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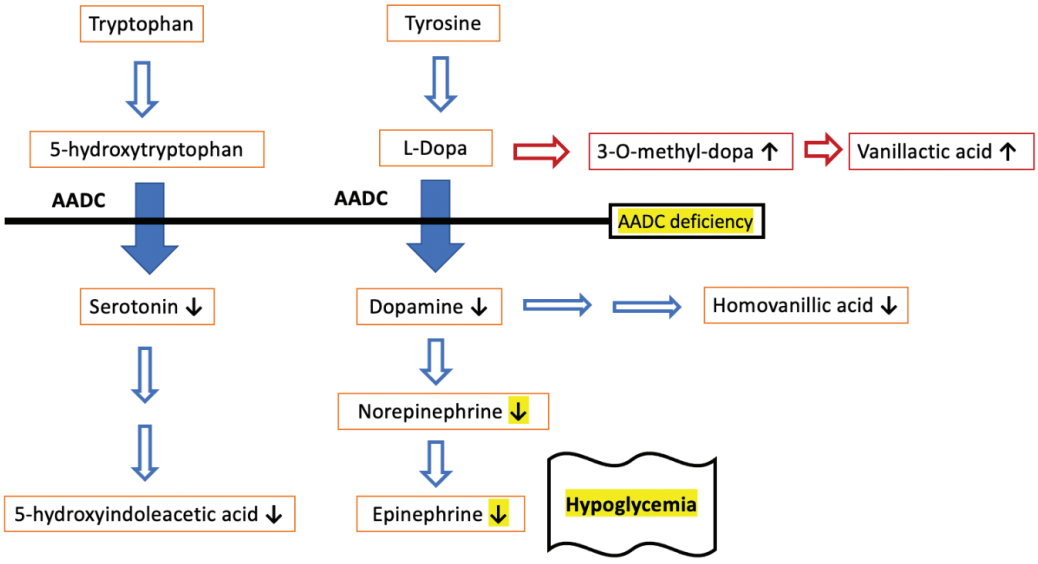

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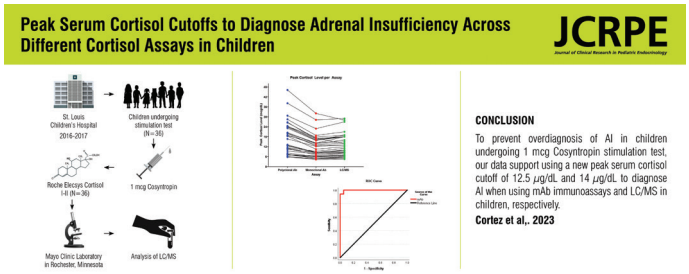

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REVIEW

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J Clin Res Pediatr Endocrinol 2024;16(3):245-255

Current Management of Type 1 Diabetes in Children: Guideline-based Expert Opinions and Recommendations

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Abstract

Successful management of type 1 diabetes (T1D) requires not only optimal glycemic outcomes, but also a holistic approach that encompasses all aspects of life and recommendations to address needs. Current goals include optimal glycemic values, quality of life and life expectancy similar to peers, prevention of long-term complications, prevention of severe hypoglycemia as far as possible and facilitation of glucose management. The International Society for Pediatric and Adolescent Diabetes (ISPAD) has been updating its guidelines for diabetes care every four years since 1995, covering more and more topics. For optimal metabolic outcomes, diabetes teams need to follow these current recommendations, adapt them to their clinical practice and provide guidance to people with T1D and their families. In this review, in the light of ISPAD 2018-2022 guidelines and clinical experiences, "10 Key Recommendations", emphasizing the importance of teamwork and the use of technology, current T1D treatment is described for practical applications.

Keywords: Carbohydrate counting, diabetes camp, glucose target, HbA1c, insulin carbohydrate ratio, insulin sensitivity, glycemic outcome

Introduction

Today, the management of type 1 diabetes (T1D) in children continues to pose significant challenges, particularly among preschoolers and adolescents. In many countries, mean/median hemoglobin A1c (HbA1c) levels are consistently 7.5% and above, contrary to recommendations (1). However, effective diabetes management extends beyond glucose control. Achieving successful treatment requires a holistic approach that encompasses optimal glycemic outcomes, as well as addressing lifelong needs and aspirations. To achieve this, knowledge about treatment needs to be made practical and explicit, based on evidence-based recommendations. Otherwise, routine practice may rely on concepts that are challenging to translate into daily practice, such as complex mathematical calculations or individual diabetes team member opinions with resultant lack in achieving target HbA1c levels. When diabetes teams lack current practice recommendations or have insufficient guidance, and individuals with T1D struggle to undertake a complex clinical management plan, the motivation toward long-term goals is disrupted. This can lead to inertia, characterized by inactivity, dormancy, and passivity. Treatment-related "inertia" is a condition that diabetes teams, as well as people with diabetes themselves fall into, and leads to a gradual move away from the goal of optimal metabolic control (2).

