep quality worsened in adolescents with obesity as their energy, refined grain, meat and sodium consumption increased.

Vegetable-based diets with low DAL and high amounts of antioxidants, phytochemicals, flavonoids, vitamins and minerals provide beneficial effects on mental health and sleep disorders by suppressing inflammation and reducing oxidative stress (10,20). Inflammation may trigger melancholic symptoms through the activation of inflammatory pathways in the brain (10). In addition, vegetables and fruits contain high amounts of magnesium, folate, zinc, antioxidants and flavonoids, which have been suggested to help mental disorders (10,21). Legumes and beans are high in tryptophan, the precursor of melatonin and serotonin, which play a role in sleep patterns (44). High DAL leads to an increase in the development of insulin resistance and causes higher blood pressure. Insulin resistance and high blood pressure are associated with deterioration of mental health and sleep quality in the general population (10). In the present study, both insulin resistance and high blood pressure levels in adolescents with high DAL may have contributed to the decrease in psychological resilience and sleep quality scores.

Study Limitations

This study has some limitations. The fact that a healthy control group was not included in our study could not reveal the relationship between obesity and DAL. The cross-sectional nature of the study did not allow cause and effect analyses. Categorization of DAL scores into tertiles may lead to misclassification of the data. The small sample size of the study may reduce the power of statistical tests. In addition, laboratory markers of acid-base balance were not collected in this study, which is another limitation. However, our study has some strengths. This is the first study to examine the effect of DAL on psychological resilience and sleep quality

in adolescents with obesity. In most studies on DAL, only one or two indicators were used, whereas in our study, all three indicators were evaluated, leading to more accuracy and reliability in the findings. In addition, the use of three-day food consumption records instead of food consumption frequency questionnaire and the use of sleep quality and psychological resilience scales, which are validated and reliable tools, are other strengths.

Conclusion

Our findings showed that high DAL was positively associated with body weight, fat mass and percentage, BMI, waist circumference, insulin resistance, fasting insulin, triglyceride levels, blood pressure levels, and negatively associated with psychological resilience and sleep quality. Implementation of strategies to reduce the acid load of the diet with nutritional interventions may have a positive effect on health. Adopting an adequate and balanced dietary model, as opposed to the Western-style dietary model, may be important in reducing the DAL. Thus, both cardiometabolic risk factors will be reduced and the psychological resilience level and sleep quality of the individual will increase. Therefore, it would be beneficial to provide nutrition education to children and adolescents with obesity about the consumption of healthy foods, such as vegetables, fruits, and whole grain products, instead of foods with high energy, saturated fat, sugar, and salt content and to advise them about appropriate eating habits. In addition, well-planned and long-term studies are needed to clarify the relationship between DAL and cardiometabolic risk, psychological resilience and sleep quality in children and adolescents with obesity.

Ethics

Ethics Committee Approval: Approval was obtained from the Gazi University Faculty of Medicine Ethics Committee (approval number: 230, date: 20.03.2023).

Informed Consent: Clear explanations were provided with regard to the purpose of the study, after which written informed consent was obtained from the adolescents in accordance with the Declaration of Helsinki.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: Esra Döğer, Mahmut Orhun Çamurdan, Aysun Bideci, Concept: Rukiye Bozbulut, Mahmut Orhun Çamurdan, Aysun Bideci, Design: Rukiye Bozbulut, Aysun Bideci, Data Collection or Processing: Rukiye Bozbulut, Esra Döğer, Analysis or Interpretation: Rukiye Bozbulut, Esra Döğer, Aysun Bideci, Literature Search: Rukiye Bozbulut, Writing: Rukiye Bozbulut, Aysun Bideci.

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Liraglutide Treatment Improves Glycaemic Dysregulation, Body Composition, Cardiometabolic Variables and Uncontrolled Eating Behaviour in Adolescents with Severe Obesity

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What is already known on this topic?

Liraglutide has shown beneficial effects in managing adolescent obesity in clinical trials and has therefore been approved for clinical use. Continuous glucose monitoring (CGM) is routinely used in type 1 diabetes mellitus, but not for type 2 diabetes mellitus or in patients with obesity.

What this study adds?

Significant improvements were observed in adolescents with obesity following liraglutide treatment in a routine clinical setting. These included improvements in weight, body mass index (BMI), BMI-standard deviation score, body fat percentage, fat mass, percentage time glucose levels spent within normal range, levels of glycated haemoglobin, cholesterol, and triglycerides, and less uncontrolled eating behaviour.

Abstract

Objective: Childhood obesity is associated with long-term health complications. Liraglutide is approved for use in adolescents for weight loss and has shown beneficial outcomes in clinical trials. Continuous glucose monitoring (CGM) is widely used in type 1 diabetes mellitus. To look at the effect of liraglutide treatment on cardiometabolic variables, glycaemic control (as assessed by CGM), body composition, quality-of-life and satiety levels in adolescents with severe obesity.

Methods: Patients aged 12 to 17.9 years were commenced on liraglutide in addition to lifestyle support. Pediatric Quality of Life 4.0 generic scale and Three-factor Eating Questionnaire R18 were completed at baseline and after 3-months.

Results: Twenty-four subjects (10 male: 14 female) took part. Significant improvements in weight, body mass index (BMI), BMI standard deviation scores, percentage body fat and fat mass following liraglutide treatment. A significant reduction in glycated haemoglobin, triglyceride and cholesterol levels, as well as a reduction in uncontrolled eating behaviour were observed. The time spent within normal glucose range (3.9-7.8 mmol/L; 70.2-140.4 mg/dL) was lower than in healthy peers (91.76% vs. 97.00%) at baseline but improved after liraglutide treatment. The cohort reported lower health-related quality-of-life scores and exhibited more uncontrolled eating and emotional eating behaviours, compared to the healthy population.

Conclusion: We report, for the first time, the role of CGM in identifying glycaemic dysregulation in children and young people with obesity before and after liraglutide treatment. The results have shown significant potential for liraglutide treatment in improving outcomes. Earlier identification of glycaemic dysregulation and targeted therapy could potentially reduce the long-term risk of developing type 2 diabetes mellitus.

Keywords: Liraglutide, glycaemic dysregulation, adolescents, obesity

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Introduction

Childhood obesity is a worldwide concern due to its rising prevalence and the associated complications. Type 2 diabetes mellitus (T2DM) is a chronic condition that is associated with a number of long-term issues, such as micro- and macrovascular diseases (1). It has been shown that children and young people (CYP) with obesity are at a four-fold increased risk of developing T2DM (2,3).

Treatment options for obesity within the pediatric population remain limited, with lifestyle intervention being the first line therapy. Liraglutide, a glucagon-like peptide 1 analogue, has received European Medicines Agency and FDA approval for adolescents aged 12-17 years with a body weight of at least 60 kg and an initial body mass index (BMI) corresponding to 30 kg/m^2 or greater for adults (4,5). It has been shown to have a significant effect on BMI standard deviation (SD) scores (SDS) and glycated haemoglobin (HbA1c) in adolescents (6,7,8,9). The efficacy and tolerability in adolescents have been reported as similar to those seen in adults, with no unexpected issues found (8,9,10). Liraglutide has both central and peripheral mechanisms of action, which result in slower gastric emptying, thus increased satiety, and increased insulin production (11).

In view of liraglutide's potential to improve glycaemic control and concerns of increasing prevalence of T2DM secondary to childhood obesity, there is a need for further research within this area. The introduction of continuous glucose monitoring (CGM) for patients with type 1 diabetes mellitus has been beneficial and reports in adults show the potential for its use in T2DM to improve glycaemic control and reduce complications (12). The use of CGM to investigate glycaemic dysregulation in childhood obesity is limited, and to the best of our knowledge it has not been used in normoglycaemic CYP with obesity who have received liraglutide treatment.

The aim of the present study was to investigate the effect of three months of liraglutide treatment on glycaemic control, anthropometry, metabolic outcomes, quality of life and satiety levels, in CYP with obesity.

Methods

This was a retrospective study over a period of 18 months. Patients were commenced on liraglutide (Saxenda; NovoNordisk) and followed regularly to assess progress (Figure 1). All patients were under the care of the Tier 3 multi-disciplinary, weight management service at a tertiary children's hospital and received lifestyle intervention in the outpatient setting. Anthropometric measurements and fasting cardiometabolic variables were performed on a regular basis. The CYP had a CGM device inserted and completed three questionnaires at baseline and at 3-months post liraglutide treatment to investigate the effects of the treatment in a routine clinical setting.

Measurements

The CYP had their weight, BMI and BMI SDS measurements undertaken by the same staff in the medical day case unit. The body composition was obtained using a TANITA: RD-545HR device (Manchester, M1 2HY) which reported body fat percentage (%), fat mass (kg) and free fat mass (kg).

Biochemistry

Fasted blood samples were taken at baseline and at 3 months for HbA1c, insulin, c-peptide, liver enzymes (aspartate aminotransferase and alanine transaminase) and lipid profile. If the patient was not fasted then the insulin, c-peptide and triglyceride levels were omitted from analysis.

Continuous Glucose Monitoring

CGM was used at these time periods to investigate alterations in glycaemic dysfunction following the use of liraglutide. Dexcom G6 CGM devices (Broadway, London, United Kingdom) were used for the majority of the CYP. Two CGM data sets were collected using FreeStyle Libre Pro devices (Abbott House, Vanwall Business Park, Maidenhead, SL6 4XE).

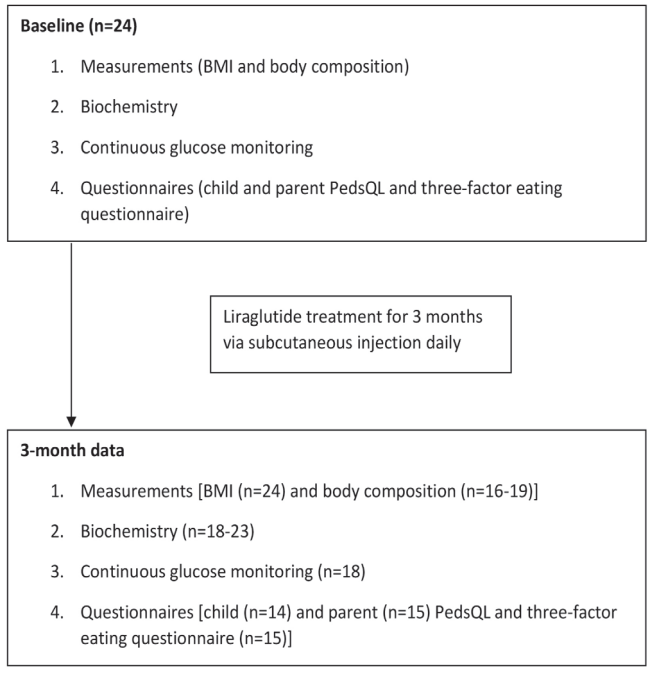

Figure 1. Methods design

BMI: body mass index, PedsQL: Pediatric Quality of Life

All devices were placed onto the upper arm of the patients and removed on day 10 for the Dexcom wearers and day 14 for Libre wearers. When analysing the data, the readings for the first 12 hours were omitted to ensure accuracy.

Questionnaires

The patients and their guardians were asked to complete the following questionnaires at baseline and 3-months.

1. Pediatric Quality of Life (PedsQL) Inventory, Version 4.0 - English (UK): Child or teenager report (13).

2. PedsQL Inventory, Version 4.0 - English (UK): Parent/ Guardian report (13).

Both the above questionnaires assess the quality of life for respondents. They include questions that investigate physical functioning, emotional functioning, social functioning, and school functioning. Transformed scores (0-100) are produced from the raw scores and the mean value is calculated. The higher the score, the better the quality of life reported. From these, the psychosocial health summary score, physical health summary score and total score are also calculated.

3. Three Factor Eating Questionnaire R18 (TFEQ-R18) (14,15).

This questionnaire was completed by the participants and investigates their levels of uncontrolled eating, cognitive restraint, and emotional eating. Each statement has a score dedicated to one of the sections and the raw score is converted to a transformed score (0-100).

Liraglutide

Liraglutide was commenced following the baseline CGM, at a dose of 0.6 mg once daily as a subcutaneous injection. It was increased in increments of 0.6 mg with at least one-week intervals up to the maximum dose of 3 mg, or highest tolerated dose, with the support of the specialist nurse.

Statistical Analysis

The measurements, blood investigations and questionnaire results were analysed using a paired sample t-test to compare the means of each time point. The average glucose and the SD from the CGM data was also analysed using a paired sample t-test. The percentage time that glucose levels were in and out of range were analysed using the Wilcoxon signed ranks test to compare the medians at baseline and 3-months post-liraglutide treatment. All statistical analysis was performed using IBM Statistical Package for the Social Sciences, version 27 (IBM Inc., Armonk, NY, USA).

Results

A total of 24 patients were included in the study. The median (range) age of the patients was 14.7 (12-17.9) years and 14 (58.3%) were female. Two patients had pre-existing T2DM, and one patient had impaired glucose tolerance. The average measurements at baseline and 3-months are shown in Table 1. A significant mean weight reduction was found (-2.95 kg, $p = 0.001$), along with a significant decrease in the mean BMI and BMI SDS (-1.38 kg/m^2; $p < 0.001$ and -0.09; $p = 0.003$, respectively). Body composition was performed, which evaluates body fat percentage, fat mass and free fat mass. A significant reduction was seen over the course of the 3-months of liraglutide treatment in mean body fat percentage (-2.09%; $p = 0.043$) and fat mass (-4.00 kg; $p = 0.011$).

The biochemistry results showed a reduction in HbA1c, insulin, liver function and lipid profile levels (Table 2). Significant reductions in HbA1c [-1.35 mmol/mol; -2.3% (DCCT), $p = 0.025$], triglycerides (-0.122 mmol/L; $p = 0.010$) and cholesterol (-0.28 mmol/L; $p = 0.029$) were noted over the 3-month treatment course. The change in high-density lipoprotein-cholesterol (HDL-C) levels were not significant, but the mean result went from being in the abnormal range to normal range over the course of the three months.

The CGM devices were worn for a median of 9.12 (2.2-13.9) days. The mean glucose level reduced slightly over the treatment course with a mean glucose of 6.56 mmol/L (118.1 mg/dL) at baseline and 6.50 mmol/L (117.0 mg/dL) at 3-months ($p = 0.828$). The SD calculated by the CGM device improved from 1.11 mmol/L to 0.97 mmol/L (20.0-17.5 mg/

Table 1. Anthropometric measurements

Mean measurement	Baseline	3-months of liraglutide treatment	Difference	p value
Weight kg (SD) (n = 24)	126.37 (+19.03)	123.42 (+19.68)	-2.95	**0.001***
BMI kg/m^2 (SD) (n = 23)	45.48 (+7.36)	44.10 (+7.68)	-1.38	**< 0.001***
BMI SDS (SD) (n = 23)	3.81 (+0.47)	3.72 (+0.54)	-0.09	**0.003***
Fat percentage % (SD) (n = 19)	52.37 (+10.06)	50.27 (+9.55)	-2.09	**0.043***
Fat mass kg (SD) (n = 16)	65.05 (+19.55)	61.06 (+18.80)	-4.00	**0.011***
Free fat mass kg (SD) (n = 16)	60.71 (+14.19)	62.28 (+13.37)	1.56	**0.260**

*Statistically significant.
BMI: body mass index, SD: standard deviation

dL), which was not significant (p = 0.066) (Table 3a). The median percentage time that the patient's glucose levels were within normal range (3.9-7.8 mmol/L; 70.2-140.4 mg/dL) significantly improved with liraglutide treatment and increased from 91.76% to 94.18% (p = 0.048). It was also noted that the percentage time that glucose levels were > 7.8 mmol/L (140.4 mg/dL) or > 10 mmol/L (180 mg/dL) also reduced during the intervention period (Table 3b). These results show that the use of liraglutide does improve glucose variation in CYP with obesity. When compared to 12-18 year olds who are healthy, with a BMI below the obesity threshold and do not have diabetes, the time spent with glucose levels within the normal range remained lower in our cohort (91.76% and 94.18% versus 97% for healthy CYP) (Table 3b) (16).

Quality of life was measured through both child and parent-reported questionnaires and the mean transformed scores were calculated. The child-reported scores showed an improvement in all sections, except for social functioning, which remained similar. None of the improvements were found to be significant (Table 4). The parent-reported scores also showed an increase in quality of life for all areas, except for school functioning. Even though an overall positive change was noted, the results remain much lower than those reported by Upton et al. (17), which represents healthy children and parents and also those with a chronic condition (Table 4). This highlights the association between childhood obesity and lower quality of life levels.

Liraglutide is known to improve satiety (11) and this was shown by the results of the TFEQ-R18 scores. A significant reduction was seen in uncontrolled eating with a mean

Table 2. Biochemistry results

Mean value	Baseline	3-months of liraglutide treatment	Difference	p value
HbA1c mmol/mol (SD) % DCCT (n = 23)	35.39 (+ 3.58) 5.4 (+ 2.5)	34.04 (+ 3.11) 5.3 (+ 2.4)	-1.35 2.3	**0.025***
Fasting insulin pmol/L (SD) (n = 19)	251.11 (+ 118.19)	241.37 (+ 129.71)	-9.74	0.723
Fasting c-peptide pmol/L (SD) (n = 19)	1480.37 (+ 462.46)	1486.16 (+ 555.17)	+ 5.79	0.950
AST IU/L (SD) (n = 22)	21.77 (+ 9.16)	21.55 (+ 11.40)	-0.23	0.877
ALT IU/L (SD) (n = 22)	33.14 (+ 28.38)	30.95 (+ 21.81)	-2.18	0.367
Triglycerides mmol/L (SD) (n = 18)	1.02 (+ 0.40)	0.89 (+ 0.38)	-0.12	**0.010***
Cholesterol mmol/L (SD) (n = 21)	4.30 (+ 1.07)	4.01 (+ 0.91)	-0.28	**0.029***
LDL-C mmol/L (SD) (n = 21)	2.67 (+ 1.01)	2.57 (+ 0.81)	-0.10	0.406
HDL-C mmol/L (SD) (n = 21)	1.13 (+ 0.20)	1.52 (+ 2.14)	+ 0.39	0.416

*Statistically significant.
SD: standard deviation, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDL-C: low-density lipoprotein-cholesterol, HDL-C: high-density lipoprotein-cholesterol

Table 3. a) Average glucose and standard deviation during continuous glucose monitoring period (n = 18)

Mean value	Baseline	After 3-months of liraglutide treatment	Difference	p value	Comparison data (USA) (16)
Glucose mmol/L (SD) mg/dL (SD)	6.56 (+ 1.18) 118.1 (+ 21.2)	6.50 (+ 1.16) 117.0 (+ 20.9)	-0.06 -1.1	0.828	5.4 97.2
Standard deviation of glucose provided by CGM mmol/L (SD) mg/dL (SD)	1.11 (+ 0.46) 20.0 (+ 8.3)	0.97 (+ 0.30) 17.5 (+ 5.4)	-0.14 -2.5	0.066	0.8 14.4
b) Percentage glucose time in and out of range during continuous glucose monitoring period (n = 18)					
Percentage time < 3.9 mmol/L (70.2 mg/dL) (IQR)	0.26 (0.03-1.64)	0.26 (0.00-0.85)	-0.052	0.959	1.7 (0.6-2.0)
Percentage time 3.9-7.8 mmol/L (70.2-140.4 mg/dL) (IQR)	91.76 (74.97-96.74)	94.18 (84.38-97.33)	-1.98	0.048*	97.0 (95.0-68.0)
Percentage time > 7.8 mmol/L (140.4 mg/dL) (IQR)	6.82 (1.07-24.72)	4.89 (0.65-15.62)	-1.72	0.085	1.2 (0.3-2.0)
Percentage time > 10 mmol/L (180 mg/dL) (IQR)	0.08 (0.00-2.06)	0.02 (0.00-0.70)	-1.50	0.133	0.0 (0.0-0.0)

*Statistically significant.
SD: standard deviation, CGM: continuous glucose monitoring, IQR: interquartile range

baseline score of 51.94 and 3-month score of 41.01 (-10.93; p = 0.006). Cognitive restraint tended to increase and emotional eating tended to reduce over the 3-months, but the differences were not statistically significant (Table 4).

Discussion

The results showed significant improvements following three months of liraglutide treatment in a routine clinical setting. Adolescents with obesity participants exhibited improvements in weight, BMI, BMI SDS, body fat percentage, fat mass, percentage time glucose within normal range, HbA1c, cholesterol, triglycerides and uncontrolled eating behaviour.

The change in weight, BMI and BMI SDS reported herein support those found in earlier clinical trials (7,18,19,20). Zhao et al. (21) recently published a systematic review and meta-analysis comparing medications for the treatment of obesity. Compared to metformin, orlistat, exenatide and topiramate, liraglutide had shown to be the most beneficial treatment for weight loss in CYP. Our results showed a significant reduction in body fat percentage and fat mass. The effect of liraglutide on body composition has been shown in adults, assessed using dual energy X-ray absorptiometry (22,23,24). Feng et al. (22) showed a significant reduction in total fat mass, trunk, limb, android and gynoid fat following a 24-week course of liraglutide treatment (1.8 mg) in adult patients with T2DM and non-alcoholic fatty liver disease. Significant reductions in total body fat, visceral adipose tissue and liver fat were seen following a median course of 36 weeks of liraglutide (3 mg) versus placebo using magnetic resonance imaging (25). These changes are thought to reduce the risk of cardiovascular disease complications (25). There are very limited studies available looking at the effect liraglutide has on body composition in the pediatric population. Our results are promising after 3-months of liraglutide treatment. Liraglutide likely has this positive effect on body composition secondary to its known mechanisms, which include lower plasma glucagon levels, delayed gastric emptying and increased satiety. It has been suggested that there may also be a weight independent effect of liraglutide on the distribution of body fat (25). Further research is needed in this area, especially in CYP.

Kelly et al. (7) did not show significant changes in glycaemic and cardiometabolic variables, but interestingly our results contradict their findings. We showed improvements in

Table 4. Quality-of-life and satiety questionnaire results

Questionnaire	Mean score at baseline (SD) (0-100)	Mean score at 3-months (0-100)	Difference	p value	Comparison data (UK) (17)
Child/Young Person reported PedsQL (n = 14)					
Physical functioning	54.99 (+24.61)	62.59 (+28.36)	+7.60	0.11	86.08 (+14.06)
Emotional functioning	50.12 (+29.07)	58.21 (+27.92)	+8.10	0.19	76.99 (+18.43)
Social functioning	60.00 (+31.74)	59.29 (+32.57)	-0.71	0.84	86.85 (+16.86)
School functioning	41.79 (+27.64)	51.07 (+26.25)	+9.29	0.16	77.29 (+16.92)
Psychosocial health summary score	50.63 (+25.93)	56.19 (+27.16)	+5.56	0.23	80.50 (+14.06)
Physical health summary score	54.99 (+24.61)	62.59 (+28.36)	+7.60	0.11	86.08 (+14.06)
Total score	51.73 (+24.71)	57.79 (+26.54)	+6.07	0.14	82.25 (+13.09)
Parent reported PedsQL (n = 15)					
Physical functioning	49.58 (+28.52)	50.00 (+25.58)	+0.42	0.90	84.99 (+16.08)
Emotional functioning	38.33 (+29.68)	41.00 (+20.37)	+2.67	0.50	74.67 (+17.67)
Social functioning	44.67 (+30.26)	50.67 (+35.20)	+6.00	0.08	84.62 (+17.24)
School functioning	43.33 (+23.80)	40.00 (+22.44)	-3.33	0.50	77.72 (+18.50)
Psychosocial health summary score	42.11 (+24.30)	43.89 (+21.01)	+1.78	0.52	79.00 (+14.70)
Physical health summary score	49.58 (+28.52)	50.00 (+25.58)	+0.42	0.90	84.99 (+16.08)
Total score	43.98 (+24.00)	45.42 (+21.14)	+1.44	0.54	81.12 (+13.85)
TFEQ-R18 (Child/Young Person reported) (n = 15)					**Comparison data (France) (34)**
Uncontrolled eating	51.94 (+25.77)	41.01 (+27.53)	-10.93	**0.006***	37
Cognitive restraint	39.63 (+17.31)	41.11 (+17.03)	+1.48	0.746	26
Emotional eating	57.04 (+24.81)	49.63 (+28.46)	-7.41	0.308	36

*Statistically significant.
SD: standard deviation, PedsQL: Pediatric Quality of Life, TFEQ-R18: Three Factor Eating Questionnaire R18

all the biochemical variables, except for fasting c-peptide levels. HbA1c, triglyceride and cholesterol levels reduced significantly and, even though the difference was not significant for HDL-C, it had improved from the abnormal to normal range over the three month treatment course. Similar results have also been shown previously (19,20). These improvements also support the potential cardiovascular benefits that liraglutide might have in the long run.