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However, important practices, such as carbohydrate counting, should be taught from diagnosis, and people with T1D and their families should be encouraged to be take an active role in insulin dose adjustments and nutritional management from the very beginning. Hanas (3), who has worked for many years as a pediatric diabetologist combining clinical practice with science, describes this approach as "*becoming an expert on your own diabetes*".

Guidelines, Exemplary Country Practices, and Advancements

The International Society for Pediatric and Adolescent Diabetes (ISPAD) has been publishing guidelines on various aspects of diabetes in childhood every four years since 1995, and has increased the number and scope of these texts over the years. Most recently, in late 2022, 25 guideline texts covering all aspects of diabetes in children were published. Compared to previous texts, in the last four years, the concepts of continuous glucose monitoring (CGM) (sensors), automated insulin delivery (AID) systems, technology, groups/regions with limited resources, inequalities, social determinants of health, cultural awareness, and person-centered care/personalized care are prominent (4).

However, centers in countries like Australia and Sweden have achieved a notable decrease in HbA1c levels to an average range of 6.7-6.8% over a decade, attributed to target settings (HbA1c target 6.5% since 2017), the nationwide practices, quality control programs, team goal setting and also benchmarking (5,6). Reportedly, in Sweden, the percentages of individuals with T1D whose HbA1c levels were below 6.5% (48 mmol/mol), below 7.3%, and above 8.6% nationally in 2022 were 43.3%, 76.7%, and 4.5%, respectively (7). Table 1 outlines the key features and leading practices observed in both the Sweden and Australia programs.

For the last eight years, the authors of this review have been implementing a program similar to the Sweden and Australia recommendations, the main components of which are the "10 Key Recommendations", teamwork and the use of technology, which we will detail in this article. The authors have seen 1,870 children with T1D and their families since 2016 through a clinical implementation program, including a 1-hour pediatric endocrinology visit, a 2-hour basic principles diabetes training by a diabetes educator, and 3-session nutritional training by a nutritionist. The 1-hour pediatric endocrinology visits address topics such as individual treatment recommendations, glucose targets, insulin dose calculations [insulin-to-carbohydrate ratio (ICR) and insulin correction/sensitivity factor], rules to be followed at bedtime, dawn/reverse dawn phenomena and their management, hypoglycemia management, correction dose timing, optimal carbohydrate amount, and the role of families, especially fathers. All these recommendations are given to the caregivers as a printed "Individual Treatment Plan", which is prepared for each child, tailored to the age and characteristics of the child.

To date, 60.4% of the children admitted to our department use CGM and 20% use insulin pump therapy (continuous subcutaneous insulin infusion) and 44.7% of these are using the Minimed 780G. In an evaluation of 543 children with regular follow-up in our center between 2018-2022, the median HbA1c was 7.1%, 45% of the children had

Table 1. The Characteristics of the Sweden and Australia Child Diabetes Programs*

Sweden	Australia
- Improvement of local and national guidelines. - Lowering of HbA1c target to NICE target 6.5% (48 mmol/mol) - Aiming for >50% of CGM values to be within 4-8 mmol/L (70-140 mg/dL) and <4% below 70 mg/dL (3.9 mmol/l). - Aiming for average glucose level <7.8 mmol/L (140 mg/dL) - Teamwork with weekly or bi-weekly meetings. - CGM for all patients within 1 week of onset. - Carbohydrate counting education for all patients from the onset of T1D. - Use of injection aids (i-Port) for young children from the onset of diabetes. - CSII for preschool children and encouraging CSII use in all age groups. - Downloading pump and CGM at each visit and promoting active use of the digital glucose logbook at home. - Improved technology training. - Encouragement of exercise.	- Team approach instead of individual (clinician-based) practices, reviewing team recommendations at least once a year, providing them as a written document to patients. - While insulin pump is recommended for all children <5 years of age, multiple daily injection doses at the time of diagnosis, transition to insulin pump according to family and clinician's recommendations. - Glycemic targets, correction boluses, and structuring meals are recommended. - NICE Guidance targets (<6.5%) instead of ISPAD targets. - NORMAL glucose is targeted as much as possible across all age groups. - Blood glucose measurement at least 5 times a day. - While constant snacking and snacking habits used to be assumed normal in young children until 2004, now regular main meals, minimum snacks as much as possible, and no need for snacks before bedtime have been adopted. - Postprandial insulin was administered for children who refused to eat until 2004, now always pre-meal insulin is recommended. - Previously, determination and adjustment of insulin doses were done by medical and nursing staff and insulin adjustment education was given after diagnosis when parents were "ready". Now, insulin sensitivity factor and insulin/carbohydrate ratio are taught at the time of diagnosis, families are given easy dosing schemes/cards. - Mini-dose glucagon is taught.

*Adapted from the references 3 and 5.
CSII: continuous subcutaneous insulin infusion, T1D: type 1 diabetes, ISPAD: The International Society for Pediatric and Adolescent Diabetes, HbA1c: hemoglobin A1c, CGM: continuous glucose monitoring

HbA1c <7%, and only 7% had HbA1c above 9%. Although these children might have had a better social and economic status, these encouraging findings may also have been due to the comprehensive training, teamwork, "10 Basic Recommendations" that set the basic goals, and the use of technology in our clinic.

Given the absence of a national diabetes registry in our country, the availability of metabolic control data remains limited. A 2013 study encompassing 1,032 cases across various national centers reported a mean HbA1c level of 8.5%. Similarly, a 2016 study involving 498 cases at the national level reported an average of 8.6% (8,9). In these studies, the percentage of children with an HbA1c level >9% was 36.9% and 35.7%, respectively. A more recent cohort study, spanning 2018 to 2023 and comprising 2,730 children from 42 centers, reported a median HbA1c of 8.4% (10). These statistics underscore that the average HbA1c in our country surpasses recommended targets, demonstrating a lack of improvement over the past decade. Particularly concerning is the persistently high proportion of children with an HbA1c above 9%, a threshold associated with increased risk of complications. Notably, these numbers reflect the status of approximately 30,000 children with T1D in Turkey, providing evidence for the necessity for new national initiatives.

This review article will focus on the main recommendations based on the ISPAD 2018-2022 guidelines (4), Hanas' (3) book and the authors' clinical experiences. Our aim is to bring to the attention of pediatric diabetes teams these apparently beneficial practices and to be the basis for a new countrywide program.

Current Goals in the Care of Children with T1D

Current goals in the management of T1D include not only better glycemic values and closer glycemic values to those without diabetes but also the quality of life and life expectancy similar to their peers, prevention of long-term complications, no severe hypoglycemia, mild hypoglycemia not being a burden, alleviating the fear of hypoglycemia, facilitating glucose management during/after meals, comfortable/fearless sleep for families and children, implementing CGM monitoring, ensuring adequate diabetes management at school supporting academic performance, participating in enjoyable activities, preventing diabetic ketoacidosis, weight control and prevention of eating disorders, reduction of diabetes treatment routines/burdens, reduction of glucose fluctuations and related problems (restlessness, sudden anger, etc.), facilitating the management of conditions such as sports, menstruation, pregnancy, lactation, surgical intervention, and fasting. The current targets for glucose regulation include an HbA1c target below 7% (below 6.5% for individuals with stage 3 diabetes, during the remission period, and for individuals with access to advanced technology, under the care of clinics offering advanced education and services) (11). Additionally, maintaining a coefficient of variation of less than 36% and achieving Time In Range (TIR) of 70-180 mg/dL at over 70%, with preprandial glucose levels between 70-144 mg/dL (11). Notably, recent emphasis has been placed on achieving a Time In Tight Range of 70-140 mg/dL at least 50% (12).

There exists a close relationship between set targets and corresponding actions. For instance, administering correction doses above 145 mg/dL aids in achieving the target HbA1c of <6.5%. Furthermore, effective communication within diabetes teams is important, ensuring clear articulation of proposed glycemic targets with a unified voice. Aligning the goals of the child and family with those of the diabetes team is also imperative, fostering overlapping objectives for optimal management.

Several Topics Concerning the Management of T1D and the '10 Key Recommendations'

In this section, certain themes related to the management of T1D will be addressed, providing practical recommendations. Unreferenced practices in this section are derived from Hanas's (3) book and the authors' clinical experiences. The '10 Key Recommendations,' which the authors apply in their clinical practices and are detailed below, are provided in Table 2.

Administering Meal Bolus Insulin 15-20 Minutes Before the Meal for the Best Post-prandial Glucose Profile

Mastery of matching the effect of exogenous insulin with the effect of nutrients is one of the cornerstones of T1D management. Except for fatty meals and instances of slow eating, it is recommended to administer rapid-acting insulins 15-20 minutes before meals, aiming for a physiologically appropriate bolus delivery. This practice is particularly beneficial, especially during breakfast, considering the lower insulin sensitivity until 10 am (13). In addition, this practice helps to suppress glucagon secretion at the onset of the meal. It remains applicable even in preschool-aged children, although the timing might need adjustment and implementation could be more challenging. This is also recommended for individuals using an AID system, like the Minimed 780G.