Due to its positive effect on glycaemic control, liraglutide has been approved for the treatment of T2DM in children aged 10 years and over. Fasting glucose and HbA1c levels have been analysed during liraglutide treatment and it has shown good effect compared to placebo (6). The rising rates of childhood obesity are associated with increased prevalence of T2DM in CYP. T2DM is known to be associated with multiple complications which may have a significant impact on the individual. Identifying glycaemic dysregulation in CYP with obesity, who are at increased risk of T2DM, would result in commencing interventions and potentially reducing the chance of the individual developing T2DM. The use of CGM has been shown to be a feasible and acceptable tool to investigate glycaemic control in both pediatric and adult populations (26,27). To the best of our knowledge, CYP with obesity, but not T2DM, on liraglutide treatment have not had their glucose levels analysed using CGM devices. Our patients tolerated the monitors well and the results show a significant improvement in the time that their blood glucose was within the normal range following treatment. Shah et al. (16) analysed glycaemic variation in healthy children and adults and this data was used for comparison. Their results from the 12-18-year-old participants were used as this was the same age range as our patients. At best, the median percentage time in range was 94.18%, which was still lower than the results seen in the healthy population (97.00%). Interestingly, Shah et al. (16) did not report any blood glucose levels above 10 mmol/L, whereas for our patients, they had 0.08% and 0.02% of time spent above this level at baseline and 3-months, respectively. This demonstrates that CYP with obesity already show evidence of glycaemic dysregulation, which improved with liraglutide treatment. This highlights the potential to commence at-risk individuals on treatment at the earliest point, thereby preventing them from progressing to pre-diabetes or T2DM.

Lower health-related quality of life (HR-QoL) has been shown to be associated with childhood obesity. Younger children have been reported to score lower for physical functioning compared to psychosocial functioning (28). Whereas other studies have shown that the individuals' psychosocial QoL is impacted more as they get older into adolescence and with the most severe grade of obesity (29,30). The total score for HR-QoL improved in our cohort of patients following liraglutide treatment. For both child and parent-reported questionnaires the psychosocial score was lower than the physical health score. Adult studies have shown this association (31,32), whereas Kelly et al. (7) did not find any difference in overall weight-related quality of life in adolescents. Data from Upton et al. (17) was used for comparison, and the scores from the healthy population were much higher in all sections than those reported by our cohort. This highlights the importance of psychological support within the multidisciplinary team in the management of childhood obesity.

The GLP-1 hormone acts by delaying gastric emptying and appetite suppression (7,33). We analysed the effect of this in the clinical setting by asking our patients to complete the TFEQ-R18 questionnaire. A significant reduction was seen in uncontrolled eating behaviours and both cognitive restraint and emotional eating showed improvement during the treatment course. The study of De Lauzon et al. (34) was used as the comparison healthy population. As expected, their scores for emotional and uncontrolled eating were lower than our results but interestingly, our patients scored higher for cognitive restraint. Emotional eating was the highest score, which links with the poor psychosocial scores previously mentioned. These results can be used by the psychology team to help improve eating behaviours. This questionnaire can also be used when assessing a patient with suspected hyperphagia.

Study Limitations

The limitations of our study include a small sample size, in comparison to clinical trials previously published. It is important to note that these patients were under the care of a Tier 3 multidisciplinary, weight management service, which has a specific inclusion criterion. This means that all the patients had significantly elevated BMIs (> +3 SDS) and associated complications. Therefore, these results show the effect of liraglutide on adolescents with severe obesity in the routine clinical outpatient setting. Due to the retrospective nature of data collection and patient engagement, some measurements and results were unavailable and the sample size (n) for each section has been highlighted throughout.

Conclusion

The results of the present study showed the positive effect of liraglutide on CYP with obesity following a 3-month treatment course in a routine clinical setting. Significant improvements were shown in anthropometric measurements, cardiometabolic variables and uncontrolled eating behaviour. We have also shown the potential use of

CGM in identifying glycaemic dysregulation in CYP at risk of T2DM due to obesity and the significant improvement that liraglutide has on the time that the glucose levels were spent within the normal range. Further research is needed, but there is promise for the use of CGM in identifying glycaemic dysregulation earlier, so that interventions can be targeted with the view of preventing the complications or progression to T2DM.

Ethics

Ethics Committee Approval and Informed Consent: Ethics committee approval and informed consent are not required (R&D ID 5900).

Footnotes

Authorship Contributions

Medical Practices: Louise Apperley, Jennifer Parkinson, Senthil Senniappan, Concept: Senthil Senniappan, Louise Apperley, Design: Louise Apperley, Senthil Senniappan, Data Collection or Processing: Louise Apperley, Jennifer Parkinson, Analysis or Interpretation: Louise Apperley, Senthil Senniappan, Literature Search: Louise Apperley, Writing: Louise Apperley, Senthil Senniappan, Jennifer Parkinson.

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Evaluation of Growth Characteristics and Final Height of Cases Diagnosed with Noonan Syndrome on Growth Hormone Treatment

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What is already known on this topic?

Short stature is a common characteristic of Noonan syndrome (NS), with many individuals' adult height remaining below the third percentile. Growth hormone (GH) treatment has been shown to be beneficial in improving height outcomes in patients with NS.

What this study adds?

This study presents national data on the efficacy and safety of GH in children and adolescents with NS. The findings confirm that GH treatment significantly increased final height in children and adolescents with NS, with a mean increase of approximately + 1.4 standard deviation scores. GH treatment was demonstrated to be safe for patients with NS, with no significant side effects observed and stable cardiac findings in those with hypertrophic cardiomyopathy.

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Abstract

Objective: Proportional short stature is one of the most important features of Noonan syndrome (NS), and adult height often remains below the third percentile. Although the pathophysiology of short stature in NS patients is not fully understood, it has been shown that growth hormone (GH) treatment is beneficial in NS, significantly improving height in respect to the results of short and long-term GH treatment.

Methods: In this national retrospective cohort study, patients with NS who reached final height from 14 centers were evaluated. Patients were stratified by sex and treatment with or without GH and final height outcomes were compared.

Results: The study included 67 patients with NS, of whom 53 (79.1%) with mean follow-up 5.6 years, received GH treatment. At presentation height standard deviation scores (SDS) of the subjects who were started on GH tended to be shorter than those who did not (-3.26 ± 1.07 vs. -2.53 ± 1.23). In girls mean final height and final height SDS in those using GH vs not using GH were 150.1 cm (-2.17 SDS) vs. 147.4 cm (-2.8 SDS), respectively, and for boys these values were 162.48 cm (-1.81 SDS) vs 157.46 cm (-2.68 SDS), respectively. The Δheight SDS value of the cases was significantly higher in the group receiving GH compared to those not receiving GH (1.36 ± 1.12 SD vs. -0.2 ± 1.24, $p < 0.001$). Cardiac findings remained stable in two patients with hypertrophic cardiomyopathy who received GH treatment. No significant side effects were observed in any patient during follow-up.

Conclusion: In patients with NS who reach their final height, a significant increase in height was observed with GH treatment. An increase of approximately + 1.4 SDS may be achieved. GH treatment appears to be safe and effective in NS.

Keywords: Final height, growth hormone, Noonan syndrome, treatment

Introduction

Noonan syndrome (NS; OMIM 163950) is an inherited, multisystemic disease that occurs in 1/1000-1/2500 live births and is characterized by unique phenotypic findings (1). Mutations in the RAS-mitogen-activated protein kinase (RAS-MAPK) pathway cause NS by altering protein-coding genes. In most cases, the genetic mutations that cause NS are "gain-of-function" mutations and lead to RAS/MAPK hyperactivation, which causes the NS phenotype (2).

One of the main features of NS is postnatal-onset, proportionate, short stature and is the most common reason for presentation to pediatric endocrinology clinics (1,3). Growth rate decreases in the first year of life after birth and in the first year height standard deviation (SD) scores (SDS) loss of 1.5 SD and weight increase loss by 2 SD have been reported (4,5). Compared to the general population, in adults with NS final height is generally short. In addition, a prolonged growth period due to pubertal delay and retardation in bone age are among the growth characteristics (6).

Although the pathophysiology of short stature in patients with NS is not fully understood, the cause of short stature is multifactorial, and response to growth hormone (GH) stimulation tests may be variable. In terms of endogenous GH, there may be a deficiency, neurosecretory dysfunction, or mild GH resistance although insulin-like growth factor-1 levels are at low normal ranges (7,8,9). GH treatment has been shown to be benefiical in NS, and significantly improves height in studies with both short and long-term GH administration (7,9-24). However, questions remain unanswered about some aspects of growth in NS, including the effectiveness and safety of GH treatment in NS. Furthermore, predictive models are not yet practical as they are not sufficiently reliable for predicitng "target heights" in families with a child affected by NS. This is becuase potential genetic growth in NS are not yet fully understood given the generaly different pattern of growth, which limits the utility of current models. In particular, final height studies are lacking, with inconsistent findings and variable age at last follow-up. There is no data on the final heights of NS cases with or without GH treatment in the Turkish population.

The aim of this study was to evaluate the efficacy of GH therapy in children and adolescents with NS in the Turkish population and who had attained final height. Retrospective analysis of presenting characteristics and final heights of patients, with and without GH treatment, was performed to identify factors affecting final height in this population.

Methods

Patient Selection

The study was conducted with multiple centers across Türkiye with clinical links to the Turkish Pediatric Endocrinology and Diabetes Association. Patients followed up with a diagnosis of NS based on genetic analysis and/or Van der Burgt (25) criteria and who had reached their final height were included. The study was cross-sectional and was conducted between September 2022 and January 2024. The study was designed by the Turkish Pediatric Endocrinology and Diabetes Association "Noonan Syndrome Working Group", and centers with Pediatric Endocrinology specialists were invited to participate. Centers caring for patients with NS and who had reached their final height were selected, and

demographic and clinical information was collected through the data collection form.

Patient information was obtained from patients' medical records. Anthropometry and physical examination findings at diagnosis and at follow-up, laboratory evaluations, the result of systemic disease screening, and responses to GH treatment were evaluated. At presentation and final follow-up, age, puberty stage (Tanner staging), height and height SDS, Ranke height SDS, body mass index (BMI), BMI SDS, and bone age were evaluated. Target height (cm), target height SDS, the difference between target height SDS and presentation height SDS, age at completion of puberty, final height (cm), final height SDS, the difference between target height SDS and final height SDS, and final bone age were recorded. For patients receiving GH treatment, additional variables included age at initiation of GH treatment, duration of GH treatment, timing of GH treatment dose increases, age, height (cm), and height SDS at cessation of GH treatment, and GH dose (mcg/kg/day) were recorded. ΔHeight SDS was defined as the difference between the height SDS at the first year of follow-up in those who did not receive GH and the height SDS at the time of admission, the difference between the beginning of GH treatment and after the first year of treatment in those who received GH.

Weight and height SD values were calculated according to Turkish children norms created by Neyzi et al. (26) and standard curves for NS created by Ranke et al. (27). Growth velocity was calculated, and puberty staging was assessed according to Tanner staging. Left wrist radiographs were evaluated using the Greulich-Pyle Atlas for bone age assessment.

Criteria for inclusion in the study were:

1) Age at diagnosis is < 18 years;

2) Confirmed diagnosed of NS by Van der Burgt (25) clinical criteria and/or genetic analysis;

3) Having achieved near final height (bone age reaching ≥14 years in girls and ≥16 years in boys, annual growth of less than 2 cm) and final height was defined as the point at which the growth plates in the bone age assessment had fully closed.

Exclusion criteria were:

1) Patients with suspected NS but who did not meet the Van der Burgt (25) criteria; and,

2) Patients with confirmed diagnosis of NS but who had not yet reached final height.

Ethical approval for the study was received from Ankara University Faculty of Medicine Human Research Ethics Committee (decision no: İ10-627-22, date: 21.11.2022).

Statistical Analysis

Cases were divided into groups, stratified by male/female and those receiving GH treatment or those not receiving GH treatment. Males and females in the GH-treated and non-GH-treated groups were compared. All statistical calculations were performed using Statistical Package for the Social Sciences for Windows, version 22.0 (IBM Inc., Armonk, NY, USA). The conformity of the variables to the normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogrov-Smirnov/Shapiro-Wilk test). Differences between independent groups were analyzed using the Mann-Whitney U test. Correlation analysis was performed using Spearman's method. A $p < 0.05$ was considered statistically significant.

Results

Fourteen centers participated in the study, submitting data on 67 cases, including 28 (41.8%) girls and 39 boys. In addition, the adult heights of 12 of the parents of these cases, who were diagnosed with NS, were recorded. In the evaluation of parental height and target height, affected parent were excluded. The mean age of the cases at presentation was 10.2 ± 4.1 years, with height SDS of -3.1 ± 1.1 and BMI SDS of -0.92 ± 1.3. At first presentation, 19 (28.35%) were prepubertal, and the mean bone age in all cases was 8.8 ± 3.6 years (Table 1).

All cases met the Van der Burgt (25) diagnostic criteria. In 51 of 67 (76.1%), the diagnosis was confirmed by the detection of a pathogenic variant on genetic analysis, including 43 (84.3%) in *PTPN11*, three (5.9%) in *SOS1*, two (3.9%) in *KRAS*, two (3.9%) in *RAF1*, and one (2.0%) patient had NS-related mutations in the *LZTR1* gene. Genetic analysis was not available in the remaining 16 (23.9%) patients.

The target height SDS for all cases was -1.1 ± 0.9, and there was no difference between girls (-1.2 ± 1.0) and boys (-1.0 ± 0.9) ($p = 0.25$) (Table 1).

Among the cases that reached final height, GH treatment was given to 53 cases, of whom 31 (58.5%) were boys. While the mean age at diagnosis was 10.3 ± 3.5 years, the mean age at start of GH was 11.7 ± 2.8 years. GH treatment was generally started around 1.4 years after diagnosis and follow-up continued for 5.1 ± 3.5 years. While the overall pre-treatment height SDS was -3.2 ± 1.0, the initial height SDS tended to be lower in girls (-3.7 ± 1.0 in girls vs -2.9 ± 0.9 in boys, $p = 0.02$). In cases where GH was not given, mean height SDS at diagnosis was -2.5 ± 1.2, being -3.3 SDS in girls compared to -2.0 SDS in boys (Table 2).

Table 1. Baseline characteristics of all patients with Noonan syndrome reaching final height

		Total group (n = 67)	Females (n = 28)	Males (n = 39)	p
Gender (male/female)		39/28	28	39	
Age at admission (years)		10.2 ± 4.0 11.4 [0.1; 17.0]	10.2 ± 2.9 10.5 [3.1; 13.4]	10.1 ± 4.3 11.7 [0.1; 16.5]	0.89
Puberty (yes/no)		19/48	7/21	11/28	-
Birth weight (grams)		3045 ± 707.0 3000 [1000; 4750]	2914.2 ± 689.05 3000 [1500; 4250]	3139.4 ± 714.3 3100 [1000; 4750]	0.18
Height SD at admission		-3.1 ± 1.1 -3.045 [-5.89; -0.06]	-3.6 ± 1.0 -3.565 [-5.5; -2.1]	-2.7 ± 1.0 -2.9 [-5.8; -0.01]	**0.008**
Height SD (Ranke) at admission		-0.3 ± 1.2 -0.4 [-3.1; 2.7]	-0.5 ± 1.0 -0.3 [-2.7; 0.9]	-0.2 ± 1.4 -0.5 [-3.1; 2.7]	0.07
BMI SD		-0,9 ± 1,3 -0,9 [-3.6; 2.3]	-1.1 ± 1.3 -1.0 [-3.5; 1.5]	-1.0 ± 1.5 -1.0 [-4.4; 2.3]	0.59
Bone age at admission		8.8 ± 3.6 9 [2; 18]	8.4 ± 3.9 8.8 [2; 18]	9.0 ± 3.4 10 [2; 14.6]	0.99
Target height SD		-1.1 ± 0.9 -1.1 [-2.7; 0.8]	-1.2 ± 1.0 -1.5 [-2.7; 0.8]	-1.0 ± 0.8 -1.0 [-2.5; 0.7]	0.25
Target height SD-height SD		-1.9 ± 1.1 -2.03 [-4.0; 1.7]	-2.3 ± 1 -2.1 [-3.8; -0.5]	-1.7 ± 1.1 -1.8 [-4.0; 1.7]	0.11
Growth hormone treatment (yes/no)		53/14	22/6	31/8	-
Genetic characteristics	***PTPN11***	43	18	25	-
	KRAS	2	2	0	-
	SOS1	3	3	0	-
	LZTR1	1	1	0	-
	RAF1	2	1	1	-

SD: standard deviation, BMI: body mass index

GH treatment dose ranged from 25 to 45 mcg/kg/day, with a mean of 32.9 ± 6.4 mcg/kg/day. In 29 of the cases receiving GH, the GH dose was started in the range of 25-30 mcg/kg/day, and in 24 of the cases, the dose was started in the range of 35-45 mc/kg/day. In 26 of the cases that started with the lower dose range, the dose was increased during follow-up.

When the admission characteristics of the cases receiving GH and those not receiving GH were evaluated, no difference was found in terms of age, gender, birth weight, presence of puberty, age of onset of puberty in those receiving prepubertal monitoring, target height and target height SDS. Most of the cases were prepubertal or in the early pubertal period at the time of starting GH treatment (39 were prepubertal, 12 were Tanner stage 2).

In terms of genetic characteristics of the patients receiving GH, *PTPN11* mutation was found in 36/53 (67.9%) and *KRAS* mutation was found in two (3.8%). Of the 14 cases who did not receive GH, 7 (50%) had *PTPN11*, 3 (21.4%) had *SOS1*, 2 (14.3%) had *RAF1*, and one (7.1%) had the *LZTR1* mutation. The limited number of patients with *PTPN11* made subgroup analysis unreliable.

When the growth response in the first year of follow-up was calculated as ΔHeight, this tended to be lower in those not receiving GH but this was not significantly different from those who did receive GH (Table 3).

Final Height Data

Subjects reached final height at a mean age of 17.8 ± 2.2 years. While the mean final height SDS was -2.12 ± 1.3, it was -1.96 ± 1.3 SDS in those who received GH compared to -2.7 ± 1.3 SDS in those who did not receive GH ($p = 0.84$). When data on the time of treatment discontinuation in cases receiving GH treatment was analyzed, the age at termination of GH treatment was 16.1 ± 4.4 years, and the height SDS was -2.0 ± 1.1. In these cases, there was no significant increase between the height SDS at the time of cessation of GH treatment and the final height SDS ($p > 0.05$).

When the difference between the height SDS at the start of follow-up and the final height SDS was assessed as ΔHeight SDS, this was 1.3 ± 1.1 SD in the group receiving GH and -0.2 ± 1.2 in those not receiving GH ($p < 0.001$). ΔHeight SDS (1.5 ± 1.2) in girls receiving GH and ΔHeight SDS (1.2 ± 1.2) in boys were similar ($p = 0.33$) (Figure 1). Although there was no difference between girls and boys in the group not receiving GH, the final height SDS of the boys was poorer when compared to presentation values (ΔHeight SDS in girls not given GH was 0.4 ± 0.9 and -0.6 ± 1.3 in boys, $p = 0.12$).

Table 2. Comparison of baseline characteristics of GH-treated and untreated patients

At presentation		GH-treated	GH-untreated	p
Age, (years)	Total	10.3 ± 3.5 11.4 [0.6; 16.5]	9.8 ± 5.8 11.8 [0.1; 17.0]	0.71
	Female	10.2 ± 2.9 10.5 [3.1; 13.4]	10.3 ± 5.8 11.2 [0.7; 17.0]	0.38
	Male	10.3 ± 3.8 11.7 [0.6; 16.5]	9.3 ± 6.1 11.8 [0.1; 15]	0.97
Height SDS	Total	-3.2 ± 1.0 -3.0 [-5.8; -1.4]	-2.5 ± 1.2 -2.6 [-4.5; -0.0]	0.08
	Female	-3.7 ± 1.0 -3.7 [-5.5; -2.1]	-3.2 ± 0.8 -3.0 [-4.5; -2.3]	0.20
	Male	-2.9 ± 0.9 -2.9 [-5.8; -1.4]	-2.0 ± 1.2 -1.9 [-3.7; -0.0]	0.1
Height SD (Ranke)	Total	-0.4 ± 1.2 -0.6 [-3.1; 2.7]	0.0 ± 1.1 -0.0 [-2.0; 1.7]	0.24
	Female	-0.6 ± 1.0 -0.5 [-2.7; 0.96]	-0.4 ± 1 -0.2 [-2.0; 0.6]	0.62
	Male	-0.4 ± 1.4 -0.6 [-3.1; 2.7]	0.5 ± 1.2 0.8 [-1.3; 1.7]	0.11
BMI SDS	Total	-0.8 ± 1.2 -0.9 [-3.6; 1.8]	-1.0 ± 1.6 -1.0 [-3.5; 2.3]	0.62
	Female	-1 ± 1.4 -1.0 [-2.7; 1.5]	-1.4 ± 1.2 -0.9 [-3.5; -0.4]	0.86
	Male	-0.7 ± 1.1 -0.8 [-3.6; 1.8]	-0.7 ± 1.9 -1.1 [-2.9; 2.3]	0.71
Target height SDS	Total	-1 ± 0,9 -0,8 [-2,7; 0.8]	-1.5 ± 0.8 -1.7 [-2.6; -0.3]	0.8
	Female	-1.1 ± 1.0 -1.1 [-2.7; 0.8]	-1.8 ± 1.0 -1.7 [-2.6; -0.5]	NA
	Male	-0.9 ± 0.9 -0.8 [-2.2; 0.7]	-1.36 ± 0.8 -1.3 [-2.5; -0.3]	0.25
Target height SD-height SD	Total	-2.2 ± 0.9 -2.1 [-4.0; -0.0]	-1.0 ± 1.3 -1.1 [-2.7; 1.7]	**0.002**
	Female	-2.5 ± 0.9 -2.7 [-3.8; -0.9]	-1.5 ± 0.9 -1.8 [-2.6; -0.5]	**0.06**
	Male	-2.0 ± 0.9 -2.0 [-4.0; -0.0]	-0.6 ± 1.4 -1.1 [-2.7; 1.7]	**< 0.001**
Bone age	Total	8.3 ± 3.0 8.8 [2; 13.5]	10.5 ± 5.2 12 [2; 18]	0.23
	Female	7.8 ± 2.9 8.8 [2; 12]	11.3 ± 6.4 12.7 [2; 18]	0.1
	Male	8.8 ± 3.1 9 [3; 13.5]	10.0 ± 4.6 11.5 [2; 14.6]	0.30

SDS: standard deviation (SD) score, GH: growth hormone, BMI: body mass index

Patients that reached final height were evaluated separately according to their gender. In girls the average final height and final height SDS in those using GH were 150.1 cm and -2.1 SDS, respectively. In girls who were not given GH (n = 12) the final height was 147.4 cm and final height SDS was -2.8 (p = 0.95 for height and p = 0.73 for SDS) (Table 3). ΔHeight SDS was 1.5 ± 1.2 in girls who received GH treatment and 0.4 ± 0.9 in girls who did not receive GH (p = 0.03). In terms of target height SDS, the final height-target height SDS difference (parentally adjusted height SDS) was 1.0 ± 1.4 SD in girls receiving GH treatment, while it was -1.0 ± 1.2 SD in girls not receiving GH (p = 0.008; see Table 3).

The mean final height and final height SDS in boys who reached final height and used GH were 162.4 ± 6.1 cm and -1.8 SD, respectively. The final height of boys who did not use GH was 157.4 ± 10.1 cm and final height SDS was -2.6 ± 1.4 (p = 0.34 for height and p = 0.19 for SDS). ΔHeight SDS was 1.2 ± 0.9 SDS in boys receiving GH treatment and

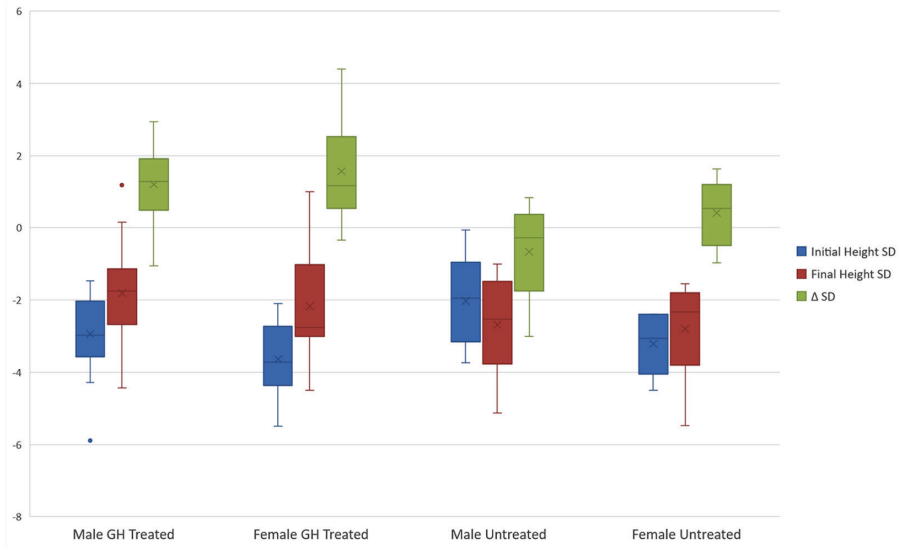

Figure 1. Initial height SDS, final height SDS, and delta height SDS of patients by GH treatment or not and gender

SDS: standard deviation score, GH: growth hormone

-0.6 ± 1.3 in those not receiving GH (p = 0.001). The final height-target height difference was 0.9 ± 1.2 SD in boys receiving GH treatment but -1.3 ± 1.6 SD in boys who did not get GH (p < 0.001; see Table 3).