The Selection, Dose, and Timing of Basal Insulin

Basal insulin requirement is higher between 10 pm and 10 am (14). The daily basal insulin rate is not more than 50%

Table 2. '10 Key Recommendations' for children with T1D

1. Administering meal bolus insulin 15-20 minutes before the meals (by deleting the last digit of the pre-meal glucose value- e.g. 162 mg/dL 16 minutes before a meal). Using abdomen and arms as injection sites for the fast-acting insulins and buttocks and thighs for slow-acting insulins.
2. Going to bed with a normal glucose level (>120 mg/dL for pre-schoolers, >90 mg/dL for older children), avoiding unnecessary carbs with the fear of hypoglycemia.
3. Avoiding snacks unless necessary, to consume carbohydrates in a moderate amount during main meals. Preschoolers may need a small snack without insulin at midmorning to avoid pre-lunch hypoglycemia.
4. Calculating the bolus dose carefully according to the carbohydrate ratio and the glucose value at the time, increasing doses if necessary to take into account the effect of fats and proteins.
5. Avoiding milk intake at night before bedtime, taking into account the glucose spike between 9 pm and 12 am after falling asleep ("reverse dawn phenomenon") in the young children; shifting milk intake to daytime active hours.
6. Using a sensor as soon as possible and an insulin pump (better yet, automated insulin delivery systems) as soon as possible after diagnosis if finances permit.
7. Administering correction boluses promptly, if the insulin sensitivity factor permits when the blood glucose is over 145 mg/dL.
8. Limiting problematic foods that may pose challenges, such as pizza and hamburgers.
9. Incorporating exercise into daily routine and taking it as a therapeutic tool, akin to a 'third type of insulin'.
10. To continue diabetes treatment according to the principle of doing our best, without turning it into a stress, and to live with diabetes in a peaceful (friendly) way.

T1D: type 1 diabetes

with exceptions including adolescence (honeymoon period, low carbohydrate diet, vigorous exercise) (13,15). When the basal insulin rate is higher than 50% of TDD (total daily dose) when using injection therapy, it causes an increased frequency of hypoglycemia during the fasting period - a kind of slope effect, which can be defined as the direction of the glucose curve pointing downwards.

It should be taken into consideration that the basal insulin requirement in young children is 25-30% of the total daily insulin dose (TDI) (12). If an insulin pump is not used, it is a good option to administer insulin glargine (Lantus) at 7 or 10 pm to benefit from its relatively strong effect in the first 12 hours. It should be kept in mind that the effect of insulin glargine begins three hours after administration. Some clinicians suggest that for doses of 15 U or less, splitting into two doses given in the morning and evening provides better coverage and less risk of fasting hypoglycaemia, and that two doses also facilitates titration for the desired glycemic effect (3).

In pre-adolescents and young children, administering insulin glargine at 7 pm before dinner can help reduce hyperglycemia occurring between 9 pm and 12 am due to the 'reverse dawn phenomenon' after falling asleep. For adolescents, administering insulin glargine at 10 pm can prevent hyperglycemia after 5:00 am. However, it can be given in the morning in very young children having hypoglycemia late at night (between 3 and 6 am). Since insulin detemir (Levemir) has 18% less receptor affinity (16) and therefore lower potency, it is usually not possible to obtain sufficient basal insulin effect with one dose/day. Insulin glargine 300 U/mL (Toujeo) or Tresiba may be preferred in children with nocturnal and/or general hypoglycemia problems. It should be noted that its effect in the first 12 hours is weaker than Lantus and the duration longer. The dose may be increased by 10-18% in transition.

The Prevention of Lipohypertrophy and Recommended Insulin Injection Sites Regarding Insulin Types

Lipoatrophy is rare, while lipohypertrophy remains a prevalent issue. To prevent lipohypertrophy, it's essential to rotate injection sites for insulin pump set/pod placement or injections, ensuring a minimum distance of 1 cm between each injection point. In Turkey, the common practice of injecting around the lower abdomen and both sides of the navel originates from the era of longer needle tips. However, using the entire abdomen is more accurate, as there is at least approximately 4 mm of fat tissue almost everywhere over the abdomen. Therefore, using both the upper and lower parts of the abdomen as injection sites is advisable. Lipohypertrophy tends to develop more easily in the arms of young children. Administering fast-acting insulins to the abdomen and arms while opting for slow-acting insulins in the hips and thighs is recommended (13). In Sweden, arms are not normally recommended for injections, except for Omnipod (3).

Early Corrections with Small Doses of Insulin

Improving time spent within the target range enhances insulin sensitivity. It's advisable to initiate corrections early and with smaller doses. This approach not only increases TIR but also mitigates the risk of hypoglycemia associated

with larger corrective doses. When the glucose level exceeds >145 mg/dL (equivalent to 6.5% in terms of HbA1c) two hours after the last bolus dose, and if the sensor glucose trend shows no decline while the insulin sensitivity factor (ISF) allows for correction, an additional insulin dose should be administered promptly. It is not advisable to wait for values exceeding >250 mg/dL before considering an additional insulin dose. Conversely, if glucose levels range between >220-270 mg/dL, it may be appropriate to administer a corrective dose according to the individual correction factor (ISF). However, a correction dose should normally not exceed 0.1 U/kg but can be repeated after 2 hours, if needed. The target glucose range for this correction should be between 100-120 mg/dL (17). For corrections made after 10:00 pm, particularly in children aged 10 years and older, it is recommended to administer half of the daytime calculated insulin dose to minimize the risk of nighttime hypoglycemia. When a correction dose is given before bedtime, glucose monitoring should be conducted around 2-3 am. In preschool-aged children, there is a heighted glucose concern between 9 pm to 12 am due to the 'reverse dawn phenomenon.' Therefore, correctional dosing should align with daytime practices until midnight. Some centers recommend correcting the night in the same way as the day for all age groups and thus expect less variability during the night (5). Another approach is to start with a half dose correction at night and then adjust individually.

The Recommended Total Daily Carbohydrate Intake and Optimal Amount of Carbohydrate in Meals

In the last 15 years, recommendations regarding the percentage of daily energy derived from carbohydrates have evolved. While the previous recommendation was to obtain 55% of energy from carbohydrates, recent guidelines from ISPAD advocate for carbohydrates to constitute approximately 40-50% of daily energy intake. Furthermore, the updated recommendations propose fats to account for <35% of energy (<10% from saturated fats) and proteins to make up 15-25% of daily energy (18). Explaining this change through calculation, consider a 6-year-old boy. If 40% of his energy intake is derived from carbohydrates, this would amount to 147 grams of carbohydrates per day. In contrast, adhering to the former recommendation of 55% from carbohydrates would necessitate an intake of 202 grams - a significant difference. Consistent with this current recommendation, carbohydrate intake should be tailored based on the child's specific circumstances, such as whether they are an athlete or not, while ensuring that daily energy intake from carbohydrates does not fall below the 40% threshold. In general, consuming more than 60 grams of carbohydrate in a single meal (different amounts may be recommended for different age groups) may lead to difficulties in achieving optimal insulin-carbohydrate matching (19). When more carbohydrate is consumed at a meal, increasing insulin doses to prevent postprandial blood glucose spikes may lead to a risk of hypoglycaemia before the next meal. A recently published study investigating varied carbohydrate amounts (20, 50, 100, and 150 grams) at breakfast showed that the glucose curve most closely resembling the effect of fast-acting insulins was observed with 50 grams of carbs. Participants given 20 or 150 grams experienced prolonged glucose elevation lasting up to three hours, suggesting the need to adjust the ICR in such cases (20). This concept parallels the 'green wave' system employed by traffic authorities on certain roads; the concept that if you drive at 50-60 km/h on those roads, you will almost never come across a red light. Thus, aiming for a total carbohydrate intake of around 60 grams per meal can lead to a more favorable postprandial glucose curve.

Low Carbohydrate Diets

If carbohydrate intake is to be reduced, this should be discussed with the dietitian and endocrinologist in the diabetes team. However, it is generally not recommended that a child with T1D should get less than 40% of their energy from carbohydrates (3) as this may make it difficult to meet nutrient requirements and may affect growth. The definition of high, moderate, low, and very low carbohydrate intake is given in Table 3 (21). Ketones will occur when carbohydrate and therefore energy intake is significantly reduced, inducing a state resembling starvation in the body. For a growing child, it's recommended that blood ketone

Table 3. High, moderate, low, and very low carbohydrate intake in childhood*

	Carbohydrate (g/day) 1-6 years	Carbohydrate (g/day) 6-10 years	Carbohydrate (g/day) 11-16 years
High carbohydrate diets (>55% energy from carbohydrates)	>170 g	>230 g	>320 g
Moderate carbohydrate diets (45% energy from carbohydrates)	140 g	200 g	280 g
Low carbohydrate diets (<26% energy from carbohydrates)	<80 g	<100 g	<150 g
Very low carbohydrate diets (<10% energy from carbohydrate)	<30 g	<40 g	<60 g