In those who were started on GH, no significant correlation was found between ΔHeight SDS and age at presentation, GH, height SDS, BMI SD, bone age at presentation and also target height SDS. Again, no difference was found in terms of ΔHeight SDS between those who started GH treatment during puberty compared to those who started prepubertally (r = -0.08, p = 0.57).

For the 12 parents with NS (9 women), the mean adult height SDS was -2.2 ± 0.9 SD. The mean BMI values were 23.1 ± 1.4 kg/m^2 and BMI SDS was 0.7 ± 0.7. No additional problems were reported in the parents. The final height SDS of the subjects who did not receive treatment was similar to the height SDS of their parents who did not receive treatment (p > 0.05).

No serious side effects were observed during follow-up with GH treatment in this cohort.There were a total of 19 cases with cardiac involvement (predominantly pulmonary stenosis) who underwent corrective surgery. Eighteen of them were in the group receiving GH treatment. Hypertrophic cardiomyopathy (HCMP) was detected in two cases receiving GH treatment, and the findings during GH treatment did not change. No additional systemic findings developed during follow-up in any case. No proliferative diseases or neoplasms were reported during the follow-up period of 5.16 ± 3.54 years.

Discussion

In this multicenter study, data of children and adolescents diagnosed with NS and who reached final height were collected and analyzed. GH treatment was not initiated in all cases with NS who reached their final height. The presenting height SDSs of the cases in which GH was started tended to be worse. When treatment was started it was around 1.4 years after the diagnosis, and that the treatment dose also tended to be lower than the GH doses recommended for NS with the same dose given to the standard GH deficiency cases. Hence, the dose was increased during the follow-up. Moreover, GH was not started in some cases despite pathological short stature. Therefore, it appears that pediatric endocrinologists may have some concerns about administering GH to patients with NS. With increasing data showing that the use of GH in NS is effective and reliable, decisions can be made more easily about administering GH to cases in need.

Several studies report short- and long-term follow-up of pediatric age patients with NS and evaluate their response to GH treatment (9,10,11,13-24). After it was shown in very early studies that patients benefited from GH, including a small number of cases with NS after short-term follow-up, the results of studies with GH in long-term use in NS began to be reported (1,8,28). In studies of NS over short-term follow-up, the GH treatment dose varied between 31-66 mcg/kg/day, and there was an increase in the growth rate and height SDS of the cases. This increase was between 0.7-1.88 SDS (9,10,11,13,14,15,16,23). Data may vary in studies on final height/near final height with GH treatment.

Table 3. Comparison of final height characteristics of GH-treated and untreated patients

At last follow-up	GH-treated		GH-untreated	p
Age at final height (years)	Total	17.82 ± 2.05 17.8 [13.33; 26]	17.94 ± 2.97 17.275 [14.7; 27.3]	0.56
	Female	17.88 ± 2.66 17.91 [13.33; 26]	17.41 ± 0.78 17.37 [16.25; 18.45]	0.73
	Male	17.78 ± 1.51 17.65 [15.12; 22]	18.33 ± 3.95 17.21 [14.7; 27.3]	0.67
Final height SD (Ranke)	Total	0.56 ± 1.11 0.52 [-1.96; 2.65]	0.07 ± 1.41 0.09 [-2.1; 3.12]	0.28
	Female	0.21 ± 1.17 -0.23 [-1.49; 2.65]	-0.38 ± 1 -0.06 [-2.1; 0.63]	0.52
	Male	0.81 ± 1 0.76 [-1.96; 2.36]	0.4 ± 1.63 0.26 [-1.91; 3.12]	0.43
Final height (cm)	Total	157.34 ± 9.21 159.4 [139.3; 175]	153.16 ± 9.92 151.8 [136.2; 170]	0.27
	Female	150.1 ± 7.84 147.4 [139.3; 169]	147.43 ± 6.52 149.4 [136.2; 153.5]	0.95
	Male	162.48 ± 6.19 162.6 [145.6; 175]	157.46 ± 10.16 160.4 [140.3; 170]	0.34
Final height (SD)	Total	-1.96 ± 1.33 -1.9 [-4.5; 1.19]	-2.73 ± 1.38 -2.34 [-5.47; -1.01]	0.84
	Female	-2.1 ± 1.4 -2.76 [-3.99; 1.01]	-2.8 ± 1.44 -2.33 [-5.47; -1.55]	0.73
	Male	-1.81 ± 1.22 -1.75 [-4.43; 1.19]	-2.68 ± 1.42 -2.52 [-5.12; -1.01]	0.19
First year ΔHeight SD	Total	0.42 ± 0.61 0.49 [-2.72; 1.72]	-0.61 ± 1.23 -0.09 [-2.01; 0.28]	0.14
	Female	0.46 ± 0.41 0.56 [-0.46; 1.21]	0.28	NA
	Male	0.39 ± 0.73 0.45 [-2.72; 1.72]	-1.05 ± 1.36 -1.05 [-2.01; -0.09]	NA
Final BMI SD	Total	-1.24 ± 1.8 -1.11 [-4.9; 2.5]	-0.87 ± 1.57 -1.285 [-3.33; 2.01]	0.53
	Female	-1.12 ± 1.93 -1.15 [-4.9; 2.33]	-0.06 ± 1.66 0.45 [-2.5; 2.01]	0.24
	Male	-1.33 ± 1.74 -1.11 [-4.72; 2.5]	-1.48 ± 1.27 -1.665 [-3.33; 0.85]	0.71
Final ΔHeight SD	Total	1.36 ± 1.12 1.28 [-1.06; 4.39]	-0.2 ± 1.24 -0.07 [-3; 1.63]	**< 0.001**
	Female	1.57 ± 1.27 1.41[-0.31; 3.98]	0.41 ± 0.94 0.55 [-0.97; 1.63]	**0.03**
	Male	1.2 ± 0.98 1.28 [-1.06; 2.94]	-0.66 ± 1.3 -0.28 [-3; 0.84]	**0.001**
Target height SD-final height SD	Total	0.99 ± 1.3 1.06 [-2.73; 4.17]	-1.2 ± 1.44 -1.02 [-3.53; 0.63]	**< 0.001**
	Female	1.05 ± 1.49 1.38 [-1.84; 3.14]	-1.02 ± 1.26 -1.02 [-2.81; 0.48]	**0.008**
	Male	0.95 ± 1.2 0.89 [-2.73; 4.17]	-1.32 ± 1.61 -1.11 [-3.53; 0.63]	**< 0.001**

SD: standard deviation, BMI: body mass index, NA: not applicable

It has been reported that height gain with GH treatment was between 0.79 and 1.5 SD (17-24). In a systematic review including articles published up to 2014 in terms of adult height, the average height gain was reported to be between 0.6 and 1.4 SDS, according to national standards (29).

Looking at the data at the time of presentation, the admission height SDS value tended to be worse in those given GH treatment than in those not given GH. Although BMI, BMI SDS, bone age, and target height SDS values were not different between the groups, the difference between

height SDS and target height SDS was greater in those given GH treatment. Moreover, the height gain with GH treatment was significantly better than in those who did not receive treatment, in spite of similar ages.

In our cohort, the target height SDS was around -1.1. It can be expected that the frequency of short stature in parents of youth with NS (since they may carry the same mutation) will be significant compared to the general population. In addition, at presentation, patients with NS were approximately -2.0 SDS more negative than their parents. The height SD of the subjects in the group receiving GH was lower than the target height SD compared to the group not receiving GH. The fact that the children were shorter than their parents may have led them to present to a physician.

Patient databases created for cases receiving GH treatment are based on observational information and ensure the accumulation of sufficient data in both number and duration. In two complementary, non-interventional (NordiNet® IOS and ANSWER) studies created from these data, the safety of recombinant human GH (rhGH) treatment in 412 patients and its effectiveness in 84 patients were evaluated. The mean height SDS of the cases was -2.76, and the mean administered GH dose was 41.6 µg/kg/day. The increase in height SDS was positive with 0.49 SD at the end of the first year, 0.79 SD at the end of the second year, and 1.01 SD at the end of the third year. In the 24 cases that reached near final height [165.61 cm (-1.79 SD) in men, 154.9 cm (-1.51 SD) in women] 70.8% of them were -2 SD or above (23). In the present study, a similar proportion (67%) of the GH-treated patients achieved a height SDS of -2 SD or above. However, the final height SDS in our cohort exhibited a wider range, (-1.96 ± 1.3), indicating that while the response to GH treatment in our cohort was effective, baseline differences, lower starting doses of GH and treatment duration may account for some variation in final height outcomes.

Within the scope of the KIGS study by Ranke et al. (21), 140 patients (74 boys/66 girls) with NS who reached near final height were evaluated. While the height SDS at the beginning of treatment was -3.8 in girls and -3.2 in boys, at the end of approximately six years of follow-up, the total height gain was 1.3 SD in girls and 1.2 SD in boys. The average rhGH dose used was 0.3 mg/kg/week in girls and 0.27 mg/kg/week in boys. In the present study the initial height SDS was similar, with a mean value of -3.1 ± 1.1 across the cohort. After approximately 6.5 years of GH treatment, the height gain observed was slightly higher, with a ΔHeight SDS of 1.3 ± 1.1 SD, indicating a comparable response to GH therapy. The GH doses used in our study were slightly lower on average but still within a similar range, which might reflect variations in treatment protocols or patient-specific factors.

Sodero et al. (7) recently evaluated 43 articles examining the effectiveness and safety of GH treatment in NS, including 3.927 patients with NS, Ages ranged from 3 to 17.5 years and clinical and genetic findings were heterogeneous. The duration of GH treatment was between one and 14 years, and the height SDS increased between 0.05 to a maximum of 3.2 SD. Most of the 43 articles reported that GH treatment helps improve target height in children or adolescents with NS. Although the range of height SDS improvement in our study was narrower than reported by Sodero et al. (7), our findings align with the overall trend that GH treatment is advantageous for height gain in young patients with NS. The variations in height SDS response across different studies may be due to differences in study design, patient demographics, and GH dosing protocols.

It has been reported that the growth response in patients with NS is better the earlier GH treatment is started and the longer it is used. The duration of GH use before puberty and the height at the time of entering puberty also affect the near-final height (1). However, in the present study, no correlation was found between GH treatment and total ΔHeight SDS or the age at which treatment started or patient pubertal status at GH initiation. Since the GH treatment dose was heterogeneous, correlation between dose and treatment response was not assessed. That the time of GH initiation in our cohort was mostly in the prepubertal or early pubertal period may have led to a lack of association with pubettal status at start of GH tretment.

In the study including twenty-five years of KIGS data, the younger the age at starting GH treatment, the better the frequency of weekly injections, birth weight and height SDS at the beginning of treatment were associated with a better response. These parameters explained 36% of the increase in growth rate in the first year of treatment. Age at starting GH treatment, growth in the first year of treatment, and gender explained 74% of the change in near-final height (21).

PTPN11 mutation was detected in the majority of the cases in our study group, and 68% of those who received GH treatment and 50% of those who did not receive GH treatment had this mutation. A small number (between one and three) of those with variants in other genes were also included in the cohort. Due to the small number of cases with variants other than in *PTPN11*, no comparison between NS-associated gene differences was attmpted.

Noordam et al. (17) in a study evaluating GH treatment in NS, reported that 22/27 (81.5%) had *PTPN11* mutation, and the average age at start of rhGH was 11 years. Before treatment, median height SDS was -2.8 compared to the healthy group and 0.0 according to NS standards. GH treatment was continued for a median of 6.4 years at a dose of 0.05 mg/kg/day and height gains were +1.3 SDS according to the standard height SDS and +1.3 according to the NS standard. The average adult height for males was 171.3 cm (median, 171.6), and the average adult height for females was 157.3 cm (median, 156.4 cm). No difference in height gain was observed in the group with *PTPN11* mutation compared to those without mutation. In our study, the baseline height SDS of patients who received GH treatment was lower (-3.2 ± 1.0), with the age of GH initiation being similar. However, in contrast to Noordam et al. (17), we observed a significant improvement in height SDS in our cohort, with a ΔHeight SDS of 1.3 ± 1.1 SD in those who received GH, compared to -0.2 ± 1.2 SD in those who did not. The height gain in our study appears to be in line with the findings of Noordam et al. (17), though the baseline SDS in our cohort was poorer, indicating a greater deficit before treatment.

It has been suggested that the severity of clinical phenotype is not important in terms of response to GH treatment. However, the relationship between genotype and growth response has been investigated and it has been suggested that cases with *PTPN11* mutation, especially, may have less growth response. In some short-term studies with a limited number of cases, it has been reported that the growth response of cases with *PTPN11* mutation may be less than that of those without *PTPN11* mutation (10,30). However, this findings was not replicated in other larger and longer-term studies (9,13,21,31). Since the cases were treated with different protocols, the answers regarding the effectiveness of the treatment are still controversial.

In patients who received GH treatment, GH treatment was discontinued when puberty was completed and the epiphyses closed. Thus, there was no increase in height after GH was discontinued. In our cases, height SDS at the time of discontinuation of GH treatment and final height SDS were not different. So, we conclude that GH was given for a sufficient period in our cohort. It is known that in NS, growth may continue until later than normal due to features of pubertal progress specific to NS. The growth spurt occurs with a delay of about 2 years compared to normal children, which leads to prolonged catch-up growth at the end of the second decade of life. However, peak height velocity is low and lower than that in normal-timed puberty (6,32). Therefore, it is important to monitor NS patients treated with GH until puberty is completed.

Different side effects have been reported with GH treatment in patients with NS. Since the underlying pathology in patients with NS is an increase in the Ras/MAPK signaling pathway, it has been reported that the occurrence of benign and malignant proliferative diseases may be higher, independent of GH treatment (33). No neoplasia was found in our cases with long-term follow-up. Although there is an increased risk in the nature of the disease, it has been stated that there is no additional increase in the frequency of malignancy in cases receiving GH treatment, and serious side effects rarely develop (21,23).

In our case series, HCMP was observed in two cases receiving GH treatment, which remained stable and did not lead to cessation of treatment. Due to the presence of structural cardiac deffects and the development of HCMP in patients with NS, questions have arisen regarding the risk of rhGH treatment that may increase the frequency of cardiac side effects. The effect of GH treatment on the heart have been studied in different studies. Data generally support that the frequency or severity of HCMP does not increase with GH treatment in patients with NS (11,26,34,35,36). In a study that included a large database, cardiac side effects were identified in only seven of 429 children with NS who received rhGH, and it was reported that there was no relationship between these cardiac events and GH treatment (14).

In the present study two patients had *RAF1* mutation and both were in the group that did not receive GH treatment. Since ventricular hypertrophy is progressive, especially in NS with *RAF1* mutation, it should be remembered that caution should be exercised regarding GH treatment (36).

Study Limitations

The strengths of the present study include having two homogeneous groups with long follow-up and so patients who did and those who did not receive GH treatment were evaluated and compared However, limitations of the present study include genetic mutation analysis not being performed in all cases. Since the number of patients with mutations in genes other than *PTPN11* was small, no comparison was attempted. Indications for dosage of GH therapy were not uniform due to data being collected retrospectively from different centers using no standardized treatment protocol for GH in NS.

Conclusion

There was a better height gain with GH treatment in patients with NS who reached their final height, compared to those who did not receive GH. Early presentation, starting GH therapy without delay in cases where necessary, and

having a better target height SDS may be associated with a better GH treatment effect. Finally, it should be noted that there were no additional adverse effects seen during GH treatment which should reassure clinicians managing NS that GH treatment in these patients is safe.

Ethics

Ethics Committee Approval: Ethical approval for the study was received from Ankara University Faculty of Medicine Human Research Ethics Committee (decision no: İ10-627-22, date: 21.11.2022).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Zeynep Şıklar, Merih Berberoğlu, Sirmen Kızılcan Çetin, Melek Yıldız, Serap Turan, Şükran Darcan, Semra Çetinkaya, Nihal Hatipoğlu, Ruken Yıldırım, Korcan Demir, Öznur Vermezoğlu, Zehra Yavaş Abalı, Deniz Özalp Kızılay, Nilay Görkem Erdoğan, Ülkü Gül Şiraz, Zerrin Orbak, İlker Tolga Özgen, Aysun Bideci, Beray Selver Eklioğlu, Esin Karakılıç Özturan, Gürkan Tarçın, Abdullah Bereket, Feyza Darendeliler, Concept: Zeynep Şıklar, Merih Berberoğlu, Feyza Darendeliler, Design: Zeynep Şıklar, Merih Berberoğlu, Fe yza Darendeliler, Data Collection or Processing: Zeynep Şıklar, Merih Berberoğlu, Sirmen Kızılcan Çetin, Melek Yıldız, Serap Turan, Şükran Darcan, Semra Çetinkaya, Nihal Hatipoğlu, Ruken Yıldırım, Korcan Demir, Öznur Vermezoğlu, Zehra Yavaş Abalı, Deniz Özalp Kızılay, Nilay Görkem Erdoğan, Ülkü Gül Şiraz, Zerrin Orbak, İlker Tolga Özgen, Aysun Bideci, Beray Selver Eklioğlu, Esin Karakılıç Özturan, Gürkan Tarçın, Abdullah Bereket, Feyza Darendeliler, Analysis or Interpretation: Zeynep Şıklar, Merih Berberoğlu, Sirmen Kızılcan Çetin, Literature Search: Zeynep Şıklar, Merih Berberoğlu, Sirmen Kızılcan Çetin, Writing: Zeynep Şıklar, Merih Berberoğlu, Sirmen Kızılcan Çetin.

Conflict of Interest: Four authors of this article, Serap Turan, Korcan Demir, Abdullah Bereket and Feyza Darendeliler, are a members of the Editorial Board of the Journal of Clinical Research in Pediatric Endocrinology. However, they did not involved in any stage of the editorial decision of the manuscript. The other authors declared no conflict of interest.

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CASE REPORT

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A Boy with 46,XX Karyotype (SRY Double-positive) and a Leydig Cell Tumor

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What is already known on this topic?

Ninety percent of the patients with 46,XX testicular disorder of sex development (DSD) are SRY positive, but double positivity is rare. To date, Leydig cell tumors have been only reported in adult cases with 46,XX, testicular DSD.

What this study adds?

We report the first pediatric case of 46,XX testicular DSD associated with a Leydig cell tumor.

Abstract

Leydig cell tumors are the most common type of testicular sex cord stromal tumors. The presence of the Y chromosome is associated with tumor risk in sex development disorders (DSD), however tumor development without Y chromosome is extremely rare. A 16-year-old boy diagnosed with Leydig cell tumor due to a mass in the right testis was referred after the right orchiectomy. On physical examination, the left testis was 10 mL, and there was a labial residue in penoscrotal region. Bilateral gynecomastia was present. The karyotype was 46,XX and SRY was double-positive on fluorescent *in situ* hybridization analysis. Ifosfamide, carboplatin and etoposide chemotherapy was initiated due to the Leydig cell tumor. Here, we report the first pediatric case having 46,XX testicular DSD with double-positive SRY and a Leydig cell tumor.

Keywords: Leydig cell tumor, sex determining region of Y-chromosome, testicular DSD

Introduction

Disorders of sex development (DSD) are defined as the incompatibility between chromosomal sex and phenotype, and DSD is seen in 1 in every 4,500 births (1). However, 46,XX DSD is usually sporadic and may be classified into three major groups; gonadal development disorders (gonadal dysgenesis, ovotesticular DSD, and testicular DSD), disorders due to androgen excess, and unclassified disorders, such as Mullerian agenesis, labial fusion and vaginal atresia (2). Furthermore, 46,XX testicular DSD is characterized by a male phenotype despite 46,XX karyotype, mainly due to *SRY* translocation, and was first reported by Delachapelle (3) in 1964. These patients usually present with gynecomastia, infertility and hypergonadotropic hypogonadism in the post-pubertal period of life. The presence of Y chromosome in

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DSD patients increases the risk of gonadal tumors. Gonadal tumors are extremely rare in 46,XX testicular DSD without Y chromosome (4). In childhood and adolescence, sex-cord stromal tumors (SCSTs) constitute approximately 5% of all testicular tumors and the remainder is of germ-cell origin (5). SCSTs originating from the supportive tissues of the testis include Leydig, Sertoli and granulosa cell tumors (6). Among these, Leydig cell tumors are the most common testicular SCSTs, and present usually with precocious puberty due to excessive testosterone secretion.

Here, we report the first pediatric case of 46,XX DSD with SRY double-positivity and with a Leydig cell tumor.

Case Report

A 16-year-old boy was admitted to a urology out-patient clinic with a mass in the right testis. The right testis was > 25 mL while the left testis was 10 mL. On initial examination, bilateral gynecomastia was present. Laboratory test results were; prolactin 51.8 µg/L (2.6-13), total testosterone 0.79 µg/L (1.7-7.8), estradiol 12 ng/L (< 15), luteinizing hormone (LH) 3.75 U/L (1.2-8.6), follicle stimulating hormone (FSH) 5.54 U/L (1.2-19.2), α-fetoprotein 2.18 µg/L and beta-hCG < 0.005 U/L. The patient underwent radical right orchiectomy, and a well-circumscribed solid tumoral tissue with a diameter of 1.5 cm was excised. On histopathology, mixed type SCST, consisting of Leydig cell tumor in 99% of the area, and granulosa cell tumor in 1% of area, was present. No other tissue involvement, nor lymphovascular invasion, were detected (Figure 1).

Then, the patient was referred to our pediatric endocrinology out-patient clinic because of gynecomastia and hyperprolactinemia. On physical examination, height was 162.9 cm (3-10 p), weight was 49.5 kg (50-75 p), left testis was 10 mL, penis size was 7.5 cm (6.4 ± 1.1), and pubarche was Tanner stage 5. Severe gynecomastia with glandular tissue of about 6x6 cm in both breasts, and a labial residue in the penoscrotal region were detected (Figure 2). His mental development was normal, and he had no syndromic features. Laboratory results showed that while he was euthyroid, prolactin was 8.2 µg/L, total testosterone was 0.7 µg/L, estradiol was 19 ng/L, LH was 4.67 U/L and FSH was 3.78 U/L. Pituitary magnetic resonance imaging was normal. However, karyotype was 46,XX, and *SRY* was double-positive on fluorescent *in situ* hybridization analysis (Figure 3). Psychiatric evaluation found no gender dysphoria. Subsequently, a chemotherapy regimen consisting of ifosfamide, cisplatin and etoposide was initiated because of the diagnosis of stage 3 Leydig-cell tumor.

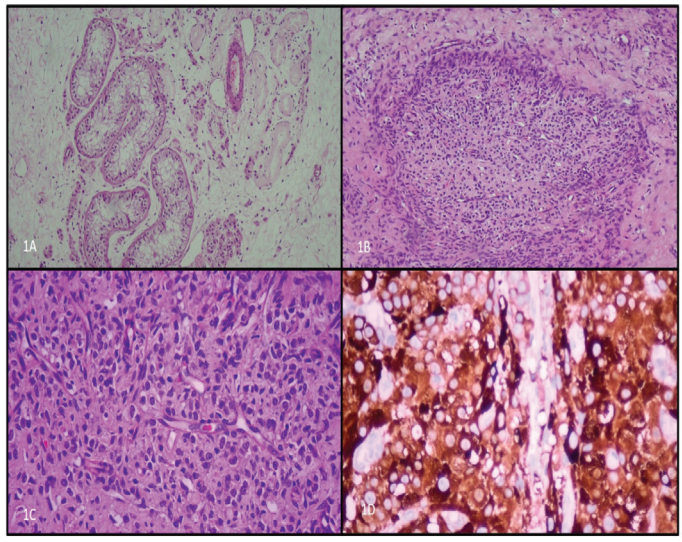

Figure 1. Histological findings of the testicular biopsy specimen. 1A) Testicular tissue containing Leydig cells and seminiferous tubules (hematoxylin-eosin stain x10). 1B) The tumor with nodular growth pattern in fibrotic stroma (hematoxylin-eosin stain x4). 1C) Tumoral tissue (hematoxylin-eosin stain x20). 1D) Strong positive immunohistochemical staining with inhibin in tumoral tissue (x10)

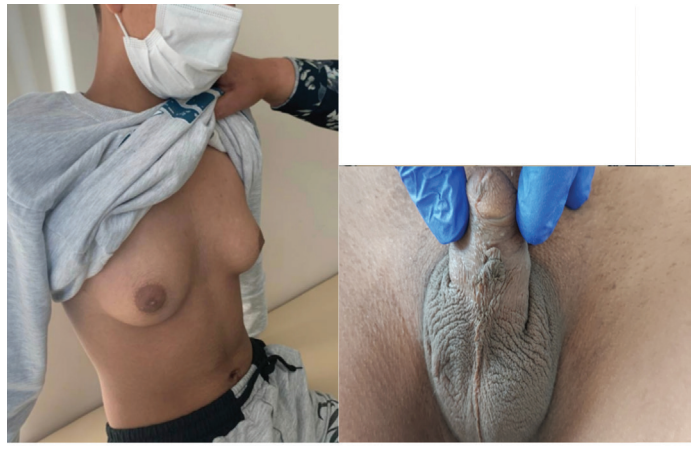

Figure 2. Bilateral gynecomastia and labial residue in penoscrotal region

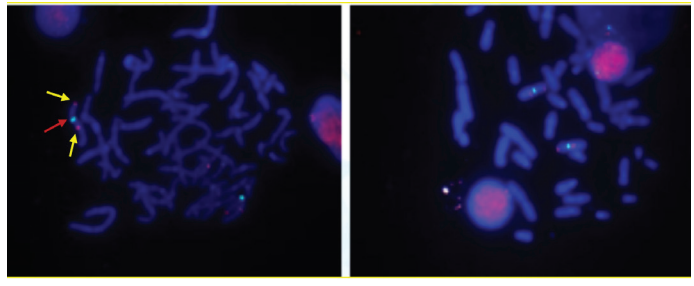

Figure 3. Fluorescent *in situ* hybridization analysis with double-positive SRY (DXZ1x2, DYZ1x0, SRYx2, [200]), in 46,XX karyotype (yellow arrow; SRY and red arrow; X chromosome)

At his last follow-up visit, at 17.6 years of age, the left testis was 5 mL and a glandular tissue of about 3x3 cm in both breasts was present. His blood test results showed euthyroidism, prolactin was 10.1 µg/L, total testosterone was 2.8 µg/L, estradiol was 24 ng/L, LH was 51.8 U/L, and FSH was 110.4 U/L. Subsequently, testosterone enanthate therapy (250 mg, im/monthly) has been initiated due to hypergonadotropic hypogonadism.

Discussion

There are at least three mechanisms for the etiology of 46,XX testicular DSD; occult mosaicism of Y chromosome only present in gonads, translocation of *SRY* gene to the X chromosome or autosomal chromosomes, or X-linked mutation/overexpression in the genes causing testis differentiation or mutation/overexpression in the autosomal genes (7). *SRY* gene at the distal end of the Y chromosome has an important role in male gender differentiation, and is effective in the differentiation of bipotential gonad towards testis. The task of this gene is to synthesize SRY protein that will ensure the formation of testicles. If the *SRY* gene does not synthesize SRY protein, ovary is formed instead of testis (8).

Ninety percent of cases with 46,XX testicular DSD are *SRY* positive. This condition is not usually hereditary, as it results from unbalanced Xp;Yp translocations during paternal meiosis resulting in the presence of *SRY* on the X chromosome. In contrast, *SRY* negative 46,XX testicular DSD originates from the rearrangements or changes in copy number in *SOX9* or *SOX3* genes, and specific heterozygous pathogenic variants in *NR5A1* or *WT1* (1,9).

Nearly, 85% of the individuals with 46,XX testicular DSD present with small testicles, gynecomastia and infertility due to azoospermia after puberty, and they usually have normal genital hair development and normal penile size. Only 15% of the cases present with ambiguous genitalia (9). While testosterone levels are normal at pubertal ages, it declines after puberty due to impaired synthesis. If untreated, osteopenia, low body muscle strength with high fat mass, decreased secondary sex characteristics, erectile dysfunction and impaired libido may occur.

The length of the translocated SRY has a role in variations in the secondary sex characters. If translocated Yp materials are smaller than 100 kb, genitalia is under masculinized due to X-inactivation into *SRY* or compromised *SRY* expression according to change in the *SRY* position relative to chromosomal environments (position effect). On the contrary, if large Yp materials are translocated onto Xp, *SRY* is protected from both the position effect and X-inactivation. Exceptionally, some patients are under masculinized under translocation of large Yp materials, or *vice versa* (10).

In the presented case, karyotype was 46,XX, SRY was double-positive, and he presented with gynecomastia, labial residue in the penoscrotal region, a small normal left testis (the right testis contained tumoral tissue) and low testosterone level for age, while his genital hair development and penile size were normal. The signals from the *SRY* region on each X chromosome indicate that there were two *SRY* genes. Therefore, we speculate that *SRY* double-positivity causes the presence of abundant Yp materials permitting near-normal male phenotype. In addition, because one X chromosome is inactivated, double *SRY* positivity has no dosage affect. Currently, in the literature, there is insufficient data to confidently understand the clinical effect of *SRY* double positivity (11).

In childhood and adolescence, SCSTs constitute approximately 5% of all testicular tumors and the remainder are germ-cell in origin (5). SCSTs originating from the supportive tissues of testis include Leydig, Sertoli and granulosa cell tumors, as well as malignant mesothelioma of tunica vaginalis (6). Among these, Leydig cell tumors are the most common testicular SCSTs, and usually present with painless testicular mass and/or precocious puberty due to excessive testosterone secretion. Although Leydig cell tumors are very rare, they develop at any age of life, but are usually seen between 5 to 10 years of age (12). Unlike in adults, Leydig cell tumors in prepubertal patients do not metastasize and can be treated with radical orchiectomy or testis-sparing surgery (13). Although Leydig cell tumors are generally benign in childhood, in the present case, radical orchiectomy was performed and a chemotherapy regimen has been initiated due to a risk of malignancy in 10% of adult male cases (14).

In ovotesticular or 45,X/46,XY DSD, there is an increased risk of germ cell tumors. Non-germ cell tumors are rarely seen. The first and only case having *SRY* positive 46,XX testicular DSD with Leydig cell tumor was reported by Osaka et al. (2) in 2020. The Leydig cell tumor was detected incidentally in a male patient during tests performed for infertility. This case was an adult patient having unilateral mass with a benign course (2). The presented case is important as he is the first pediatric case with a Leydig cell tumor having *SRY* double-positive, 46,XX testicular DSD.

Conclusion

In conclusion, we presented this first published case of 46,XX testicular DSD with double-positive *SRY*. This case is

even more unusual because it is the first case of 46,XX, *SRY* positive testicular DSD with a Leydig cell tumor.

Ethics

Informed Consent: Consent form was filled out by all participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Doğa Türkkahraman, Sultan Aydın, Merve Güllü, Tangül Pınarcı, Tarkan Kalkan, Concept: Doğa Türkkahraman, Sultan Aydın, Merve Güllü, Design: Doğa Türkkahraman, Sultan Aydın, Merve Güllü, Data Collection or Processing: Doğa Türkkahraman, Sultan Aydın, Merve Güllü, Analysis or Interpretation: Doğa Türkkahraman, Sultan Aydın, Merve Güllü, Tangül Pınarcı, Tarkan Kalkan, Literature Search: Doğa Türkkahraman, Sultan Aydın, Merve Güllü, Writing: Doğa Türkkahraman, Sultan Aydın, Merve Güllü.

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CASE REPORT

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Diagnostic Pitfalls of a Newborn with Congenital Nephrogenic Diabetes Insipidus

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What is already known on this topic?

Nephrogenic diabetes insipidus (NDI) is caused by antidiuretic hormone (ADH) resistance in the principal cells of the renal collecting ducts which results in impaired water reabsorption. NDI is a rare cause of hypernatremic dehydration in the neonatal period.

What this study adds?

Early partial but transient response to ADH is possible in NDI.

Abstract

Congenital nephrogenic diabetes insipidus (NDI) is a rare cause of hypernatremia in newborns. Central diabetes insipidus (CDI) is the main differential diagnosis in NDI, however NDI responds poorly to desmopressin acetate (DDAVP) treatment, while this is the mainstay of CDI management. Therefore, early and correct diagnosis of NDI is important to avoid the complications of inappropriate therapy. We report a newborn with hypernatremia and hypotonic polyuria. The patient was initially responsive but subsequently unresponsive to intranasal DDAVP treatment in terms of both urine output and serum sodium levels. A novel hemizygous missense mutation (c.632T > C, p.L211P) in the *AVPR2* gene was found in both the baby and his mother, and the diagnosis of congenital NDI was established. After hydrochlorothiazide treatment and hypo-osmolar formula were given, urine volume was decreased, and serum sodium levels were normalized. Early recognition and appropriate management of NDI may prevent complications of hypernatremic dehydration in young infants.

Keywords: Nephrogenic diabetes insipidus, neonate, hypernatremia, AVPR2

Introduction

Nephrogenic diabetes insipidus (NDI) is a disorder of water reabsorption, caused by the resistance in the principal cells of renal collecting ducts to antidiuretic hormone (ADH). Genetic forms of NDI are most commonly seen in early life and 90% of cases are caused by a mutation in the *AVP2R* gene that is located on the X chromosome (1). The other 10% is inherited autosomal recessively or dominantly due to a mutation in the gene encoding the aquaporin-2 water channel (*AQP2*) (1). Although more than 200 mutations of the *AVPR2* gene have been described that cause complete ADH resistance (2), only a few have caused partial ADH resistance (3,4).

In this case report, we present a newborn who was admitted to the neonatal intensive care unit (NICU) with hypernatremic dehydration and was eventually diagnosed

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with NDI due to a novel missense mutation (c.632T>C, p.L211P) in the *AVPR2* gene. The aim of this report is to highlight and discuss potential pitfalls in the management of neonatal NDI.

Case Report

A nineteen-day-old male was transferred to our unit with a clinical suspicion of diabetes insipidus (DI). He was born to a 22-year-old mother in the 39th week of gestation with no complications. The birth weight, height, and head circumference were 4160 g, 52 cm, and 37 cm, respectively. The parents were third-degree cousins and a maternal aunt had died at one month of age because of an unknown cause (Figure 1A). The mother had no complaints during pregnancy; however, mild fetal hydronephrosis had been detected on antenatal ultrasound in her third trimester of gestation. The baby was discharged with no problem and a recommendation for breastfeeding. He had been taken to another medical center at the age of 11 days with fever and restlessness. The serum sodium concentration was measured as 155 mEq/L. The patient had been admitted to NICU for hypernatremic dehydration, which had been interpreted as related to neonatal sepsis. The serum sodium level had increased to 161 mEq/L under rehydration treatment with 1/3 isotonic saline. The urine output was as high as 10-12 mL/kg/hour with a simultaneous plasma ADH level of 16 pg/mL (normal range: 2-12 pg/mL for a serum osmolality of >290 mOsm/kg). His volume resuscitation treatment had been made by large volumes of intravenous fluids (240-260 mL/kg/day) to compensate for the increased urinary output (>10 mL/kg/hour) and insensible fluid loss. The concentration of the intravenous fluid had gradually been decreased to 1/8 of normal saline and then switched to 5% dextrose with no sodium. On the nineteenth postnatal day, the baby was referred to our NICU for further investigation.

On physical examination, he appeared well and active with no remarkable pathologic findings. The weight was 4290 g. His serum sodium level was 149 mEq/L while he was receiving a 5% dextrose solution as 100% replacement volume for urinary output. Serum urea, creatinine, potassium, calcium, phosphate, alkaline phosphatase and magnesium, and capillary blood gas levels were all in normal ranges. Renal ultrasound showed grade 2 pelvicaliectasis in the right and grade 1 in the left kidneys. Urine output was measured as 11 mL/kg/hr on the first day in our unit.

An intranasal desmopressin test using 10 µg desmopressin acetate (DDAVP, Minirin® nasal spray, Ferring GmbH, Kiel, Germany) (a synthetic arginine vasopressin analogue) was performed to make the differential diagnosis of DI. Since the urine density increased from 1005 to 1022 six hours after the first administration of desmopressin and urine output decreased to 9.9 mL/kg/hr, suggesting central DI (CDI), treatment with 7.5 µg/day DDAVP (Minirin® Melt tablet) was started. However, urine output continued to be as high as 10-12 mL/kg/hour with a density of <1003 in the follow-up, despite gradually increasing doses of DDAVP up to 120 µg/day in the following four days. Based on this clinical observation, DDAVP was discontinued and hydrochlorothiazide was started at a dose of 1 mg/kg/day, a hypo-osmolar formula was given, and genetic analysis was planned for NDI. After rearrangement of treatment the urine volume decreased from 9.2 mL/kg/hour to 6.7 mL/kg/hour, and serum sodium levels were stabilized between 135-145 mEq/L (Figure 1B).

Meanwhile, it was learned that the mother also had polyuria and polydipsia. She used to drink about 15 to 20 litres of water per day, but she had never attended a doctor for this symptom. The genetic analyses revealed that both the mother and the infant had heterozygous and hemizygous missense mutations (c.632T>C, p.L211P), which had not been previously reported in large population databases, including ExAC, 1000 Genomes, 6500ESP and gnomAD. The pathogenicity of the variant was predicted using *in silico* tools (Polyphen-2, Sort Intolerant from Tolerant 'SIFT', and MutationTaster). Leucine (Leu; L) at position 211 in the AVPR2 protein which is located in the fifth helix in the cytoplasmic domain (Figure 2A, 2B). This change of Leu211 to Pro211 is predicted to be pathogenic and impair the interaction of AVPR2 and ADH by changing the three-dimensional structure of AVPR2 protein (Figure 2C).

Discussion

The diagnostic process and management of a newborn with NDI caused by a novel missense mutation (c.632T>C, p.L211P) in the *AVPR2* gene is described. The initial diagnosis was CDI, due to a positive response to DDAVP. However, the failure of ADH treatment during the clinical follow-up led to consideration of a diagnosis of NDI, which has been termed "AVP resistance" (5). The patient was successfully treated with hydrochlorothiazide and hypo-osmolar formula.

Hypernatremic dehydration is common in the neonatal period. Three pathophysiologic mechanisms may underlie the etiology: 1) decreased water intake; 2) increased water loss; or 3) increased intake of sodium. Treatment depends on the severity of dehydration and hypernatremia and consists of fluid therapy to replace fluid loss, maintenance fluids, and insensible loss. Due to the risk of brain edema, it

is strongly recommended that serum sodium should not be decreased more than 10 meq/L in 24 hours (1).

Hypernatremia in DI develops due to impaired water reabsorption and increased water loss. The symptoms are non-specific and may be confused with other disorders. Restlessness, vomiting, fever, lethargy, dehydration and polyuria are common (6,7). Unlike adults, neonates are unable to access water themselves when thirsty, which makes them prone to hypernatremia. Despite dehydration, frequent and heavy nappies suggesting polyuria are important clues for DI, but are usually overlooked by both the family and health workers (8,9). Once the diagnosis is established, treatment is the same as the other aetiologies

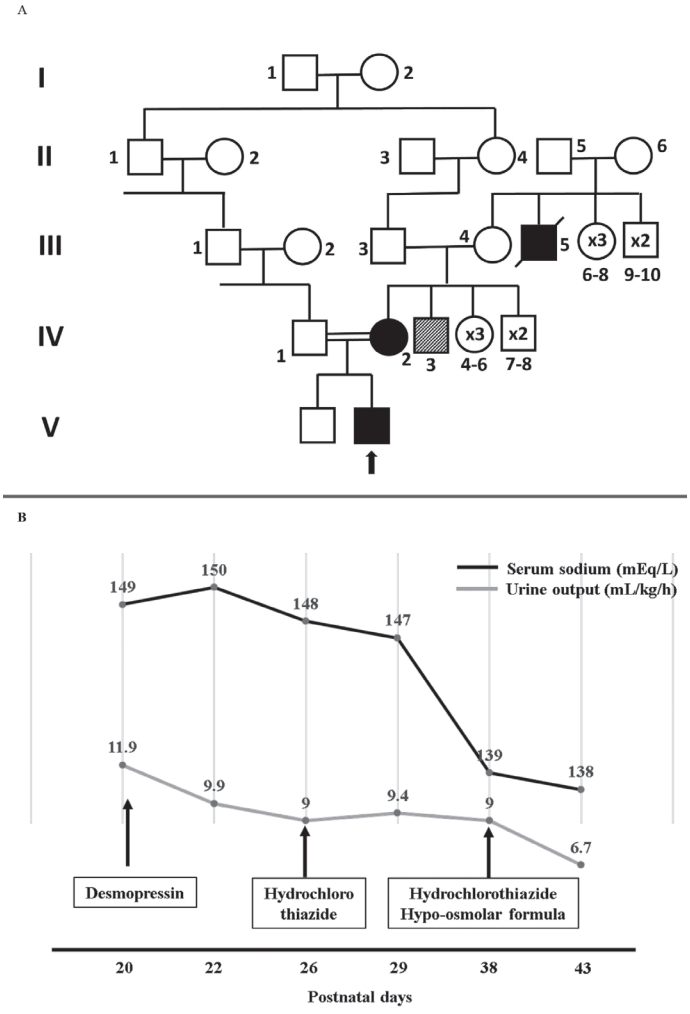

Figure 1. Clinical characteristics of the patient with nephrogenic diabetes insipitus due to mutation in *AVPR2* gene. A) Pedigree of the patient and his family. Individual IV.2 is heterozygous for *AVPR2* c.632T > C. Individual V.2 is hemizygous for AVPR2 c.632T > C. Genetic analysis could not be performed in II.6, III.4, III.5, and IV.3. Slash-line square (IV.3) indicates maternal uncle with polyuria, polydipsia and mental retardation. B) The urine output and serum sodium concentrations of the patient with nephrogenic diabetes insipidus during the clinical follow-up

of hypernatremic dehydration except the concentration of fluid should be more hypotonic than for neonates with deficient intake. Another important aspect is that the total volume of fluid replacement cannot be easily decreased in these patients because of extremely high urine output which sometimes reaches to 10 mL/kg/hour and continues indefinitely unless treated with effective drugs. After stabilizing serum sodium and water homeostasis, discharge of these babies warrants family education about the disease, emergency situations and compliance to therapy.

ADH functions to control reabsorbtion of water in both volume depletion or increased serum osmolality. Therefore, impaired ADH production or resistance to its effect causes central or nephrogenic DI, respectively. A water deprivation test is not suggested in neonates and very young infants (8). Thus, a desmopressin test may be used to decide whether DI is central (ADH-responsive), or nephrogenic (ADH-not responsive) with close follow up of urinary density and amount of urinary output. Partially responsive NDI cases have been reported and treatment with DDAVP may be successful (3,4,7,10,11,12).

Ninety percent of congenital NDI is caused by a mutation in *AVPR2*, located on the X chromosome (1). Therefore, mainly males are affected. Female carriers may have NDI depending on the extent of genomic inactivation of the healthy X chromosome, which we suggest may have occurred in the mother of our patient (13). We could not perform studies showing X chromosome inactivation in the mother. The other 10% of NDI cases are due to aquaporin gene mutation, which is inherited in both autosomal recessive or dominant patterns (1). Congenital CDI is rarely seen and usually present after one year of age (6). History of polyhydramnios or fetal hydronephrosis, and family history of an X-linked pattern of inheritance, as in the presented case, should suggest NDI.

We have identified a novel missense mutation in *AVPR2*, in both the patient and his mother as the cause of NDI. To date, over 200 mutations in the *AVPR2* gene have been described, but only a few of the reported mutations cause partial NDI (3). The AVPR2 protein has 371 amino acids, three extracellular and three intracellular loops with seven transmembrane domains (2). The severity of NDI depends on the type of mutation (2,14,15,16,17). Some mutations lead to partial response to ADH, whereas the others cause complete ADH resistance. In some mutations the AVP protein is produced but trapped in the endoplasmic reticulum without being transferred to the cell membrane (14). In the presented case, the change of Leu211 to Pro211 is predicted to impair the interaction of AVPR2 and ADH by changing

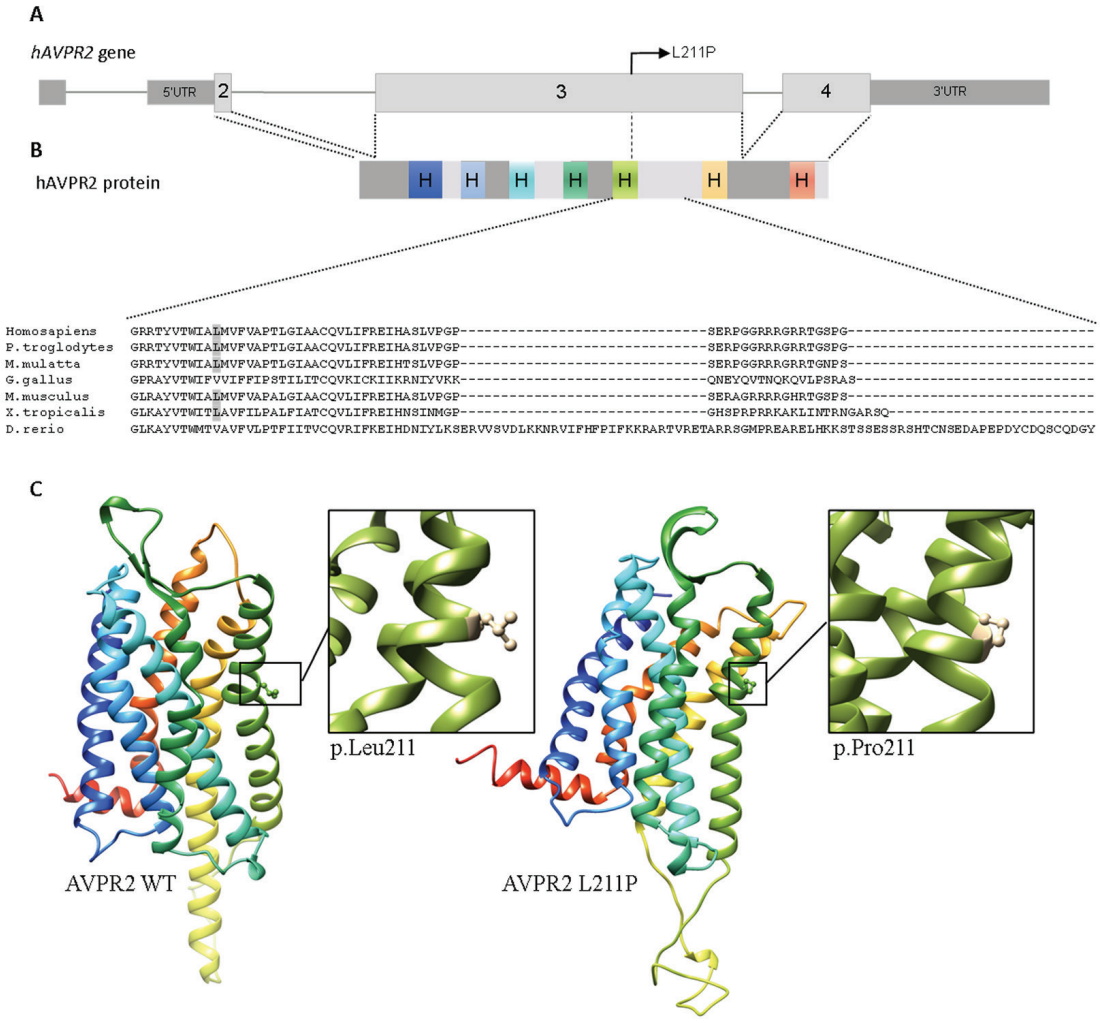

Figure 2. Molecular characteristics of wild type and mutant *AVPR2* gene and AVPR2 protein. A) Diagram of h*AVPR2* gene (NM_000054.6): Arrow shows novel missense variant (L211P) identified in the patient and his mother. B) Structure of AVPR2: Dark grey and light grey indicates extracellular and cytoplasmic components of AVPR2, respectively. H: Transmembrane helical components of AVPR2; painted with corresponding colors of the helixes in three-dimensional structure of protein. Partial alignment of AVPR2 protein sequences, generated by Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/), showing conservation of leucine (Leu; L) at position 211, highlighted in grey. C) Three-dimensional protein structures for wild-type and mutant proteins were obtained with Swiss-Model and UCSF Chimera 1.10.2 servers, and rainbow-painted from dark blue for N-terminal to red for C-terminal. The Leu211 and Pro211 residues are presented in a magnified frame for viewing at a higher quality and indicated in yellow

the three-dimensional structure of the AVPR2 protein. This may have caused an initial response to DDAVP but later the patient became unresponsive, which can be explained by residual mutant ADH receptor activity responsible for the partial ADH response. Severe ADH resistance may also cause over-expression of AVPR2 on the membrane surface of the principal cells of the renal collecting ducts, which may also lead to a partial ADH response.

The medical therapy for NDI includes the use of diuretics and non-steroidal anti-inflammatory drugs (NSAIDs). In volume depletion states, thiazide diuretics reduce urine output by blocking the sodium-chloride co-transporter in the distal convoluted tubule and thus increase the reabsorption of sodium and water in the proximal tubule (1,10). Hydrochlorothiazide at 2 to 4 mg/kg/day in twice-daily doses is the initial treatment for NDI. It can decrease urine output by as much as 50% (1). The loss of potassium, which is induced by thiazide diuretics, may require adding of potassium-sparing diuretics, such as amiloride, to the treatment. NSAIDs, such as ibuprofen and indomethacin, can be used in combination with diuretics in NDI. Prostaglandin inhibitors reduce urinary output with a mechanism independent of vasopressin,

and renal function must be closely monitored in patients using prostaglandin inhibitors (18). In patients who cannot tolerate indomethacin because of gastric side effects, selective inhibitors of cyclooxygenase-2 might be helpful.