*Adapted from reference 22

levels remain below 0.5 mmol/L - the same upper limit seen in healthy children - to avoid any negative impact on growth. In young adults who have reached their final height, a blood ketone level of up to 1.0 mmol/L may be acceptable if the reduction in carbohydrate intake is overseen by an endocrinologist and dietitian. Strict low-carbohydrate high-fat diets (LCHF) are not recommended for people with T1D as no studies indicate their benefit over more moderate carbohydrate intakes. Typically, blood ketone levels exceed 3 mmol/L during ketoacidosis, and individuals strictly following a LCHF diet may experience ketone levels ranging from 3-5 mmol/L. For individuals with diabetes, this narrow margin increases the likelihood of ketoacidosis, a potentially life-threatening condition, which is particularly concerning in children. The most important potentially negative effect of low carbohydrate nutrition is on growth (22). Given that insulin has an important effect on growth, once children with T1D are compelled to follow a low-carbohydrate diet paired with inadequate insulin, it leads to a marked slowdown in growth in a short period of time.

Diets with low to very low carbohydrate intake can disturb the natural relationship that children and adolescents have with food. Over time, these diets can gradually impact the child's relationship with food, for example food restricton can cause disordered eating which can lead to binge eating, potentially leading to the development of disordered eating (23). This scenario can be likened to an overstretched spring, losing its tension and functionality. Some adolescents, after an extended period on a low-carbohydrate diet, encounter difficulties reverting to their previous diet and may face challenging issues like depression.

Calculating Empirical ICR and Addressing Low Morning ICR

The conventional method for calculating the ICR has been dividing the number 500-450 by the TDI as an empirical practice. However, recent reports highlight that this formula often results in insufficient bolus dose calculation, particularly in children. Instead, it has been found more effective to utilize the average number 315 for this calculation (24). Recently, the ISPAD guidelines for insulin treatment have emphasized a revised approach to this calculation. Specifically, it is recommended to divide by 330 or 250 for young children. In the case of preschool children, a division by 150 is advised during breakfast (12,13). Typically, insulin sensitivity decreases due to the influence of anti-insulin hormones until 10 am. This underscores the need to divide the total insulin dose into a smaller number for morning ICR calculation in all age groups. In the 2022 edition of his book, Hanas (3)recommends using the formulas 200/TDI at breakfast and 400/TDI at other meals in preschool children and 300/TDI at breakfast and 500/TDI at other meals in older children and adolescents for empirical ICR calculation (3). Ideally, these empirical calculations should be individualized and reviewed in line with carbohydrate intake. Breakfast typically involves a high carbohydrate intake, posing a challenge to administer sufficient insulin before lunch without the risk of hypoglycemia. Hence, it might be prudent to recommend a lower carbohydrate intake during mornings compared to other meals. Additionally, a concern arises regarding the ICR to apply when waking up later than usual. The dawn phenomenon consistently occurs at a specific time daily, yet if one stays up late, the cortisol response upon waking will happen later. Consequently, even with a very delayed breakfast, it's recommended to use the breakfast ICR (3).

Time to Start Carbohydrate Counting and Use of "Carbohydrate-Bolus Calculator Application"

Presently, precise carbohydrate counting is crucial for calculating the bolus doses. However, the primary challenge families encounter in diabetes management is often determining the appropriate food portions. Carbohydrate counting allows for more flexible carbohydrate intake as well as understanding that there is a mathematical basis for insulin dose calculation. It is inaccurate to claim, as often suggested, that learning carbohydrate counting is premature or overly challenging at the time of diagnosis. Carbohydrate counting ought to be introduced right at the time of diagnosis and ideally during hospitalization. Many mothers, closely linked to food management, find valuable assistance through the Carbohydrate-Bolus Calculator Application to navigate this process (25). The "sliding scale method" for determining the insulin doses according to glucose value ranges based on fixed carbohydrate administration prevents families from participating in and mastering insulin dose calculations from the onset. The sliding scale method falsely implies that the insulin dose is determined solely based on the glucose level. Determining the pre-meal insulin dose primarily depends on the carbohydrate amount. The sliding scale method may be likened to fastening the first button incorrectly, so all the following buttons are also incorrect. This method may hinder the proper understanding and adjustment of insulin doses.