More recently, AVPR2 receptor antagonists and agonists, vasopressin analogues, prostaglandin receptor agonists, secretin receptor agonists and cGMP phosphodiesterase inhibitors have been found beneficial in model organisms, which activate secondary intracellular messengers through alternative pathways (1,19).

In infants, early recognition of NDI and treatment is very important as the proper treatment can avert the physical and mental retardation that results from repeated episodes of dehydration and hypernatremia. The presented patient is still under follow-up at pediatric endocrinology and nephrology. At the last examination, he was 4 years and 10 months old. His weight and height were 17.5 kg [0.04 standard deviation score (SDS)] and 103 cm (-0.73 SDS), respectively. Neuromotor development was normal.

Conclusion

In conclusion, hypernatremic dehydration with hypotonic polyuria in a newborn should evoke the suspicion of DI. Characteristics suggesting antenatal onset and X-linked inheritance are important clinical clues for the diagnosis of congenital NDI. However initial or partial DDAVP response may complicate the diagnostic process of NDI, as in our case who was found to harbor a novel missense (c.632T>C, p.L211P) *AVPR2* mutation. Early recognition and appropriate management of NDI may prevent potentially life-threatening hypernatremic dehydration in young infants.

Ethics

Informed Consent: The patient's parents provided informed consent for publication of this case report.

Footnotes

Authorship Contributions

Concept: Ömer Güran, Design: Ömer Güran, Data Collection or Processing: Ömer Güran, Serçin Güven, Heves Kırmızıbekmez, Özlem Akgün Doğan, Leyla Karadeniz Bilgin, Literature Search: Ömer Güran, Serçin Güven, Heves Kırmızıbekmez, Özlem Akgün Doğan, Leyla Karadeniz Bilgin, Writing: Ömer Güran, Leyla Karadeniz Bilgin.

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CASE REPORT

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A Rare Cause of Hypergonadotropic Hypogonadism: Transaldolase Deficiency in Two Siblings

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What is already known on this topic?

Transaldolase deficiency is a multisystemic disease that is characterized by intrauterine growth restriction, dysmorphism, cytopenia, hepatosplenomegaly, liver cirrhosis, endocrine problems, and skin, renal and cardiac abnormalities. Several endocrine system problems, such as abnormal external genitalia, primary hypothyroidism, short stature, bone abnormalities and hypergonadotropic hypogonadism may occur in transaldolase deficiency.

What this study adds?

Gonadal dysfunction with hypergonadotropic hypogonadism may occur in both girls and boys with transaldolase deficiency. Hypergonadotropic hypogonadism may become hormonally apparent in adolescence in girls with transaldolase deficiency although puberty starts on time. Transaldolase deficiency should be included in the differential diagnosis of cryptogenic cirrhosis and multisystemic involvement, especially if concomitant hypergonadotropic hypogonadism is present. Patients with transaldolase deficiency should be evaluated for gonadal functions, especially during puberty.

Abstract

Transaldolase deficiency is a rare inborn autosomal recessive disorder caused by biallelic mutations in the *TALDO1* gene. It is characterized by intrauterine growth restriction, dysmorphism, cytopenia, hepatosplenomegaly, liver cirrhosis, endocrine problems, and skin, renal and cardiac abnormalities. We present two siblings of Turkish origin with an early-onset form of transaldolase deficiency and hypergonadotropic hypogonadism in both sexes. The girl (index) was followed-up for cryptogenic cirrhosis, leukopenia and thrombocytopenia, skin abnormalities, congenital heart defects, hypercalciuria, nephrolithiasis, proteinuria, and chronic kidney disease throughout childhood. She developed hypergonadotropic hypogonadism in adolescence. Whole exome sequencing due to the multisystemic involvement revealed a previously described homozygous, inframe deletion in *TALDO1*. Her brother was born small for gestational age and was also followed-up with cryptogenic cirrhosis from infancy, together with cytopenia, congenital heart defects, bilateral cryptorchidism, short stature, hypercalciuria, proteinuria and chronic kidney disease in childhood. He presented with testicular microlithiasis and hypergonadotropic hypogonadism in adolescence. Sanger sequencing of *TALDO1* confirmed the presence of the same homozygous deletion as his sister. The mother was found to be a heterozygous carrier for this deletion. We describe two patients with multisystemic involvement since the neonatal period who presented with additional hypergonadotropic hypogonadism in adolescence. The diagnosis of transaldolase deficiency should be kept in mind for these patients, and they must be evaluated for gonadal functions, especially during puberty.

Keywords: Transaldolase deficiency, hypergonadotropic hypogonadism, whole exome sequencing, TALDO1

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Introduction

Transaldolase is a key enzyme in the pentose phosphate pathway (PPP), an alternative route for glucose oxidation. Glucose metabolism through PPP has two important functions: formation of ribose 5-phosphate for the synthesis of essential biomolecules, such as adenosine triphosphate (ATP), RNA, and DNA; and formation of NADPH for biosynthetic reactions and neutralization of reactive oxygen intermediates. In the absence of this enzyme, some intermediate products, such as polyols and seven-carbon sugars, accumulate in body fluids, mostly in the urine (1). Transaldolase deficiency was first described in 2001 in a Turkish girl with prominent liver involvement during early infancy (2). It is a rare, inborn, autosomal recessive disorder caused by biallelic mutations in the *TALDO1* gene and characterized by intrauterine growth restriction, dysmorphism, abnormal skin (telangiectasia, dryness, thinness), cytopenia, hepatosplenomegaly, liver cirrhosis, endocrine problems, and renal and cardiac abnormalities (3). Patients may exhibit either an early-onset presentation (prenatally or before one month of age) with severe symptoms during the neonatal period or a relatively milder late-onset presentation.

It has been reported that evaluation of the endocrine system in patients with transaldolase deficiency may show abnormal external genitalia, primary hypothyroidism, short stature, bone abnormalities and/or gonadal dysfunction with hypergonadotropic hypogonadism (3). Here, we present two siblings of Turkish origin with the early-onset form of transaldolase deficiency and hypergonadotropic hypogonadism with an overview of multisystemic manifestations throughout childhood in both sexes. Molecular diagnosis was established with whole exome sequencing (WES) in the index due to the multisystemic involvement, just before transition.

Case Reports

Patient 1

A $7^{6/12}$ year-old girl (index) was referred to the pediatric endocrinology clinic due to development of pubic hair. She was born after an uneventful pregnancy at term, with a birth weight of 2800 g [-1.44 standard deviation score (SDS)] and birth length of 49 cm (-0.51 SDS). She was the second child of healthy, consanguineous parents of Turkish origin. There was no family history of infertility. Soon after the newborn period, she underwent diagnostic work-up due to splenomegaly, elevated transaminase levels, direct hyperbilirubinemia, and prolonged coagulation tests. She was diagnosed with chronic liver disease of unknown cause. At the age of $2^{9/12}$ years, the diagnosis of cryptogenic liver disease was established after liver biopsy and etiological investigations. She was followed up for portal hypertension and managed conservatively for gastrointestinal bleeding. Bone marrow aspiration for evaluation of possible storage diseases showed hypercellular and heterogenous bone marrow associated with hypersplenism, but no evidence of storage cells. She was also regularly followed-up due to secundum atrial septal defect (ASD), mitral valve prolapse, mild dilatation of the aortic root, and aortic regurgitation since infancy. She had primary nocturnal enuresis, history of a kidney stone due to hypercalciuria, and proteinuria. Furthermore, she was under medication due to attention-deficit/hyperactivity disorder. Endocrine pancreas functions were normal, with blood glucose, insulin and HbA1c in normal ranges. Clinical manifestations are given in Table 1.

On physical examination at referral she had a body weight of 19.8 kg (-1.34 SDS), height of 122.6 cm (-0.32 SDS) [target height 160 cm (-0.53 SDS)], a small triangular face, microretrognathia, flat nasal bridge, long eyelashes, high palate, diffuse telangiectasias, thin and dry skin, hemangiomas, and a 3 cm palpable splenomegaly. Neurodevelopmental milestones and systemic examination were otherwise normal. She had Tanner stage 2 pubic hair but no breast development or clitoromegaly. Pubertal examination findings, and pubertal hormones at admission and subsequently are given in Table 2. Laboratory investigation for premature adrenarche found normal levels of dehydroepiandrostenedione sulphate and total testosterone. Slightly elevated 17-hydroxyprogesterone (1.29 ng/mL) and 1.4-delta androstenedione (1.5 ng/mL) levels prompted a 250 µg adrenocorticotropic hormone stimulation test which resulted in normal cortisol response and adrenal precursor concentrations. Thyroid hormone levels were within normal limits. Bone age was consistent with $8^{10/12}$ years, and slightly advanced.

During follow-up, breast development started at $9^{5/12}$ years and pubertal development proceeded with menarche at $12^{7/12}$ years. Inappropriately increased follicle stimulating hormone (FSH) levels for her pubertal stage were consistent with a hypergonadotropic state with menarche. Subsequently, primary ovarian insufficiency (POI) became clinically apparent, with secondary amenorrhea and hormonally apparent because of low anti-Mullerian hormone levels (<0.08 ng/mL). Pelvic ultrasound imaging revealed a pubertal uterine volume of 8 mL (13x23x53 mm) and ovarian volumes of 2.4/1.7 mL. Diagnostic work-up for the etiology of POI resulted in a normal 46,XX karyotype, negative genetic testing for the fragile X mental retardation

Table 1. Clinical manifestations of two siblings throughout childhood

Clinical features	Patient 1 (index)	Patient 2
Gender	Female	Male
Molecular diagnosis	Homozygous p.Ser171del c.512_514delCCT	Homozygous p.Ser171del c.512_514delCCT
Birth weight	2800 g (-1.44 SDS)	2500 g (-2.94 SDS)
Dysmorphism	+	+
Skin abnormalities	Telangiectasia, hemangiomas	Telangiectasia
Hepatological problems	Splenomegaly, cryptogenic cirrhosis, portal hypertension	Splenomegaly, cryptogenic cirrhosis, portal hypertension
Liver transplantation	+	-
Impaired coagulation tests	+	+
Cytopenia	Leukopenia and thrombocytopenia	Leukopenia and thrombocytopenia
Urinary system problems	Primary nocturnal enuresis, hypercalciuria, nephrolithiasis, proteinuria, chronic kidney disease	Primary nocturnal enuresis, hypercalciuria, proteinuria, membranous glomerulopathy, chronic kidney disease
Cardiac problems	Secundum ASD, MVP, aortic regurgitation	Secundum ASD (surgically corrected)
Endocrine system problems	Hypergonadotropic hypogonadism	Bilateral cryptorchidism (orchiopexy), short stature, hypergonadotropic hypogonadism
Mental problems	ADHD	None
At transition	Adult height: 165.7 cm Regular menses with HRT	Adult height: 165 cm On testosterone treatment

ASD: atrial septal defect, MVP: mitral valve prolapse, ADHD: attention-deficit/hyperactivity disorder, HRT: hormone replacement therapy, SDS: standard deviation score

Table 2. Clinical and hormonal profile of the cases at admission and pubertal milestones

	Tanner stage	FSH (mIU/mL)	LH (mIU/mL)	Estradiol (pg/mL)	Total testosterone (ng/mL)
Patient 1					
Admission ($7^{6/12}$ years)	P2, T1/1	0.6	0.1	18.5	-
Pubertal onset ($9^{5/12}$ years)	P2, T1/2	7.1	1.6	21	-
Menarche ($12^{7/12}$ years)	P4, T4/4	18	11.1	29.6	-
Secondary amenorrhea ($13^{10/12}$ years)	P5, T5/5	56.2	33.5	15.6	-
Patient 2					
Admission (9 years)	P1, bilateral NP testis	3.4	0.23	-	< 0.01
At the time of pubertal delay (14 years)	P2, TV 0.5/0.5 mL	128	88.8	-	0.28

FSH: follicle stimulating hormone, LH: luteinizing hormone, P: pubic hair, T: thelarche, NP: non-palpable, TV: testicular volume

1 premutation (29/32 CCG repeats), negative 21-hydroxylase antibody testing, and negative screening for reducing substances in urine. There had been no sign of autoimmune or adrenal disease, nor history of a cytotoxic treatment to the ovaries. She was evaluated for the consequences of estrogen deficiency. Dual energy X-ray absorptiometry scan revealed a low L1-4 bone mineral density of 0.782 g/cm^2 (Z score -2.5). Since there had been no history of any bone fracture, she was put on calcium and vitamin D prophylaxis. Transdermal estrogen and an oral progesterone regimen was preferred as hormone replacement therapy because of her well-established chronic liver disease.

At the age of 15 years, she was diagnosed with chronic kidney disease. At the age of 17.5 years, a hypoechoic nodular mass on cirrhotic liver was detected on ultrasonography and magnetic resonance imaging (MRI). Due to suspicion of hepatocellular carcinoma, she underwent living related liver transplantation from her father. The histopathological assessment of the explanted liver did not confirm the diagnosis of hepatocellular carcinoma. The features of nodular cirrhosis, focal dysplastic changes, and macro-microvesicular steatosis were reported after histopathological examination. Owing to the multisystemic involvement, chromosomal microarray and WES was perfomed. Xgen® Exome Research Panel v1.0 (Integrated DNA Technologies, USA) in Novaseq Platform (Illumina, USA) for exome sequencing and Cytoscan 750K Array kit in Affymetrix Platform (Thermo-Fisher Scientific, USA) for

microarray analysis were used. A previously described, homozygous, inframe deletion in exon 5 of *TALDO1* was detected (NM_006755.2; c.512-514delCCT; p.Ser171del). This mutation was previously reported in a Turkish girl (2).

Patient 2

Patient 2, the older brother of Patient 1, was referred to the pediatric endocrinology clinic due to bilateral cryptorchidism at the age of 9 years. He was born after an uneventful pregnancy at term, with a birth weight of 2,500 g (-2.94 SDS), and thus was classified as small for gestational age. He was also investigated for chronic liver disease since the newborn period, and the diagnosis of cryptogenic cirrhosis was established at the age of $4^{9/12}$ years. He had portal hypertension, esophageal varices, leukopenia, and thrombocytopenia. He was operated for secundum ASD. He had primary nocturnal enuresis, hypercalciuria, and proteinuria. Clinical manifestations of both siblings are given in Table 1.

Physical examination at referral revealed a body weight of 20.8 kg (-2.23 SDS), height of 122 cm (-1.83 SDS) [target height 173 cm (-0.52 SDS)], sitting height/height ratio 0.54, small triangular face, micrognathia, diffuse telangiectasia, thin and dry skin, 3/6 systolic murmur, and a 4 cm palpable splenomegaly. Neurodevelopmental milestones were normal. His pubertal development was consistent with Tanner stage 1, both testes were non-palpable, and stretched penile length was 4 cm (normal penile length for age: >4.72 cm). FSH, luteinizing hormone (LH) and testosterone concentrations were within prepubertal ranges. Testicular ultrasonography revealed bilateral small testes (0.5/0.5 mL) in the proximal inguinal canal. Human chorionic gonadotrophin (hCG) stimulation test (intramuscular hCG 1,500 IU/day, for 3 days) revealed no testosterone response. He underwent bilateral orchiopexy due to bilateral undescended testes soon after admission. Pubertal examination findings, and pubertal hormones at admission and subsequently for both siblings are given in Table 2.

During follow-up, he was evaluated for short stature and low annual growth rate, when he was 13 years old. Thyroid hormone levels were within normal limits. Insulin-like growth factor-1 (IGF-1) was 8.08 ng/mL (-2.3 SDS), IGF binding protein-3 (IGFBP-3) was 1790 ng/mL (-2.1 SDS), and bone age was 11 years. Growth hormone (GH) stimulation tests revealed GH deficiency with peak GH levels of 4.96 ng/mL and 5.37 ng/mL. MRI scan of his hypophysis was normal. During the investigations for delayed puberty at the age of 14 years, hypergonadotropic hypogonadism was detected. Inhibin-B level was 13 ng/L (100-444 ng/L). Testicular ultrasonography revealed atrophic testes with testicular microlithiasis. Monthly testosterone replacement was started. A normal 46,XY karyotype excluded chromosomal anomaly.

At the age of 13 years, he was diagnosed with membranous glomerulopathy and, after adolescence, with chronic kidney disease. He did not have hepatic decompensation throughout the childhood period and liver transplantation was not needed. Sanger sequencing of *TALDO1* confirmed the presence of the same homozygous deletion as his sister. The mother was found to be a heterozygous carrier for this deletion. She had not exhibited any evidence of relevant manifestations of the disease and was still having normal menses at the age of 48. Genetic analysis could not be performed for the father.

Discussion

In this report two patients with multisystemic involvement since the neonatal period are described, who presented with additional hypergonadotropic hypogonadism in adolescence. After years of diagnostic work-up, a homozygous *TALDO1* gene mutation causing transaldolase deficiency was detected with WES. The clinical diagnosis was considered as an early-onset form of transaldolase deficiency. Both patients had displayed normal prepubertal concentrations of gonadotropins before puberty. With the onset of puberty, hormonal status became hypergonadotropic in a few years.

Hypergonadotropic hypogonadism was reported in 18% of transaldolase deficient patients in a study performed by a retrospective questionnaire and literature review of 34 patients from 25 families (3). All these six reported patients also had early-onset phenotype. Recently, a boy with a late-onset presentation of transaldolase deficiency was reported with the prominent clinical finding of hypergonadotropic hypogonadism for the first time (4). Several hypotheses have been proposed to explain the mechanism of gonadal dysfunction in patients with transaldolase deficiency. As cirrhosis has been suggested to result from increased cell death of hepatocytes, gonadal insufficiency was thought to occur due to cell damage. Enzyme-activity and metabolic studies of transaldolase deficient lymphoblasts had revealed coordinated changes in mitochondrial homoeostasis, oxidative stress, and Ca^{2+} fluxing (5). Shortage of NADPH and antioxidant glutathione lead to decreased mitochondrial transmembrane potential and reduced ATP/ADP ratio in the liver of mice lacking transaldolase (*TALDO1 -/-*) (6). Increased levels of reactive oxygen intermediates and depleted neutralization, together with toxic accumulation of C5 polyols and seven-carbon sugars may lead to apoptosis,

and direct damage to gonadal cells in these patients (1). On the other hand, decrease in the ratio of NADPH/NADP may cause abnormal gonadal steroid hormone biosynthesis (5). *TALDO1* is significantly expressed in almost all tissues in the body. It has relatively higher expression in bone marrow and the gastrointestinal tract, while it is also expressed to some extent in the ovary and testis (7). Therefore, oxidative stress due to dysfunction of PPP to metabolize glucose could account not only for defects in liver tissue or bone marrow but also for gonadal damage. Patients should also be assessed carefully in terms of other system dysfunctions during follow-up.

The phenomenon of accumulation of metabolites, such as polyol and a sugar phosphate due to an enzyme deficiency in a pathway is also seen in galactosemia due to galactose-1-phosphate uridyltransferase deficiency (8). Ovarian failure in galactosemia is suggested to be due to direct toxic effect of galactose or its metabolites on ovarian parenchyma. Gonadal dysfunction is acquired and varies in severity with the age of the patient at onset (9). However, clinically significant gonadal dysfunction is not reported in boys, except for cryptorchidism. There has been evidence for both mild Sertoli and Leydig cell dysfunction in the testes, but these would have little impact on fertility (10). In contrast, gonadal dysfunction and hypergonadotropic hypogonadism has been reported in both sexes with transaldolase deficiency (3). Fertility of spermatozoa depends on the maintenance of the mitochondrial transmembrane potential and is regulated by an oxidation-reduction equilibrium of reactive oxygen intermediates. In a murine study, *TALDO1 -/-* male mice exhibited defective forward motility of spermatozoa, thus associating transaldolase deficiency with sperm dysmotility and potential male infertility (11). In Patient 2, low inhibin B concentrations suggested Sertoli cell dysfunction and potential subsequent infertility. However, the pathogenesis of gonadal dysfunction in males has not yet been entirely elucidated.

In this report, the girl (Patient 1) with transaldolase deficiency had spontaneous pubertal onset within the expected time frame with gonadotropins in the normal range. She had normal pubertal development but hypergonadotropic hypogonadism became hormonally apparent at the time of menarche, and several years prior to her liver transplantation. In contrast, puberty of the boy (Patient 2) was delayed, and no testicular enlargement was observed, as in the previously reported boy with late-onset presentation (4). This might be either due to the primary cellular damage in testis or damage to gonadal tissue due to late orchiopexy. Furthermore, coexistence of these two conditions may have exacerbated the clinical presentation. The onset and timing of the damage causing gonadal dysfunction in transaldolase deficiency in both sexes remains unclear, as it is for ovarian dysfunction in galactosemia (12). Further studies are needed to understand if transaldolase expression in ovary and testis differ, and if gonadal cells are affected in a different manner from the increased levels of reactive oxygen intermediates and oxidative stress.

Short stature was described in some patients with transaldolase deficiency, with a concomitant IGF-1 deficiency in a few (3). It has been suggested that IGF-1 deficiency may be due to delayed puberty, malnutrition, or liver disease. The presence of chronic systemic diseases in transaldolase deficiency could further contribute to poor growth. Short stature and IGF-1 deficiency observed in Patient 2 was possibly due to the combination of all these mechanisms. GH therapy has not been considered due to underlying chronic diseases of unknown etiology in young patients.

The mutation in the presented patients was same as in the first reported Turkish girl with transaldolase deficiency (2). This girl was evaluated for an enlarged clitoris, but dehydroepiandrosterone, androstenedione, and testosterone in serum were normal. Our index case was also evaluated for signs of hyperandrogenism, but the final clinical diagnosis was premature adrenarche with slightly elevated adrenal precursors. The condition may exhibit variable expressivity or intrafamilial phenotypic variability, as previously reported (13). Follow-up of these patients is extremely important since defects in a range of organ systems may appear at various times due to ongoing oxidative stress.

Conclusion

In conclusion, patients with cryptogenic cirrhosis and multisystemic involvement should be evaluated for gonadal function, especially during puberty. Transaldolase deficiency may be included in the differential diagnosis of these patients, especially if concomitant hypergonadotropic hypogonadism is present. In patients with transaldolase deficiency, puberty may begin spontaneously but managing clinicians should be aware of the possibility of developing hypergonadotropic hypogonadism during follow-up. Cryptorchidism may be an alarming symptom. Early diagnosis of these patients may present an opportunity for tissue cryopreservation to preserve fertility in the long term. The presented cases only had definitive diagnosis of transaldolase deficiency with WES after years of follow-up with multisystemic involvement throughout childhood. Testing for pathogenic variants in *TALDO1* gene may be considered earlier in these patients.

Ethics

Informed Consent: Written informed consent was obtained from the parents for publication of this case report.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: Melek Yıldız, Zerrin Önal, Tuğçe Göksu Kabil, Güven Toksoy, Şükran Poyrazoğlu, Özlem Durmaz, Concept: Melek Yıldız, Tuğçe Göksu Kabil, Güven Toksoy, Şükran Poyrazoğlu, Özlem Durmaz, Design: Melek Yıldız, Tuğçe Göksu Kabil, Şükran Poyrazoğlu, Data Collection or Processing: Melek Yıldız, Gözde Yeşil, Firdevs Baş, Özlem Durmaz, Feyza Darendeliler, Literature Search: Melek Yıldız, Zerrin Önal, Gözde Yeşil, Tuğçe Göksu Kabil, Güven Toksoy, Şükran Poyrazoğlu, Firdevs Baş, Feyza Darendeliler, Writing: Melek Yıldız, Zerrin Önal, Gözde Yeşil.

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CASE REPORT

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The First Case of 4H Syndrome with Type 1 Diabetes Mellitus

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What is already known on this topic?

4H syndrome is a rare, autosomal recessive disorder characterized by hypomyelination, hypodontia, and hypogonadotropic hypogonadism. Biallelic pathogenic variants in *POLR3A, POLR3B, POLR1C*, and *POLR3K* gene cause 4H syndrome. There is no obvious genotype/phenotype correlation. In addition to the three classic features, patients may present with other system involvements.

What this study adds?

We report two siblings with bi-allelic pathogenic variants of the *POLR3A* gene. This is the first case of 4H syndrome accompanied by type 1 diabetes mellitus, but in only one of the siblings, in the literature. It is not exactly known whether this is coincidental or an expansion of the phenotype.

Abstract

4H syndrome is a rare, progressive, hypomyelinating leukodystrophy. Hypomyelination, hypodontia, and hypogonadotropic hypogonadism are the three classic features of 4H syndrome. Biallelic pathogenic variants in *POLR3A*, *POLR3B*, *POLR1C*, and *POLR3K* gene cause 4H leukodystrophy. Herein, we present clinical features in two siblings with 4H syndrome. The first patient (16 years) presented with hypogonadotropic hypogonadism, euthyroid Hashimoto's thyroiditis and type 1 diabetes mellitus (DM). The second patient (13.5 years) showed normal physical, biochemical and hormonal examination at presentation. The second patient was followed up for epilepsy between the ages of 6 months and 6 years, when his epilepsy medication was discontinued, and he did not have seizure again. T2-weighted magnetic resonance images showed increased signal intensity secondary to hypomyelination in both. They were subsequently found to have a homozygous variant in the *POLR3A* gene. 4H syndrome may present with neurological and non-neurological findings in addition to classic features of 4H syndrome. Progressive neurological deterioration may occur and endocrine dysfunction may be progressive. Although multiple endocrine abnormalities associated with this disorder have been reported to date, a case accompanied by type 1 DM has not previously been published. We do not know if this was a coincidence or an expansion of the phenotype. However, reporting such cases helps to determine the appropriate genotype-phenotype correlation in patients.

Keywords: 4H leukodystrophy, *POLR3A*, hypogonadotropic hypogonadism, type 1 diabetes mellitus

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Introduction

Leukodystrophies constitute a heterogeneous, rare, inherited group of diseases that mainly affect the white matter of the central nervous system (1). The clinical signs of the condition are generally nonspecific and may occur at different ages, from the neonatal period to late adulthood (2). Patients may present with non-neurological findings as well as neurological findings. Non-neurological symptoms have been used to categorize leukodystrophies more accurately (3). Ophthalmological, dental, musculoskeletal, gastrointestinal, and skin problems have also been reported, in addition to endocrine problems, such as adrenal insufficiency, hypogonadism, hypothyroidism, growth hormone deficiency, and ovarian insufficiency (2).

4H syndrome inherited in an autosomal recessive manner is a rare, progressive, hypomyelinating leukodystrophy. It was first described in 2006 by Timmons et al. (4) and is characterized by hypomyelination, hypodontia, and hypogonadotropic hypogonadism. Its clinical course is highly variable. In addition to cases with severe neurological signs, some cases presenting with only idiopathic hypogonadotropic hypogonadism in late adolescence have been reported (5,6). While the most common endocrine abnormalities in 4H syndrome have been reported as hypogonadotropic hypogonadism, we present two siblings, both of whom had 4H syndrome and one of whom had diabetes mellitus (DM).

Case Reports

Case 1

A 16-year-old Turkish female (II-1) was referred to our hospital with the complaint of secondary amenorrhea. After menstrual bleeding twice with an interval of 1 month at the age of 13.5 years, there had been no subsequent menstrual bleeding. She was born at term, with a birth weight of 4,750 g, from a first-degree consanguineous marriage. Her neuromotor development was consistent with her peers. She started to walk at the age of 10 months, walked without support by 12 months of age, started speaking single meaningful words by 12 months of age and spoke in short sentences by 2 years of age. After the age of 12, she could not continue school due to the gradual decrease in her academic success and the increase in forgetfulness.

When she attended endocrinology, her body weight was 50.7 kg [-0.88 standard deviation score (SDS)], height was 161.1 cm (-0.22 SDS), and body mass index was 19.5 kg/m² (-0.8 SDS). Other systemic and detailed neurological examinations of the patient with Tanner stage 5 were normal. The laboratory examination results were found to be compatible with impaired fasting glucose, impaired glucose tolerance, euthyroid Hashimoto's thyroiditis, and hypogonadotropic hypogonodism (Table 1).

Table 1. The laboratory findings of siblings on admission

	Case 1	Case 2	Reference ranges
Fasting plasma glucose (mg/dL)	104	73	< 100
2. hour glucose during an OGTT (mg/dL)	142	NA	< 140
Fasting insulin (mU/L)	14.4	6.7	3-25
2. hour insulin (mU/L)	36.6	NA	22-79
C peptide (µg/L)	1.1	1.1	0.8-3.8
HbA1c (%)	5.6	5.4	< 5.7
Anti-GAD (IU/mL)	61.2	5.7	< 17
ICA (U/mL)	54.9	3.8	< 28
FSH (U/L)	4.6	6.1	
LH (U/L)	0.5	2.8	
Estradiol (ng/L)	< 11.8		11.8-36.6
Testosterone (ug/L)		1.51	0.23-7.42
LHRH peak LH (U/L)	3.6	NA	
TSH (mU/L)	1.6	3.3	0.5-4.9
fT4 (ng/dL)	1.09	1.01	0.83-1.43
Anti-TG (IU/mL)	31.1	< 1.3	< 4.5
Anti-TPO (U/mL)	32	< 28	< 60
ACTH (pg/mL)	14	16	< 46
Cortisol (µg/dL)	10	11	5.2-22
Prolactin (µg/L)	2.3	4.8	4.3-23 (female) 3.2-13.5 (male)

OGTT: oral glucose tolerance test, Anti-GAD: glutamic acid decarboxylase antibody, ICA: islet cell antibody, TSH: thyroid stimulating hormone, HbA1c: hemoglobin A1c, Anti-TG: anti-thyroglobulin, Anti-TPO: anti-thyroid peroxidase, LH: luteinizing hormone, FSH: follicle-stimulating hormone, LHRH: luteinizing-hormone releasing hormone, ACTH: adrenocorticotropic hormone, NA: Not available

The patient, whose antibody levels (Table 1) for type 1 DM were found to be positive, was initially planned to be followed-up without insulin by adjusting her diet. On brain magnetic resonance imaging (MRI) pathological hyperintensity on T2-weighted images secondary to hypomyelination was seen in periventricular white matter and centrum semiovale (Figure 1). Mild atrophy of the cerebrum, cerebellum, and corpus callosum was also detected. Metabolic investigations involving very long-chain fatty acids, free carnitine, urinary organic acids, urinary and plasma amino acids, lactic and pyruvic acids, arylsulfatase A, b-galactocerebroside and total hexosaminidase were normal. Since our patient did not have findings, such as fatty and oily stools, diarrhea, gas, bloating, abdominal pain, or unexplained weight loss, no evaluation was made in terms of pancreatic exocrine functions. Her psychometric evaluation with the Wechsler Intelligence Scale showed that her IQ score was 70-79. There were no signs of hypo-oligodontia, or any other dental anomaly. Ophthalmic examination showed no abnormality. The possible diagnosis of 4H syndrome was considered due to the presence of hypogonadotropic hypogonadism and hypomyelination.

In genetic analyses, genomic DNA was extracted from the patient's peripheral blood lymphocytes (QIAGEN Inc., Hilden, Germany) by obtaining an informed consent form from the patient's parents. All 31 exons and exon-intron boundaries of the *POLR3A* (NM_007055.4) gene were analyzed with a Next Generation Sequencing system according to manufacturers' instructions (Myseq, Illumina Inc., San Diego, CA, USA). The homozygous c.2005C > G (p.R669G) (p.Arg669Gly) missense variant on exon 15 of the *POLR3A* gene was detected and evaluated as likely pathogenic according to the guidelines (7). The variant was not found in any healthy population (GnomAD) and *in silico* analyzing tools predicted pathogenicity (7). The variant was reported previously and registered as a disease-causing variant in the Human Genome Variation Database (CM141442). Segregation analyses of the variant were performed with QIAseq® FX DNA Library Kit (Qiagen, Hilden, Germany) in all of the family members and the results are shown in Figure 2.

In the follow-up after 3 months, her fasting plasma glucose level measured 400 mg/dL, while her insulin was 3 mU/L, C-peptide was 0.53 µg/L, and HbA1c was 10%. Therefore, intensive insulin therapy was started.

Case 2

The younger brother of the proband was evaluated at age 13.5 years (II-3). Body weight was measured as 57.4 kg (0.38 SDS), height was measured 167 cm (0.65 SDS). Between the ages of 6 months and 6 years, he was followed with the diagnosis of epilepsy in another hospital. MRI and electroencephalography (EEG) findings from that period could not be re-evaluated. Epilepsy treatment was completed at the age of 6 years and he did not have epileptic seizures afterward. We were able to obtain the MRI findings when he was 7 years old, as the oldest date. Increased signal intensity was also detected in the MRI at that time. However, since the diagnosis was unknown, further investigation was recommended in terms of metabolic disease or hypoxic ischemic encephalopathy. It was learned that his school success was bad and he had a problem of forgetfulness. IQ score was 68 by Wechsler Intelligence Scale. He was at Tanner stage 3-4 in terms of puberty progression.

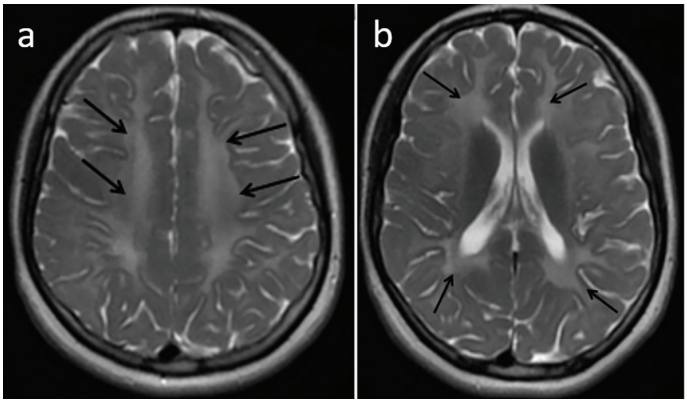

Figure 1. On axial T2-weighted MRI images, hyperintense areas (black arrows) secondary to hypomyelination are seen in bilateral centrum semiovale (a) and periventricular white matter (b)

MRI: magnetic resonance imaging

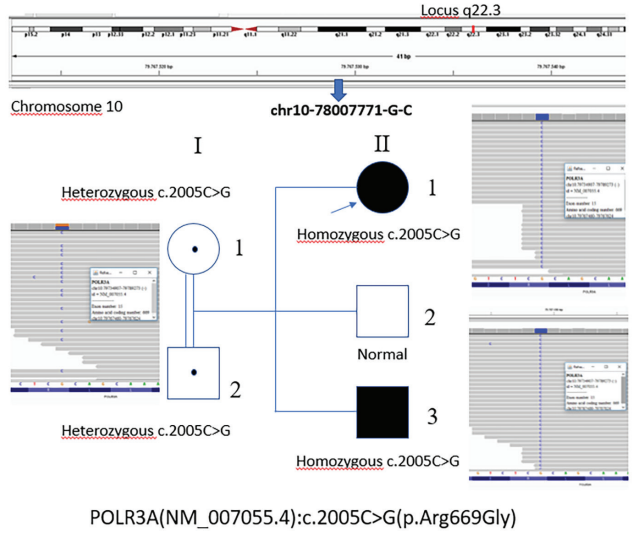

Figure 2. Schematic presentation of the genomic locus of the *POLR3A* gene on chromosome 10, and the results of segregation analysis of *POLR3A* gene c.2005C > G variant on the pedigree of the family

The patient's biochemical and hormonal examinations were evaluated as normal and are shown in Table 1. EEG monitoring was normal. T2-weighted images showed increased signal intensity secondary to hypomyelination in bilateral periventricular white matter (Figure 3). The same homozygous missense variant as in his sister was confirmed with genetic testing. The consanguineous parents of these siblings were found to be heterozygous carriers.

Discussion

RNA polymerase III (POLR3) related leukodystrophy, also known as 4H leukodystrophy, are both terms accepted for five overlapping clinical phenotypes described previously, which comprise 1) hypomyelination, hypodontia, hypogonadotropic hypogonadism (4H syndrome); 2) ataxia, delayed dentition, and hypomyelination; 3) tremor-ataxia with central hypomyelination; 4) leukodystrophy with oligodontia; and 5) hypomyelination with cerebellar atrophy and hypoplasia of the corpus callosum (8). Biallelic pathogenic variants in *POLR3A, POLR3B, POLR1C*, and *POLR3K* gene cause 4H leukodystrophy (9,10,11,12). Variants in these genes either disturb the proper assembly of the RNA POLR3 enzyme or impair its ability to bind to DNA (13,14). Disruption of this function is very important for the maintenance and development of myelin, which can affect the development and function of many parts of the body (14). However, the molecular basis of the pathophysiology of the disease is not fully understood. It remains a mystery how variants in *POLR3* lead to disorders with clinical features largely restricted to the central nervous system, and a few other tissues, all of which originate from neural crest cells (15). The variants are spread throughout the gene and there is no obvious genotype/phenotype correlation.

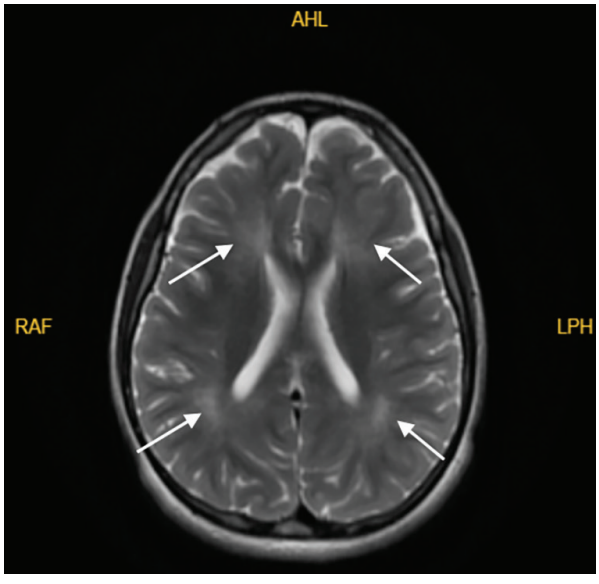

Figure 3. Axial T2-weighted MRI shows (white arrows) increased signal intensity secondary to hypomyelination in bilateral periventricular white matter

MRI: magnetic resonance imaging

Classical clinical findings with typical brain MRI features are helpful in making the diagnosis of 4H leukodystrophy. While hypomyelination, hypodontia, and hypogonadotropic hypogonadism are the three classic features, patients may also present with neurological findings, such as ataxia, dysarthria, dysmetria, tremor, and eye movement abnormalities, while non-neurological features include cataract, progressive myopia, dental abnormalities, and various endocrine abnormalities (6,12,16,17). Diagnostic MRI findings include cerebellar atrophy, progressive thinning of the corpus callosum, and high-intensity areas in the white matter on the T2-weighted images (18,19). The disease progresses insidiously and may result in early death.

In a study examining the endocrine problems of 150 patients with 4H leukodystrophy, delayed puberty and short stature were the most common endocrine problems. Most of the patients who underwent luteinizing hormone (LH) releasing hormone stimulation test had abnormally low levels of LH and follicle stimulating hormone (FSH). Moreover, immunohistochemical analysis of the anterior pituitary gland in the same study revealed that there was no immunostaining for anti-FSH and anti-LH antibodies. All of these findings implied that the hypogonodism was hypophyseal. A delay in puberty was detected more frequently in patients with *POLR3A* gene variant, which was followed by patients with *POLR3B* variants. Patients with 4H leukodystrophy have short stature compared to the general population. So that growth and height should be evaluated at least once a year. In 41% of patients, prolactin levels were found to be abnormal, either elevated (18%) or deficient (23%). Hypothyroidism was reported in only 4% of patients. No problems were detected in the cortisol axis (12).

The cases (II-1) and (II-3) have mild neurological manifestations. Patients are able to walk independently. They have no cerebellar, pyramidal and extrapyramidal signs. Cognition began to deteriorate slowly after 12 years old, but language comprehension and nonverbal communication are present at the time of writing. *POLR3A* variants tend towards a more severe disease course compared to *POLR3B* variant but the disease starts slightly later in patients harboring *POLR3A* variants in contrast to *POLR3B*-harboring patients (6). A 38-year-old Turkish male patient with *POLR3A* associated leukodystrophy was previously reported. His first neurological complaints started at the age of 25 years. Signs of endocrine dysfunction and dental

anomaly was not detected (20). The patients in the current report with *POLR3A* variant have exceptionally mild clinical courses. Dental abnormalities are not present. In addition to hypogonadotropic hypogonadism, hypoprolactinemia, type 1 DM and euthyroid Hashimoto's thyroiditis were detected in the sister. Other anterior pituitary hormones were normal. On questioning, no other family member suffered from type 1 DM and the autoimmune thyroid antibodies of the parents were negative. To date, more than 100 patients have been reported to have POLR3A or POLR3B variants in the literature. To the best of our knowledge, this is the first case of 4H syndrome due to *POLR3A* variant accompanied by type 1 DM in the literature. However, given the younger brother has exactly the same homozygous variant and has no sign of type 1 DM (yet), it is unclear if this was coincidental. In the literature, no relation was found between the *POLR3A* gene and pancreatic abnormalities.

Conclusion

In conclusion we are still far from understanding the pathogenesis of 4H leukodystrophy. It is important for radiologists, endocrinologists and neurologists to recognize the clinical and imaging characteristics of this disorder. One of the presented patients showed not only hypogonadotropic hyogonadism, but also some other endocrine disorders. Reporting such cases will contribute to the genotype-phenotype relationship of the disease.

Ethics

Informed Consent: Informed consent form was obtained from the patient's parents.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Gönül Büyükyılmaz, Büşra Erozan Çavdarlı, Keziban Toksoy Adıgüzel, Fatih Gürbüz, Concept: Gönül Büyükyılmaz, Büşra Erozan Çavdarlı, Mehmet Adıgüzel, Fatih Gürbüz, Esra Gürkaş, Design: Gönül Büyükyılmaz, Büşra Erozan Çavdarlı, Mehmet Adıgüzel, Fatih Gürbüz, Esra Gürkaş, Data Collection or Processing: Gönül Büyükyılmaz, Büşra Erozan Çavdarlı, Keziban Toksoy Adıgüzel, Mehmet Adıgüzel, Çiğdem Seher Kasapkara, Fatih Gürbüz, Analysis or Interpretation: Gönül Büyükyılmaz, Büşra Erozan Çavdarlı, Literature Search: Gönül Büyükyılmaz, Çiğdem Seher Kasapkara, Fatih Gürbüz, Writing: Gönül Büyükyılmaz, Büşra Erozan Çavdarlı, Keziban Toksoy Adıgüzel, Çiğdem Seher Kasapkara, Fatih Gürbüz, Esra Gürkaş.

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CASE REPORT

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Pituitary Stalk Interruption Syndrome – clinical Presentation and Management of a Potentially Life-threatening Disease in Newborns

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What is already known on this topic?

Pituitary stalk interruption syndrome (PSIS) rarely manifests immediately after birth. The first clinical signs are elusive but delayed diagnosis and treatment may lead to life-threatening complications.

What this study adds?

Hypoglycaemia, hyponatraemia, jaundice, cholestasis, sucking weakness and micropenis should be regarded as early leading symptoms of neonatal PSIS suggesting endocrine testing. If findings are suspicious, cerebral magnetic resonance imaging should be performed early during postprandial sleep. This is the first report to describe a persistent substitution-dependent thrombocytopenia together with a new variant in *GLI2* in PSIS.

Abstract

Pituitary stalk interruption syndrome (PSIS) is a rare congenital disease resulting in hypopituitarism of variable degree. Serious courses, due to severe combined pituitary insufficiency, are even rarer and associated with very early manifestation immediately after birth. The first clinical signs are elusive and lead to delayed diagnosis and treatment, often resulting in life-threatening complications. The objective was to highlight early leading symptoms and key issues of PSIS in neonates to increase awareness, improve clinical management and thereby enable an early diagnosis and treatment to prevent further complications. This report presents and compares the clinical course and management of two male neonates with PSIS. Early leading symptoms were the same in both patients, including recurrent hypoglycaemia, hyponatraemia, jaundice, cholestasis, sucking weakness and genital abnormalities. Patient 1 developed an infection-induced adrenal crisis, persistent substitution-dependent thrombocytopenia and convulsions due to severe hypoglycaemia because of delayed PSIS diagnosis. In patient 2, with recognition of the leading symptoms, endocrine testing and a subsequent cerebral magnetic resonance imaging were performed early and he was diagnosed and treated before major complications occurred. Genetic testing was performed in both patients. A heterozygous variant in *GLI2* [NM_005270.5:c.2537del; p.(Pro846Argfs*66)] was detected in patient 1. No potential PSIS-associated variant has been found in patient 2. In conclusion, the early diagnosis of neonatal PSIS is key to prompt treatment and prevention of potential severe clinical manifestation of this orphan disease. Therefore, increased awareness of early leading symptoms among clinicians caring for neonates will lead to improved care.

Keywords: Pituitary stalk interruption syndrome, hypopituitarism, neonatal manifestation, clinical management in newborns, neonatal cerebral magnetic resonance imaging, haematological abnormalities, *GLI2* mutation

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Introduction

Pituitary stalk interruption syndrome (PSIS) is a rare congenital disease characterised by a thin or absent pituitary stalk, associated with anterior pituitary hypoplasia/aplasia and an ectopic posterior pituitary. Hypoplasia of the pituitary is diagnosed by cerebral magnetic resonance imaging (cMRI) and, depending on the extent, is associated with variable timing of onset and degree of hypopituitarism (1). In most cases, PSIS results in a growth hormone insufficiency. Thus, persistent short stature in the course of child and adolescent development is the main presentation (2,3). Infants suffering from a combined pituitary insufficiency including compromised adrenocorticotropic and thyroid stimulating hormone secretion are usually more severely affected. Since early symptoms during the neonatal period are non-specific and current literature remains sparse, life-threatening complications may arise in delayed diagnosis (1,2). The following report focuses on the neonatal onset of PSIS in two cases, highlighting early leading symptoms and key issues in the clinical manifestation and management of neonatal onset PSIS. The objective is to increase awareness of this rare syndrome and to facilitate early diagnosis.

This report describes two, full-term, male infants with neonatal onset PSIS, treated at the Department of Paediatrics II, Neonatology, Medical University of Innsbruck. Clinical characteristics, biochemical analyses including endocrine hormone levels, and cMRI findings were obtained and are listed in Table 1. Selected cMRI scans are shown in Figure 1. In both patients, whole exome sequencing was performed. 150 bp paired end sequencing was performed using an Illumina HiSeq4000 platform (Illumina, Inc., San Diego, CA) after exon enrichment with the Agilent Sureselect V6 Exome kit (Agilent Technologies, Santa Clara, CA). Identified variants were filtered for autosomal recessive mode of inheritance and minor allele frequency of $<0.5\%$, X-chromosomal and autosomal dominant mode of inheritance with minor allele frequency of $<0.1\%$, and analysed in public databases (Database for Single Nucleotide Polymorphisms and Other Classes of Minor Genetic Variation, Exome Sequencing Project, and Exome Aggregation Consortium). OMIM-listed variants in disease-associated genes were taken into consideration. Findings are also shown in Table 1. Written informed consent for publication of the case reports including images was obtained from the caregivers.