Going to Bed with a Normal Glucose Level and Fear of Hypoglycemia

Several factors contribute to the challenge of achieving optimal control, such as the lack of residual beta cell function, non-physiological insulin treatments, burnout, increased strain on families, outdated methodologies, limited access to technology, and other disparities (26). In addition, fear of

nocturnal hypoglycaemia may lead families to ignore their goals, given that more than 50% of severe hypoglycemic episodes in children and adolescents occur during sleep (27). The fear of hypoglycemia often leads people with T1D to consume unnecessary carbohydrates and go to bed with high glucose levels (sometimes exceeding 250 mg/dL). However, it is a more accurate approach to enter the night with normal glucose (>120 mg/dL is safe for the preschool period, and >90 mg/dL is safe for older children) (11). If glucose levels exceed 160-180 mg/dL, it is advisable to administer an additional dose, provided that there is no downward trend in the glucose level. The previous suggestion to avoid administering an extra dose of insulin at bedtime was due to the possibility that the extra dose might coincide with the the glucose lowering effect of NPH and result in low blood glucose levels 4-5 hours later. However, this issue is not anticipated in individuals using insulin glargine or an insulin pump. Between the hours of 3 and 6 am, the amount of insulin requirement decreases (12), which can lead to a risk of hypoglycemia. To avoid this, it is important to allow certain hormones like glucocorticoids, adrenaline, and glucagon to be activated when they are needed during this period. This helps to maintain a normal balance in the body. It is not advisable to rely on unnecessary carbohydrates before bedtime to protect against hypoglycemia during the night. This approach can lead to high glucose levels in the first half of the night. This is especially true for young children, who may experience the "reverse dawn phenomenon" between 9 pm and midnight. When a person sleeps with high glucose levels at night, insulin sensitivity decreases due to hyperglycemia, which can lead to high glucose levels both during the night and in the morning. This same problem may also occur after 4 pm, or during afternoon nap-time, which is called the "dusk" phenomenon.

Routine/Obligatory Snacks and Milk Before Bedtime

In the past, using NPH and regular insulins together increased the risk of hypoglycemia. As a result, it became routine to have snacks between the main meals, three times a day. Using multiple injections of rapid-acting insulin or an insulin pump reduces the need for snacks between meals. These types of insulins have a similar effect to the way blood glucose increases after a meal, resulting in lower insulin levels between meals. Therefore, it is suggested to minimize snacking throughout the day and only consume snacks if necessary (18). Unregulated snacking habits often lead to the consumption of high-glycemic index carbohydrates without insulin, causing elevated glucose levels during the day (5,28). In preschoolers, frequent or irregular snacking can cause early fullness, making it difficult to predict the required amount of carbohydrates in their main meals. Similar concerns arise with night-time snacks, particularly the "milk feed" before bedtime. The carbohydrates in milk, slowed by fats and proteins, cause a glucose curve that insulin doesn't effectively match. This may also be related to insufficient bolus or any food/liquid consumed after dinner with multiple daily injections (MDI). This impact is noticeable at all times but more pronounced in young children experiencing elevated glucose levels between 9 pm and midnight due to the "reverse dawn phenomenon" (29). Consequently, it's advantageous to consider shifting milk consumption to earlier in the day, preferably before daytime physical activities rather than right before bedtime, whenever feasible.

The Impact of Fats on Insulin Sensitivity and the Issue of Problematic Foods

High-fat meals are one of the major causes of post-prandial glucose elevation and even AID systems are not successful in this regard. Fats delay the post-prandial rise time of glucose by delaying the gastric emptying time, decreasing insulin sensitivity, and increasing hepatic glucose production, preventing the matching of insulin and carbohydrates, and causing glucose elevation within 3-5 hours after the meal (30,31). This leads to the problem of inability to manage "problematic foods" such as pizza, pasta and hamburgers and results in out of target glucose levels (32). High glucose levels due to fats leads to decreased insulin sensitivity caused by high glucose and a "vicious cycle".

T1D guidelines recommend consideration of fat and protein in the meal-time insulin strategy, but optimal adjustments are still unknown. Data support an insulin dose increase of up to 30% for carbohydrate meals containing more than 30 g fat or 15 g fat + 25 g protein (33).

Combined boluses in pump therapy favorably influence the glycemic course and at least 60% of the calculated bolus should be given 15 minutes before a meal. With MDI, the efficacy of a split insulin strategy in improving postprandial glucose control is uncertain. Consequently, navigating the impact of fats is challenging, requiring dietary guidelines that prioritize reducing fats by avoiding "problematic foods" (34).

Diabetes Care at School

Children typically spend over 30 hours per week at school. Optimal diabetes management at school including help with carbohydrate counting and insulin dosing at lunch is closely related to successful academic performance and preventing complications. Diabetes care should be

maintained at the same high standard at home and school, with consistent daily blood glucose targets, regardless of the setting (35). Besides national programs, pediatric diabetes teams should provide an "Individual Treatment Plan for School" for each child and make diabetes care at school a part of their routine practice. Recently, the aim has been to ensure that the glucose levels are kept within the target range for a full 24 hours, not just at home but also when at school. To achieve this, pediatric diabetes teams need to be complemented by "diabetes teams at home (the family or caregivers of a child with diabetes)" and teachers need to be part of pediatric diabetes teams. (https://okuldadiyabet.meb.gov.tr/anasayfa).