Case Reports

Fetal ventriculomegaly and hexadactyly were conspicuous in patient 1. Birth took place with an emergency caesarean section under general anaesthesia after pathological cardiotocography. The infant presented as floppy, bradycardic and apnoeic. Despite initial sustained inflations, the apnoea persisted and the patient was ventilated for approximately 4 minutes, resulting in respiratory stability without breathing support. Initial blood glucose level was decreased (33 mg/dL) and returned to normal after a total glucose administration of 4.5 mg/kg/min on day 1. Partial parenteral nutrition was continued due to sucking weakness and vomiting after feeding. Physical examination confirmed hexadactyly with bilateral hypoplastic sixth finger and a postaxial polydactyly with a sixth toe on both sides. Furthermore, there was a cleft uvula, glandular hypospadias with presence of micropenis and microorchidism. Fetal ventriculomegaly regressed in time and postnatal cerebral sonography showed normal ventricular sizes. Blood testing revealed progressive leukocytopenia/neutropenia and thrombocytopenia. Recurrent electrolyte analysis showed persistency of mild hyponatraemia. On day 4, he developed jaundice requiring phototherapy for 24 hours. On the sixth day of life, he developed a *Klebsiella oxytoca* infection resulting in a systemic inflammatory response syndrome with fluid- and catecholamine-resistant septic shock, severe hypoglycaemia (21 mg/dL) and transient multiple organ failure. Cardiovascular function improved and blood pressure normalized after administration of hydrocortisone at a dose of 55 mg/m²/day. Empiric antibiotic therapy was started and adapted in accordance to the antibiogram. Blood and coagulation factors, including fresh frozen plasma, antithrombin III, platelet and erythrocyte concentrates, immunoglobulins and granulocyte colony stimulating factor were administered during the critical phase of sepsis. Whereas leukocytopenia/neutropenia recovered, a low thrombocyte count persisted and platelets had to be substituted regularly until the 25th day of life, as illustrated in Figure 2. Possible further underlying pathologies, including neonatal alloimmune thrombocytopenia, coagulation disorders and Wiskott-Aldrich syndrome, were excluded. As soon as the patient remained clinically stable, hydrocortisone was tapered and discontinued at day 11. Substitution of thyroid hormones was started due to decreased levels of free triiodothyronine, thyroxine and inadequately low thyroid stimulating hormone level (Table 1). At week five, the patient presented with convulsions due to severe hypoglycaemia of 18 mg/dL. Endocrine hormone testing revealed a combined pituitary insufficiency with secondary adrenal insufficiency. Hydrocortisone at a dose of 18.75 mg/m²/day was restarted and levothyroxine at a dose of 6 µg/kg/day was continued, whereupon the patient rapidly improved and was discharged a few days later. Genetic analysis revealed a heterozygous *GLI2* variant [GLI family zinc finger 2; variant: NM_005270.5:c.2537del;

Table 1. Clinical characteristics, biochemical analyses, cerebral magnetic resonance imaging findings and genetic data of both cases

		Patient 1	Patient 2
Clinical characteristics			
Gender		Male	Male
Birth weight, grams (percentile)		3,545 (29)	2,730 (24)
Gestational age, weeks		41.1	37.4
Mode of delivery		Caesarean section	Caesarean section
Indication for caesarean section		Pathological cardiotocography	Breech position
Apgar 1/5/10 minutes		5/8/10	5/7/8
Umbilical cord pH		7.26	7.28
Umbilical cord base excess, mmol/L		-0.6	-1.4
Respiratory distress		No	Yes
Hypoglycaemia/with convulsions		Yes/yes	Yes/no
Hyponatraemia		Yes	Yes
Jaundice/cholestasis		Yes/yes	Yes/yes
Hematologic risk factors for jaundice		No	No
Cytopenia		Thrombocytopenia, leukocytopenia/neutropenia	No
Sucking weakness		Yes	Yes
Addisonian crisis		Yes	No
Micropenis/microorchidism		Yes/yes	Yes/no
Associated malformations		Polydactyly, hypospadias, cleft uvula	No
Biochemical index	Normal		
Glucose, mg/dL (day of life)	45-180	33 (1)	19 (1)
Total bilirubin, mg/dL (day of life)	0-1	15 (5)	13.19 (5)
Direct bilirubin, mg/dL (day of life)	0-2.1	-	2.85 (5)
Gamma-glutamyltransferase, U/L (day of life)	8-178	256 (5)	770 (5)
Serum-sodium, mmol/L (day of life)	134-144	130 (2-7)	131 (9-12)
Cortisol, µg/L (day of life)	48.2-195.0	< 1.1 (41)*	5.6 (12)
ACTH, ng/L (day of life)	10-48	< 7 (41)*	< 5 (16)
GH, µg/L (day of life)	0.09-6.29	0.11 (41)*	1.78 (3)
IGF1, µg/L (day of life)	18-179	26 (41)*	-
IGFBP3, mg/L (day of life)	1.4-4.2	0.6 (41)*	-
FSH, U/L (day of life)	0.1-1.4	< 0.1 (214)	< 0.1 (12)
LH, U/L (day of life)	0.8-4.2	< 0.1 (214)	< 0.1 (12)
TSH µU/mL (day of life)	0.7-18.1	3.7 (19)	2.58 (3)
FT3, pmol/L (day of life)	4.6-10.1	3.0 (19)	3.77 (3)
FT4, pmol/L (day of life)	8.5-30.5	6.7 (19)	13.1 (3)
Cerebral magnetic resonance imaging			
Pituitary stalk		Not visible	Not visible
Anterior pituitary		Hypoplastic (severe)	Hypoplastic (mild)
Posterior pituitary		Ectopic	Ectopic
Genetic data		GLI2 mutation	No suspicious mutation

*During hypoglycaemia.
ACTH: adrenocorticotropic hormone, FSH: follicle stimulating hormone, FT3: free triiodothyronine, FT4: free thyroxine, GH: growth hormone, IGF1: insulin-like growth factor 1, IGFBP3: insulin-like growth factor binding protein 3, LH: luteinizing hormone, TSH: thyroid stimulating hormone

p.(Pro846Argfs*66)] and the cMRI scan at the age of three years identified pituitary hypoplasia as seen in PSIS, presented in Figure 1A.

Patient 2 was conceived by *in vitro* fertilisation. Incipient preeclampsia and breech position at the end of pregnancy resulted in an induced birth by caesarean section. Due to respiratory distress, the patient received continuous positive airway pressure support for two hours. The first blood test one hour after birth showed hypoglycaemia of 19 mg/dL, serum glucose levels stabilised after a glucose administration of 4.5 mg/kg/min on day 1. Despite frequent feeding a gradual withdrawal of continuous parenteral substitution was not successful until the 4th day of life. Physical examination revealed isolated presence of a micropenis, without further clinical abnormalities. On day 3, he presented with jaundice and received phototherapy for 24 hours. Laboratory analysis after treatment detected elevated parameters of cholestasis, seen in Table 1, yet abdominal sonography showed no abnormalities of the biliary tract. From the ninth day onward, laboratory results showed mild hyponatremia. Hypoglycaemia did not reoccur after day 4. Nevertheless, presentation of the patient revealed continuous muscular hypotonia and sucking weakness. Endocrine hormone testing and the cMRI (Figure 1B) identified a PSIS with combined pituitary insufficiency, secondary adrenal insufficiency and an incipient secondary thyroid dysfunction. Administration of hydrocortisone at a dose of 14.5 mg/m²/day on day 13 resulted in good feeding and adequate weight gain. Serum sodium returned to normal and parameters of cholestasis decreased, so that the patient could be discharged on day 23. Blood analysis shortly before discharge showed a progressing thyroid dysfunction, which was treated by the administration of levothyroxine at a dose of 6.5 µg/kg/day. Genetic testing was performed, yet no mutation was found in clinical exomes.

Discussion

This report illustrates the clinical course of two patients with rare neonatal onset PSIS. Leading symptoms were similar with early hypoglycaemia, persistent mild hyponatraemia, unconjugated hyperbilirubinemia in absence of haematological risk factors, cholestasis and sucking weakness. Clinically evident genital abnormalities were indicative for potential gonadotroph pituitary insufficiency. There may be a male gender predominance of neonatal PSIS, as previously suggested (1), as our two patients also were male, yet it should be noted that the phenotype of neonatal male hypogonadism is more easily recognised. In patient 1, diagnosis was delayed and he developed an infection-triggered, life-threatening adrenal crisis, persistent substitution-dependent thrombocytopenia and convulsions due to recurring hypoglycaemia. With hormonal substitution, he recovered. Conversely, patient 2 was diagnosed and received hormone substitution on day 13, thus very early and before major complications arose. cMRI is vital for the diagnosis and anatomical dimension of PSIS (1), and this can be performed safely in neonates during postprandial sleep without the risk and burden of anaesthesia (4).

PSIS remains a rare disorder with an unknown prevalence (1). Manifestation during infancy is even rarer with only 15% of PSIS patients becoming symptomatic during

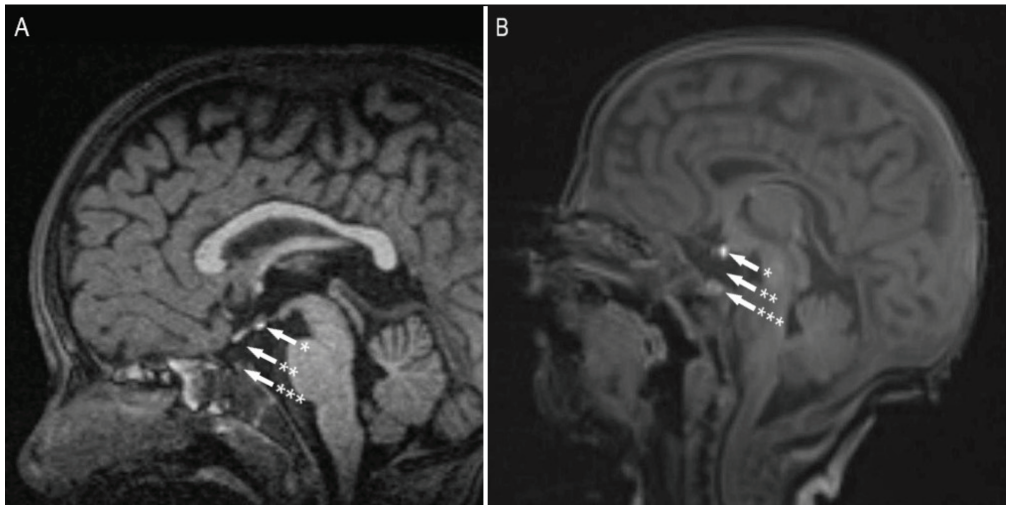

Figure 1. Cerebral magnetic resonance imaging (cMRI) of patient 1 (A) and patient 2 (B). Patient 1 was imaged at the age of 3 years and 3 months, in patient 2 MRI was performed in the 15th day of life. The sagittal T1-weighted image revealed an ectopic posterior pituitary (*), absence of pituitary stalk (**), and anterior pituitary hypoplasia (***)

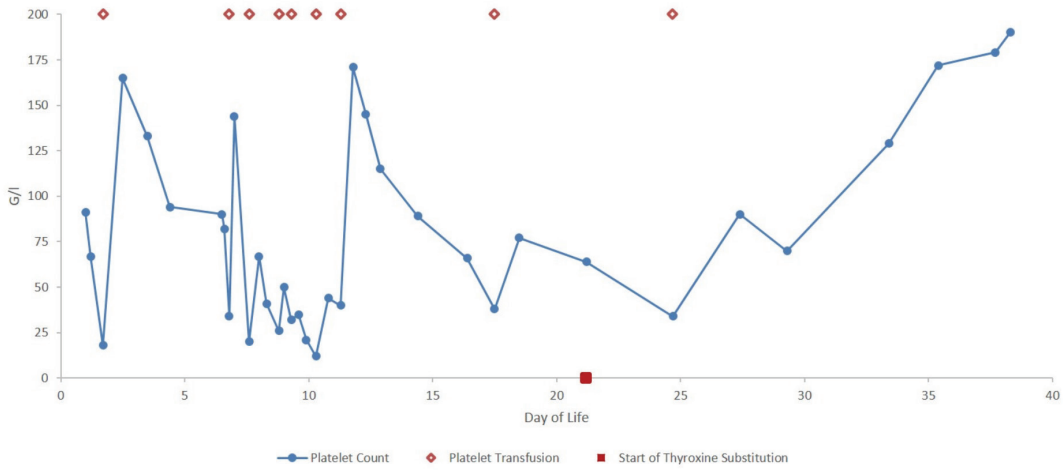

Figure 2. Platelet count of patient 1 from the first day to the 38th day of life. Indication for platelet transfusion was a count of < 50 G/L. This occurred nine times, particularly during severe infection from the sixth day of life. Thyroxine substitution started at day 21. Four days later, platelet transfusion was required for the last time, and subsequently platelet count increased to normal

the neonatal period (2). Case studies regarding neonatal manifestation are still lacking, yet case reports describe a correlation between onset of symptoms and the degree of anatomical disorder. Distinct anatomical phenotypes cause severe combined pituitary insufficiency and result in an earlier clinical manifestation (2,5). Our cases confirm these findings as the cMRI revealed an absent pituitary stalk, a hypoplastic anterior pituitary and an ectopic posterior pituitary. Based on this hypoplasia, both patients manifested with a combined pituitary insufficiency with hypoadrenocorticism, hypothyroidism and hypogonadism, further leading to hypoglycaemia, hyponatraemia, jaundice, cholestasis, sucking weakness and genital abnormalities (5). Of importance, secondary adrenal insufficiency led to an infection-triggered adrenal crisis in patient 1, starting with nonspecific symptoms of recurrent vomiting progressing to hypotension and shock (6). After unsuccessful therapy attempts with catecholamines, administration of stress-dose hydrocortisone led to stabilisation of the patient.

Mendelian inheritance is present in less than 5% of PSIS cases, and so digenic and/or polygenic inheritance is likely (1,7). Variants in *GLI2*, coding for zinc-finger proteins, are associated with PSIS (7,8) and, as seen in patient 1, mutations are often correlated with other malformations, such as polydactyly and midline defects (9). To the best of our knowledge, the variant of *GLI2* mutation found in patient 1 has not been described in public databases or other reports (10). Thus, this is the first report of this specific variant of *GLI2* being associated with a hypopituitarism phenotype.

In literature research on PSIS and *GLI2* mutations, thrombocytopenia was not reported, but an association between abnormal Gli2-signalling and megakaryocytic differentiation is possible (11). In patients with Sheehan syndrome, it was shown that anterior pituitary hormones affect bone marrow function and that cytopenia in various combinations are frequent (12). Leukocytopenia/neutropenia has been observed in 20-50% of patients with Addison disease (13). Elmelhat and Khadora (14) described the presence of congenital hypothyroidism with leukocytopenia/neutropenia and thrombocytopenia during the neonatal period, which improved after thyroxine administration. Regarding the incidence of congenital hypothyroidism (15), this isolated case is perhaps negligible, yet should be noted due to the similarity to patient 1. He suffered from the same bicytopenia and recovered a few days after beginning with thyroxine treatment (Figure 2).

Conclusion

To conclude, the presentation of these two cases of neonatal PSIS emphasises the importance of early diagnosis to avoid life-threatening complications. It is important to implement hormone analysis as soon as a newborn presents with recurrent hypoglycaemia, hyponatraemia, jaundice, cholestasis, sucking weakness and micropenis in males. In the case of conspicuous endocrine findings, cMRI should be performed promptly to identify PSIS and to confirm diagnosis. During the neonatal period, it can be performed safely during postprandial sleep without the risk and burden of anaesthesia.

Ethics

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

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Footnotes

Authorship Contributions

Surgical and Medical Practices: Ira Winkler, Elisabeth Steichen, Klaus Kapelari, Peter Wöckinger, Ursula Kiechl-Kohlendorfer, Elke Griesmaier, Concept: Ira Winkler, Elisabeth Steichen, Peter Wöckinger, Design: Ira Winkler, Data Collection or Processing: Ira Winkler, Elisabeth Steichen, Ursula Kiechl-Kohlendorfer, Elke Griesmaier, Analysis or Interpretation: Ira Winkler, Elisabeth Steichen, Klaus Kapelari, Peter Wöckinger, Vera Neubauer, Literature Search: Ira Winkler, Elisabeth Steichen, Writing: Ira Winkler, Peter Wöckinger.

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CASE REPORT

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Diazoxide-unresponsive Hyperinsulinemic Hypoglycaemia in a Preterm Infant with Heterozygous Insulin Receptor Gene Mutation

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What is already known on this topic?

Homozygous or compound heterozygous mutations in *INSR* gene cause severe insulin resistance syndromes, such as Donohue syndrome (also known as leprechaunism) and Rabson-Mendenhall syndrome, whereas heterozygous *INSR* gene mutations result in a milder phenotype, known as type A insulin resistance syndrome (type A-IR). Adults with type A-IR commonly demonstrate abnormal glucose homeostasis with fasting and postload hyperglycaemia, as well as high testosterone levels compared to age-matched controls. Phenotypes and clinical course in children, especially infants, with heterozygous *INSR* gene mutations have been reported infrequently and there is little evidence for optimal management of these infants.

What this study adds?

We report a preterm infant who presented with diazoxide-unresponsive hyperinsulinemic hypoglycaemia. Whole-exome sequencing identified a heterozygous *INSR* variant in the infant and her father. We postulate that use of diazoxide exacerbated post-prandial glucose excursion by inhibiting insulin release, while subsequent hypoglycaemia may be explained by reduced degradation or clearance of insulin due to the underlying mutation. This case highlights that in situation where mutations could not be identified by targeted sequencing of *ABCC8/KCNJ11* or *GCK* genes in an infant with suboptimal response to diazoxide, sequencing of the *INSR* gene should be considered. It is proposed that the *INSR* gene should be included in a targeted gene panel for workup of hyperinsulinism.

Abstract

Homozygous or compound heterozygous mutations in insulin receptor gene (*INSR*) lead to marked insulin resistance and hyperglycaemia in Donohue syndrome and Rabson-Mendenhall syndrome, conditions which are associated with significant morbidity early in life. In contrast, heterozygous INSR variants result in a milder phenotype, known as type A insulin resistance syndrome. While presentation in adults with this condition is well reported, phenotypes in infant are less well-characterized. Herein, we report an infant presenting with hyperinsulinemic hypoglycaemia who did not respond to diazoxide therapy. She was subsequently found to have a heterozygous *INSR* gene mutation. The patient was a female infant born at 29 weeks of gestation who developed recurrent hypoglycaemia in early infancy. Workup showed hyperinsulinism and she was started on first-line therapy with diazoxide and high-calorie feeds. However, continuous blood glucose monitoring showed post-prandial hyperglycaemia followed by rapid fall to hypogylcaemia. Whole exome sequencing was performed to investigate for diazoxide-unresponsive hyperinsulinism, which revealed a likely pathogenic mutation in the *INSR* gene, c.1246C>T p. (R416X). This nonsense mutation was inherited from the father. With the molecular diagnosis, diazoxide was stopped and she followed a diet with low glycaemic-index food. Subsequent monitoring showed stable glucose profile. This case highlights the importance of considering type A insulin resistance syndrome when no mutation is found in the *ABCC8/KCNJ11* genes in diazoxide-unresponsive hyperinsulinism. With autosomal dominant inheritance, cascade screening should be performed in family members to identify those harbouring the mutation as they are at risk of early onset diabetes.

Keywords: Hyperinsulinism, hypoglycaemia, insulin receptor

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Introduction

Mutations in the insulin receptor (*INSR*) gene are known to cause insulin resistance and hyperinsulinemia. Homozygous or compound heterozygous mutations in *INSR* gene cause the severe insulin resistance syndromes, Donohue syndrome (DS, also known as leprechaunism) and Rabson-Mendenhall syndrome (RMS), whereas heterozygous *INSR* gene mutations result in a milder phenotype, known as type A insulin resistance syndrome (type A-IR). In both DS and RMS, patients have marked hyperinsulinemia with fluctuating blood glucose levels, impaired muscle and adipose tissue development, growth failure, characteristic facial features and intellectual disability. Significant hyperglycaemia ensues when β-cells decompensate. Patients with DS, the most severe insulin resistance syndrome, seldom survive beyond infancy whereas patients with RMS can survive into early adulthood and usually die of diabetic ketoacidosis or advanced microvascular complications in the second decade of life (1). In contrast, patients with type A-IR live beyond middle age and usually present with hypoglycaemic symptoms, hypertrichosis, acanthosis nigricans and hyperandrogenism in the absence of obesity or lipoatrophy. Biochemically, these adults commonly demonstrate abnormal glucose homeostasis with fasting and postload hyperglycaemia, as well as high testosterone levels compared to age-matched controls (2).

Phenotypes and clinical course in children, especially infants, with heterozygous *INSR* gene mutations are less commonly reported. There is also a lack of evidence on how these infants can be best managed. Herein, we report a preterm infant who presented with diazoxide-unresponsive hyperinsulinemic hypoglycaemia. Whole-exome sequencing identified a heterozygous *INSR* gene mutation in the infant and her father, which helped to guide further investigation and management.

Case Report

Our proband was a Chinese female infant, born at 29 weeks of gestation for threatened preterm labour, weighing 1 kg (25th centile) and measuring 37 cm (25th centile) in length. Antenatal history was unremarkable with no gestational diabetes in the mother and there was no family history of endocrine disorders. There was no birth asphyxia and her neonatal course was relatively smooth with mild respiratory distress syndrome requiring one day of invasive ventilation, feeding intolerance and grade 1 intraventricular haemorrhage. Newborn screening for inborn errors of metabolism was normal. Parenteral nutrition was given according to standard protocol and enteral feeding was gradually stepped up.

She presented with recurrent hypoglycaemia from 1 month of life when full enteral feeding was established. Investigations showed repeated pre-feed hyperinsulinemic hypoglycemia with low serum levels of free fatty acids and ketones (Table 1).

Thyroid function, growth hormone, cortisol and ammonia levels were normal and lactate was not elevated. After cardiac assessment, diazoxide 15 mg/kg/day and hydrochlorothiazide were commenced for neonatal hyperinsulinism. Glucose polymer (Polycal) was added to feeds. However, her glucose profile worsened with more frequent episodes of pre-feed hypoglycemia. The baby was then put onto a continuous glucose monitoring system (CGMS) which revealed frequent post-prandial hyperglycaemia, ranging from 178.2 mg/dL to 309.6 mg/dL, followed by a rapid fall to the hypogylcaemic range with a nadir of 37.8 mg/dL. Insulin was still detectable (26.9-69.0 pmol/L) during these episodes of hypoglycaemia. Bolus feeding was thus halted and she was commenced on continuous milk feeding with dextrose infusion. To further investigate for diazoxide-unresponsive hyperinsulinism, genetic analysis was performed for the infant and her parents. Whole exome sequencing revealed a likely pathogenic mutation c.1246C>T p. (R416X) in exon 5 of the *INSR* gene, which resulted in a change of codon 416 from arginine to a premature termination. This nonsense mutation resulted in a truncated protein product. Her father carried the same mutation.

With the molecular diagnosis, diazoxide and Polycal supplement were gradually tapered. Dextrose infusion was weaned and bolus feeding was re-introduced on a three-hourly basis. Less post-prandial excursion, followed by a less severe plunge in blood glucose level, was observed. She finally passed an 8-hour fasting challenge with a blood glucose of 82.7 mg/dL at the end of the test and was discharged with bolus feeding at 4 months old. Regular home blood glucose monitoring showed no hypoglycaemia and she adopted a weaning diet with low glycaemic-index food. Subsequent assessment at 20 months old showed normal neurological development.

Table 1. Biochemical parameters during hypoglycaemia

	Day 37 of life	Day 82 of life
Insulin (pmol/L)	104.2	50.7
Blood glucose (mg/dL)	25.0	43.2
FFA (mEq/L)	0.11	0.13
β-OHB (mmol/L)	0.05	0.1

FFA: free fatty acid, β-OHB: beta-hydroxybutyrate

We also evaluated our proband's father in view of the mutation identified. In retrospect, he reported dizziness and tiredness after large carbohydrate meals but had never required medical attention. Physical examination showed a BMI of 26.6 kg/m^2 with no acanthosis nigricans. His fasting blood glucose, hemoglobin A1c, lipid profile and liver function tests were normal. His homeostatic model assessment for IR (HOMA-IR) was 3.0 which was $>95^{th}$ centile cut-off for normal glucose tolerance in southern Chinese (3). A 6-hour oral glucose tolerance test (OGTT) with 75 grams oral anhydrous glucose solution was performed (Table 2). He had normal glucose tolerance, but fasting hyperinsulinemia and elevated insulin-to-C-peptide ratio of 0.42 (normal range for fasting <0.1) (4). At 210 minutes, he developed asymptomatic hypoglycaemia with blood glucose of 46.8 mg/dL when paired insulin was 132 pmol/L. He was subsequently referred to the adult endocrine unit for follow up.

The parents of this infant, and the father himself, gave written consent to the writing of this manuscript. The study has been approved by the Ethics Committee of the Hong Kong West Cluster Clinical Research Ethics Review Board (HKWC-2022-249).

Discussion

We report an infant with heterozygous mutation in the *INSR* gene who presented with hyperinsulinism in the neonatal period, highlighting the need to consider this entity, especially in the setting of excessive post-prandial glucose excursion followed by reactive hypoglycaemia. Apart from the implications for treatment, cascade screening for family members is also important for the early identification of individuals at risk of young-onset glucose intolerance and insulin-resistant diabetes. These mutation carriers may benefit from dietary modification and use of insulin-sensitizing drugs, such as metformin and glitazones (2,5).

Hypoglycaemia associated with heterozygous *INSR* mutations has been described in a few adult studies (4,6,7,8). Most patients experienced hypoglycaemic during fasting and more characteristically, after a meal, which was similar to the presented case. Diagnosis in these adult studies was made with fasting hyperinsulinism, elevated fasting insulin to C-peptide ratio and hypoglycemia on prolonged OGTT. Further hyperinsulinemic-euglycaemic clamp studies showed markedly reduced insulin sensitivity and lowered metabolic clearance rate for insulin compared to controls. As a result, there is excessive insulin secretion after meal loading, which persists at high concentrations even with a falling blood glucose level, resulting in suppressed hepatic glucose output and postprandial hypoglycaemia (4,6). This phenomenon was also observed in our proband's father who demonstrated fasting and postload hyperinsulinemia, as well as hyperinsulinemic hypoglycemia (concurrent blood glucose of 46.8 mg/dL mmol/L and insulin 132 pmol/L) at 210 minutes of OGTT. Notably, he also exhibited elevated insulin to C-peptide ratio. In normal physiological conditions, insulin and C-peptide are co-secreted by the pancreas, with insulin rapidly metabolized by the liver and C-peptide slowly eliminated by the kidneys (9). Hence, elevated insulin to C-peptide ratio was suggestive of decreased clearance of endogenous insulin as a result of the underlying *INSR* mutation. While he has not developed frank diabetes, long term follow up of his metabolic profile will be necessary and avoidance of high glycaemic index food may help to ameliorate symptoms of post-prandial hypoglycaemia.

The *INSR* gene, located on chromosome 19, consists of 22 exons and 21 introns. Exons 1-11 (and part of exon 12) encode the extracellular α-subunits of the receptor that bind insulin, whereas exons 12-22 encode the β-subunits that span the plasma membrane and have an intracellular tyrosine kinase domain. Mutations in the α-subunits lead to decrease in the number of mature INSR or defective insulin binding, while mutations in the β-subunits impair autophosphorylation and subsequent activation of downstream signaling transduction. Longo et al. (10) demonstrated that mutations markedly impairing insulin binding resulted in the most severe phenotype with early demise, while mutations leaving residual insulin binding activity were associated with longer survival. However, while there is no definite genotype-phenotype correlation due to the rarity of these syndromes, mutations affecting the α-subunit of the receptor are generally associated with a more severe phenotype than those affecting the β-subunit

Table 2. Extended 6-hour OGTT of proband's father

Time (min)	0	30	60	90	120	150	180	210	240	270	300	330	360
Glucose (mg/dL)	72.0	180.0	144.0	118.8	104.4	122.4	75.6	46.8	66.6	75.6	81.0	82.8	88.2
C-peptide (pmol/L)	280	2500	2020	1920	1630	1520	830	420	310	280	210	230	280
Insulin (pmol/L)	118	1750	1229	1236	819	854	305	132	97	97	76	90	118
Insulin-to C-peptide ratio	0.42	0.70	0.61	0.64	0.50	0.56	0.37	0.31	0.31	0.35	0.36	0.39	0.42

OGTT: oral glucose tolerance test

(10,11). Hence, the majority of patients with DS have mutations in the α-subunit, while type A-IR syndrome is more frequently associated with mutations in the tyrosine kinase domain of the β-subunit (4,6,7,8,11). The nonsense mutation c.1246C>T identified in our proband and his father is located in the second leucine-rich repeat domain (L2) of the extracellular ligand-binding α-subunit. This variant was previously reported in a boy diagnosed with DS at 1 month old. Interestingly, this boy only carried a single mutation, as did our proband, but presented early with severe phenotype (12). Unfortunately, we were not able to perform functional analysis which could possibly explain the milder phenotype in our case. Nevertheless, this is the first report of a heterozygous α-subunit mutation causing neonatal hyperinsulinism with a mild presentation.

Neonatal hyperinsulinimic hypoglycaemia linked to heterozygous *INSR* mutation was first reported in four infants from three families by Sethi et al. (13). All these infants had mutations located in exon 20 of the *INSR* gene, which encode the β-subunit of the receptor. They were all born small for gestational age and developed hypoglycaemia on the first day of life. In contrast to our proband, they showed good response to diazoxide therapy (at a dose between 3-7.5 mg/kg/day) and were able to wean off the medication before 1 year of age. Diazoxide acts to open pancreatic β-cell ATP-sensitive potassium (K_{ATP}) channels and inhibit insulin secretion. The mechanism in which hyperinsulinism in these infants responds to diazoxide is not clear. In contrast to the reported cases, the glucose profile in our infant worsened after diazoxide. We postulate that use of diazoxide exacerbated post-prandial glucose excursion by inhibiting insulin release while the hypoglycaemia that follows could be explained by reduced degradation or clearance of insulin due to the underlying mutation.

The most common form of monogenic hyperinsulinism is caused by inactivating mutations in the *ABCC8* or *KCNJ11* genes, which encode subunits of the K_{ATP} channel. These mutations also account for almost 90% of diazoxide-unresponsive hyperinsulinism cases, followed by activating mutations of the glucokinase (*GCK*) gene (14,15). In contrast to infants with *INSR* mutation, those who harbour *ABCC8* or *KCNJ11* mutations typically present with fasting hypoglycaemia rather than post-prandial hypoglycaemia. In addition, post-prandial hyperglycaemia, a prominent feature in patients harbouring *INSR* gene mutations, helps to differentiate these conditions. Birth weight also provides another important clue to help identify patients with diazoxide-unresponsive hyperinsulinism due to K_{ATP} channel mutations, as these babies are usually born macrosomic. Therefore, careful history taking and biochemical phenotyping in the evaluation of hyperinsulinism are very helpful in recognizing patients with possible *INSR* gene mutations. CGMS was used to monitor the glucose profile in our proband. While accuracy might be an issue in young infant, CGMS offers great value in the evaluation of glucose fluctuation, thereby helping clinicians consider the diagnosis of *INSR* gene mutations.

Conclusion

In conclusion, the present case report details the clinical and biochemical features of an infant with hyperinsulinimic hypoglycaemia caused by heterozygous *INSR* gene mutation. Response to diazoxide therapy was poor and resulted in even more severe post-prandial hyperglycaemia. While further accumulation of clinical experience in managing this group of pediatric patients is required, accurate genetic diagnosis of the condition is essential to ensure regular monitoring of metabolic control and prompt initiation of intervention when necessary.

Ethics

Informed Consent: The parents of this infant, and the father himself, gave written consent to the writing of this manuscript.

Presented in: Abstract of this case report has been presented in the 12th Biennial Scientific Meeting of the Asia Pacific Paediatric Endocrine Society.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Sarah Wing-Yiu Poon, Brian Hon-Yin Chung, Mabel Siu-Chun Wong, Anita Man-Ching Tsang, Concept: Anita Man-Ching Tsang, Design: Sarah Wing-Yiu Poon, Data Collection or Processing: Sarah Wing-Yiu Poon, Brian Hon-Yin Chung, Anita Man-Ching Tsang, Analysis or Interpretation: Brian Hon-Yin Chung, Mabel Siu-Chun Wong, Literature Search: Sarah Wing-Yiu Poon, Brian Hon-Yin Chung, Anita Man-Ching Tsang, Writing: Sarah Wing-Yiu Poon, Brian Hon-Yin Chung, Anita Man-Ching Tsang.

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CASE REPORT

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Continuous Glucose Monitoring Systems and the Efficacy of Acarbose Treatment in Cystic Fibrosis-related Dysglycemia

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What is already known on this topic?

Dysglycemia is common in patients with cystic fibrosis (CF). Insulin is the first choice for treatment, especially in cases of hyperglycemia.

What this study adds?

In the early detection of CF related diabetes (CFRD), screening with oral glucose tolerance test after the age of 10 years may be inaccurate. Therefore, routine use of the continous or intermittant glucose monitoring systems should be considered. In CFRD with severe hypoglycemia, acarbose may be an important alternative in the high and increased dose range.

Abstract

Early detection of glycemic dysregulation and optimization of glycemic control in cystic fibrosis (CF) related diabetes (CFRD) is associated with improved pulmonary function and decreased mortality. The standard 2-hour oral glucose tolerance test (OGTT) is the current routine screening test for CFRD. However, hyperglycemia can be detected by continuous glucose monitoring systems (CGMS) in patients with normal OGTT evaluation. High-dose acarbose is an important alternative in the treatment of glycemic dysregulation especially accompanied by hypoglycemia. A 7-year-old boy with CF presented with hyperglycemia. Hypoglycemia (29 mg/dL) and hyperglycemia (400 mg/dL) were demonstrated by OGTT and intermittent CGM (iCGMS). Thickener was added to nutritional solutions and acarbose was initiated as 3x12.5 mg/dose and increased to 6x25 mg without any side effects. On the twentieth day of treatment, glycemic dysregulation resolved. In the early detection of CFRD, screening with OGTT after the age of 10 years may be inaccurate. Therefore, routine use of CGMS or iCGMS should be considered. In addition, in CFRD with severe hypoglycemia, acarbose may be an important alternative in the high and increased dose range.

Keywords: Acarbose, CFRD, CGMS, cystic fibrosis

Introduction

The incidence of cystic fibrosis (CF) related diabetes (CFRD) has increased as more effective clinical management of CF has developed and the life expectancy of patients with CF extended. The prevalence of CFRD increases markedly with age, affecting approximately 2% of children, 19% of adolescents, and 40% to 50% of adults with CF (1). Early detection of glycemic dysregulation and optimization of glycemic control is associated with improved body weight and pulmonary function, reduced frequency of pulmonary exacerbations, and decreased mortality (1). Therefore, early detection of glycemic dysregulation in patients with CF is important. The issue of how to screen for glycemic dysregulation in these patients is still contentious. The standard 2-hour oral glucose tolerance test (OGTT) is currently

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the routine screening test for CFRD and is recommended annually after 10 years of age. However, hyperglycemia (>200 mg/dL) may be detected by continuous glucose monitoring systems (CGMS) in patients with normal OGTT evaluation (2). Therefore, CGMS has been suggested for use in follow-up. Treatment of CFRD is complicated because of the presence of both insulin deficiency and resistance, a high energy requirement, nocturnal feeding to ensure adequate energy intake, fasting and postprandial hypoglycemia as well as hyperglycemia. Insulin therapy is often required in these patients. However, different treatment methods are used in the management of early glucose dysregulation and in patient groups with prominent hypoglycemia (3,4,5).

Case Report

A 7-year-old boy with CF (homozygous c.2183AA>G variant was detected in the *CFTR* gene) and pancreatic insufficiency presented with hyperglycemia during resolution of a pulmonary exacerbation.

Since the age of four years, routine annual hemoglobin A1c (HbA1c) had remained below 6.5% (NR 5.4% to 6.4%). OGTT was performed with frequent sampling (at minutes -15, 0, 10, 20, 30, 45, 60, 90, 120, 150, 180) and symptomatic severe fasting hypoglycemia (29 mg/dL), severe hyperglycemia at 60 minutes (400 mg/dL), impaired glucose tolerance at 120 minutes (180 mg/dL) and severe hypoglycemia at 180 minutes (26 mg/dL) was detected (Table 1). He had no history of polyuria, polydipsia, or chronic glucocorticoid treatment. He had a history of frequent acute exacerbations and hospitalization, poor weight gain, and underwent a gastrostomy at the age of four years. His weight was 20 kg (-1.18 standard deviation score (SDS)], height 124 cm (-0.21 SDS) and body mass index (BMI) 13.1 (-2.39 SDS). He was on a high energy diet by oral and gastrostomy route with continuou infusion overnight and pump assisted infusion during the day.

Anti-insulin and anti-glutamic acid decarboxylase antibodies were negative. Sensor glucose readings above 350 mg/dL and below 50 mg/dL were detected with intermittent CGMS (iCGMS) (FreeStyle Libre system; Abbott Diabetes Care) (Figure 1). Due to frequent acute exacerbations, history of hospitalization, and poor weight gain, the need for gastrostomy was accepted as a symptom of CFRD for our patient. The patient was diagnosed with CFRD according to the International Society for Pediatric and Adolescent Diabetes (ISPAD) 2022 CFRD guideline with hyperglycemia exceeding 200 mg/dL, which was detected both by OGTT and CGMS data.

Glucose dysregulation was attributed to the negative effect of CF on gastric emptying and insulin-glucagon action. Postprandial hyperglycemia and reactive hypoglycemia were also associated with delayed and prolonged hyperinsulinemia.

To prevent postprandial rapid glucose rise and reactive hypoglycemia, enteral nutrition formula content was adjusted and thickener was added. Since hyper- and hypoglycemia continued after these changes, 3x12.5 mg of acarbose (alpha-glucosidase inhibitor) was added due to the effect of slowing down the hydrolysis and absorption of carbohydrates before the meal and the initial dose was gradually increased. Although glucose fluctuations decreased, hyperglycemia persisted during meals without

Table 1. Frequently sampled oral glucose tolerance test

	-15. min	0. min	10. min	20. min	30. min	45. min	60. min	90. min	120. min	150. min	180. min
Glucose (mg/dL)	29	55	65	130	208	342	415	308	181	45	26
Insulin (mU/L)	0.772	4.15	2.21	5.21	5.86	76.4	220	174	67.3	17.9	5.88
C-peptide (mg/L)	0.232	1.04	1.21	2.7	2.36	7.64	18.1	17.3	14.7	7.01	3.25

min: minutes

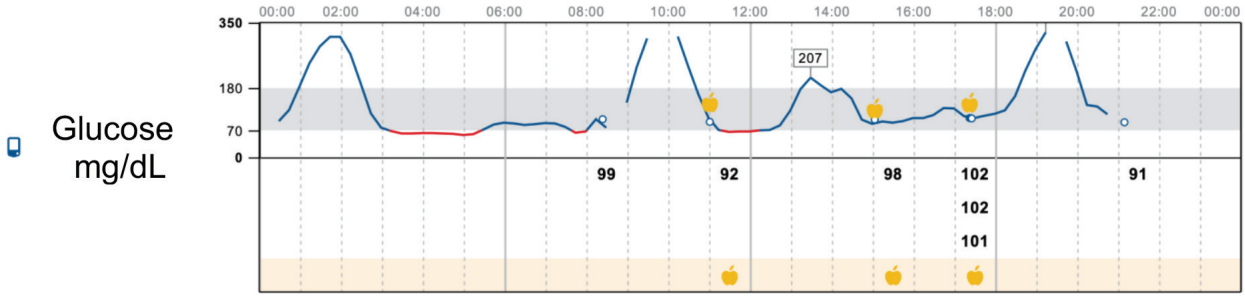

Figure 1. Pre-treatment continuous glucose monitoring systems

acarbose treatment and so treatment was increased to 6x12.5 mg and then gradually to 6x25 mg without any side effects (Figure 2). On the twentieth day of treatment, no hyper- or hypoglycemia was detected. The CGMS data of the case before and after treatment is summarized in Table 2. Weight gain improved after treatment, and at the sixth month of treatment, improvements in weight 22.5 kg (-0.62 SDS), height 127 cm (-0.14 SDS) and BMI 13.95 (-1.56) were noted.

Discussion

CFRD shares some characteristics with both type 1 and type 2 diabetes, yet also has unique pathophysiologic considerations. CFRD is not an autoimmune disease as diabetes autoantibodies and diabetes-associated HLA types are not different from the general population (6). Specific features of CFRD include partial loss or dysfunction of pancreatic islets leading to deficiency of insulin secretion, insulin resistance caused by chronic inflammation that increases and fluctuates periodically during infection, a very high energy diet in order to achieve weight gain, and disruption of the incretin system.

The incretin axis is involved in the etiology of the development of CFRD. Incretins, such as glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1 (GLP-1), are intestinal hormones that are secreted after meals, primarily triggered by carbohydrates. Insulin release, inhibition of glucagon and somatostatin, preservation of β-cells, delay of gastric emptying, and suppression of appetite are important biological effects of incretins. Kuo et al. (7) showed that nondiabetic CF patients with exocrine pancreatic insufficiency had faster gastric emptying after a high-fat/high-carbohydrate meal compared with healthy controls. This was accompanied by profound disruption of GLP-1 secretion and these authors suggested that this is one of the causes of postprandial glycemic variability

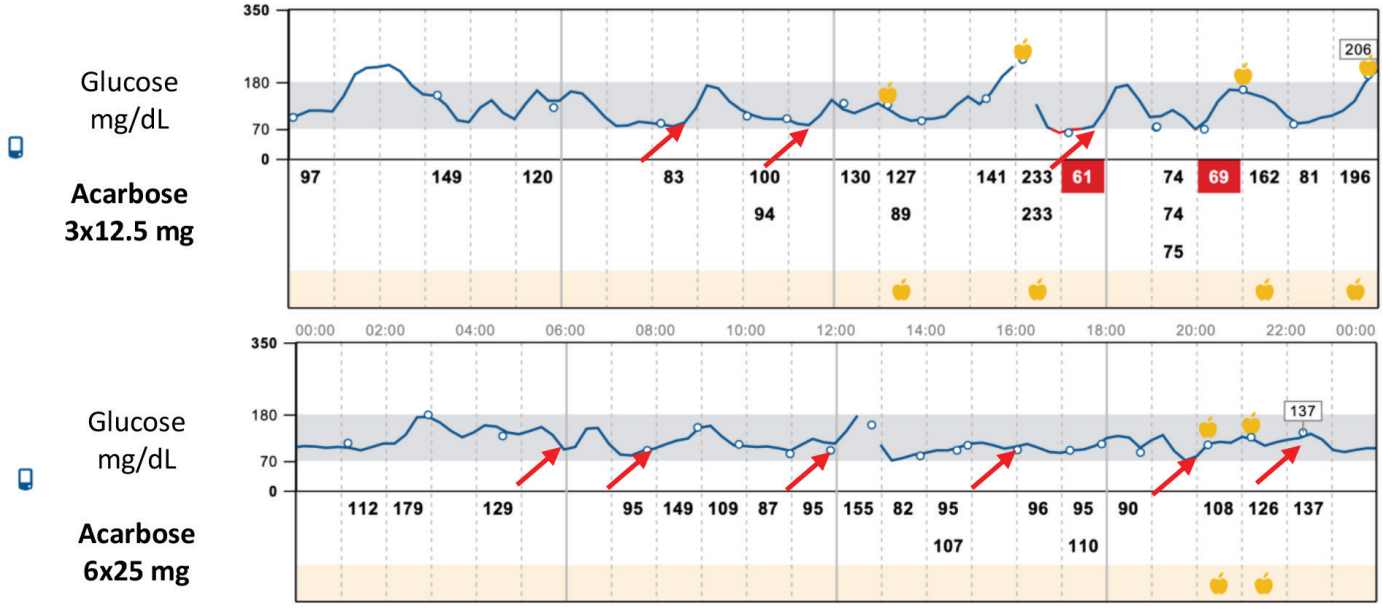

Figure 2. After adding acarbose to meals

**Red arrows indicated with meals added acarbose*

Table 2. CGMS data before and after treatment changes

	Before treatment	With thickener	Acarbose 3x12.5 mg	Acarbose 6x25 mg
GMI, %	7.2	6.8	6.8	6.0
Mean glucose, mg/dL	164	145	144	114
CV, %	40.7	38.7	36.2	22.9
Time in range, %	60	74	80	96
Low glucose*, %	6	4	2	1
Very low glucose**, %	2	1	1	0

* < 70 mg/dL, ** < 55 mg/dL.
CGMS: continuous glucose monitoring systems

in patients with CF (7). This hypothesis is consistent with the fact that the first step in the progression to CFRD is impaired first-phase insulin secretion (6). Compatible with this mechanism, in the presented case there was a rapid increase in serum glucose level in the postprandial first hour due to fast gastric emptying, followed by delayed and exaggerated hyperinsulinemia and reactive hypoglycemia.

The diagnosis of CFRD may be more challenging than the classical diagnosis of diabetes, and HbA1c in the diagnosis is questioned. In the ISPAD CFRD guideline, diagnosis of CFRD is made according to American Diabetes Association criteria during a period of stable baseline health:

a. 2-hour blood glucose on OGTT ≥11.1 mmol/L (≥200 mg/dL),

b. Fasting blood glucose ≥7.0 mmol/L (≥126 mg/dL),

i. Fasting blood glucose ≤7.0 mmol/L (≤126 mg/dL) does not rule out diabetes in CF,

c. HbA1c ≥ 48 mmol/mol (≥6.5%),

ii. HbA1c < 48 mmol/mol (< 6.5%) does not rule out diabetes in CF,

d. Random blood glucose ≥11.1 mmol/L (≥200 mg/dL) with classic symptoms of diabetes (2).

Although it has been demonstrated that the sensitivity of HbA1c in the diagnosis of CFRD is low, ISPAD 2022 CFRD guideline recommends classical diabetes criteria for diagnosis of CFRD. However, the guideline also states that a normal or low HbA1c value does not exclude CFRD. Consequently, it cannot be asserted that HbA1c is no longer commonly utilized in the monitoring of patients with CF.

Glucose abnormalities demonstrated by CGM are common in CF, including in very young children, however there are as yet no established criteria using CGM for either screening or diagnosing diabetes. Retrospective and cross-sectional single-center studies have associated glucose abnormalities on CGM with ß-cell dysfunction on OGTT, weight decline, lower lung function, and elevated inflammatory markers. However, evidence from larger multi-center studies are lacking to support the benefits of treating intermittent elevations in blood glucose concentrations prior to a diagnosis of diabetes (2).

Many studies have questioned the adequacy of the OGTT for the early detection of impaired glucose regulation, emphasizing that the decline in the patient's weight and pulmonary function would have started much earlier if the diagnosis was based solely on OGTT (8). Mainguy et al. (9) reported that the capacity of an OGTT to diagnose CFRD was weak and pathological glucose fluctuations were frequent, even in the early stages of life. Hameed et al. (10) showed that peak glucose occurred earlier than the routinely measured 120-minute sample, occurring within 30 minutes in 18% of patients, 60 minutes in 45%, 90 minutes in 33%, and 120 minutes in only 3%.

Many studies have shown that CGM is a useful clinical tool in CF, and many studies continue to be conducted on the interpretation and predictiveness of CGMS (11). It should be kept in mind that weight loss and poor weight gain may also be predisposing to CFRD, as in our case. Thus it should not be overlooked that close CGMS monitoring should be performed in patients with CF who need continuous enteral nutrition. As recommended in the ISPAD 2022 CFRD guideline, these patients should have their blood glucose checked in the middle and at the end of feeding. Furthermore, these patients are suggested to be suitable candidates for follow-up with CGMS.

Another important problem in CF patients with abnormal glucose tolerance is reactive hypoglycemia. It has been reported that the prevalence of reactive hypoglycemia may be as high as 29% during OGTT, especially if the test is performed > 2 hours (6).

In the presented case, CFRD was diagnosed with both OGTT and CGMS. However, it should be noted that we did not perform the standard OGTT, which is currently recommended in the CFRD guidelines. Since we used OGTT with frequent sampling, hyperglycemia was detected reaching 400 mg/dL in the first hour and hypoglycemia after 120 minutes. Had the standard OGTT been employed, a hyperglycemic level of 181 mg/dL would have been identified at the 120-minute mark; however, hypoglycemia occurring after 120 minutes would not have been recognized. Consequently, the patient would receive a diagnosis of impaired glucose tolerance exclusively. Therefore, we suggest that the standard OGTT is not applicable for safe diagnosis when CFRD is suspected. We recommend that all patients with CF be screened with CGMS before the age of 10 years, if possible. More studies are needed for the initiation of the age of screening but it has been suggested that screening should start after the age of 6 years (12).

Acarbose is an alpha-glucosidase inhibitor and a competitive inhibitor of pancreatic α-amylase and intestinal brush border α-glucosidases and delays the hydrolysis of polysaccharides, oligosaccharides, and disaccharides to monosaccharides, blunting and prolonging the postprandial increase in plasma glucose, which reduces insulin secretion. In addition, acarbose has been shown to increase postprandial GLP-1 levels and regulate insulin secretion in both normal and diabetic patients. This increase

in GLP-1 has been attributed to the decrease in carbohydrate absorption in the proximal part of the small intestine and the corresponding increase in nutrient load in the distal intestine, where GLP-1 secretion is higher.

In the multicenter study of Sels et al. (13) a significant decrease in postprandial hyperglycemia and HbA1c by 0.4% was reported after adding acarbose to the treatment in patients with type 1 diabetes. Riccardi et al. (14) in a study with 121 patients with type 1 diabetes showed that adding acarbose to the treatment provided a significant decrease in the 120 minute glucose level without any serious side effects. In a placebo-controlled study conducted by Kentrup et al. (5) in CF patients with impaired glucose tolerance, use of acarbose had a positive effect on glucose tolerance by providing a significant attenuation in postprandial glucose increase and a decrease in insulin secretion. However, these authors reported that the gastro-intestinal system side effects, seen in 67% of the patients using acarbose, may negatively affect the long-term continuation of the treatment. Acarbose is generally used in adults with dumping syndrome at 50-100 mg three times a day with meals. However, in children, treatment is usually started with lower doses, such as 12.5-25 mg.

Some studies have shown that acarbose can be safely taken up to 100 mg before each feeding in children without significant side effects (15). The most common side effect is gastrointestinal symptoms, such as bloating due to carbohydrate malabsorption. There is evidence that these gastrointestinal side effects are not as common as some have reported and are generally mild and it has been suggested that acarbose can be used safely (15).

We started with a low dose (3x12.5 mg) in this case and managed to gradually increase up to 6x25 mg/daily without any serious side effects related to acarbose treatment. We were able to achieve significant improvement in glucose regulation with only acarbose.

Conclusion

In the early diagnosis of CFRD, screening with OGTT after 10 years of age causes a delay in the diagnosis of CFRD and so we suggest periodic screening of patients with CF with CGMS may be a good alternative. In CFRD with severe hypoglycemia, acarbose is an important alternative in the treatment of glycemic dysregulation. We recommend keeping high dose acarbose among the treatment options, especially in patients with CF who have frequent hypoglycemia, as patients may not suffer severe side-effects, as was the case with our patient and in whom we were able to achieve improved glucose regulation.

Ethics

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

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Footnotes

Authorship Contributions

Surgical and Medical Practices: Emrullah Arslan, Deniz Özalp Kızılay, Yasemin Atik Altınok, Bahar Girgin Dündar, Arzu Jalilova, Günay Demir, Samim Özen, Şükran Darcan, Ruhsar Damla Gökşen, Concept: Emrullah Arslan, Samim Özen, Şükran Darcan, Ruhsar Damla Gökşen, Data Collection or Processing: Emrullah Arslan, Deniz Özalp Kızılay, Analysis or Interpretation: Emrullah Arslan, Samim Özen, Literature Search: Emrullah Arslan, Yasemin Atik Altınok, Samim Özen, Şükran Darcan, Ruhsar Damla Gökşen, Writing: Emrullah Arslan, Deniz Özalp Kızılay, Bahar Girgin Dündar, Günay Demir, Samim Özen, Şükran Darcan, Ruhsar Damla Gökşen.

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