Diabetes Technologies

Insulin pump therapy should be presented as an option, not a necessity, and sensors should always be prioritized (36). The authors do not start insulin pump therapy before the end of the honeymoon period, with exceptions for preschool children with small doses but they do encourage the use of sensors from the time of diagnosis. Striving for equitable access to diabetes technologies is recognized as important and education programs should be as short and structured as possible. The teams should strive for the training programs to be outpatient and individualized.

Continuous communication and support should be provided to technology users, and the establishment of groups on online platforms should be encouraged for learning and experience sharing among users.

Diabetes Camps for Children and Families

The "My friend diabetes camps" have been organized in İznik (Turkey) since 1997 to improve the education and health of children with diabetes (37). Numerous families who send their children to these camps express that they would like the camps to be organized for themselves as well. They would like to receive the same comprehensive education as their children receive and also want to share their experiences and interact with other families who have children with diabetes. The "My Friend Diabetes Family Camp" has been organized since 2018 to respond to this request, which has been voiced over the years, to help families provide better diabetes care. In addition to children with diabetes, their parents and non-diabetic siblings can participate in this camp.

The camps aim to enable children and families to find a new "normal", to be hopeful and to be friends with diabetes, and to create versatile opportunities for this. Being friends with diabetes is the first step of living with diabetes; it is not helpful to magnify the difficulties that come with diabetes, not to exhaust themselves with worries. Once diabetes is encountered, it is best to leave the past behind, look to the future, and live a life at peace with diabetes. The objectives of diabetes camps for children and families are given in Table 4.

Table 4. The objectives of diabetes camps

- To make children with diabetes and their families competent in diabetes care and treatment and to enable them to cope with problems in diabetes care.
- To gain awareness of diabetes care.
- Providing training on new treatments and technologies.
- To enable participants to share their experiences with each other and feel less alone.
- Helping them to look at their future life more positively and confidently.
- Providing an environment in which democratic parenting attitudes are valued over neglectful, authoritarian or permissive parenting attitudes.
- To enable families of children with diabetes to better understand and manage the changes/problems their children experience during adolescence.
- To strengthen the training of pediatric diabetes teams and other camp participants, to provide an environment where they can receive feedback from families and evaluate their own work/attitudes, and to train health teams to better understand children with diabetes.

"10 Behaviors to Avoid" for Better Diabetes Care and Health in Children with T1D

Improving the management of T1D in children requires education that emphasizes not only what to do but also the behaviors to avoid. In the long term, technology and behaviors will largely determine the treatment of T1D. Therefore, the "10 Behaviors to Avoid" that complement the "10 Key Recommendations" are also important.

1. Assuming that one can gauge their glucose level solely based on how they feel, without glucose testing: This behavior is more common in adolescence and leads to a gradual neglect of diabetes management, avoidance of confronting glucose values and doing what is necessary, ignoring the facts, self-deception and eventually a "vicious circle" develops. Children who exhibit such behavior usually have high HbA1c values and are at a greater risk of being hospitalized with severe hypoglycemia or ketoacidosis. Managing diabetes without glucose data, including fasting and postprandial glucose values, is like walking blindfolded or driving a car with the speedometer, fuel gauge, and warning alerts turned off. It is important to have access to 24-hour sensor data to get a complete picture of glucose levels and manage diabetes effectively.

2. "Skipping or forgetting to take pre-meal rapid-acting insulin doses and administering it after the meals": Understandably, diabetes treatment can lead to fatigue

and disruption of routines over time. However, if diabetes is neglected, it will not simply disappear. It is important to remember that missing or forgetting to take fast-acting insulin doses (also known as bolus doses) before a meal or taking them after a meal can lead to poor glucose management during the meal, insulin resistance caused by elevated glucose levels, and eventually, a prolonged period of high glucose levels throughout the day. Diabetes management relies on successfully managing fasting and post-prandial periods separately; getting enough basal insulin cannot compensate for problems caused by skipping bolus doses, as is sometimes assumed.

3. Irregular sleeping habits, going to bed late and waking up late: The dawn phenomenon, which causes high blood glucose levels in the morning, can be prevented to some extent by maintaining a regular sleep schedule. On the other hand, lack of sleep can lead to reduced insulin sensitivity, making diabetes management more challenging. It is also essential to avoid lying in bed until late morning, as this can result in skipping the morning bolus doses that help overcome the dawn phenomenon. This may cause elevated glucose levels and a difficult start to the day, even when fasting.

4. Irregular eating: Although meals can be managed with accurate carbohydrate counting and appropriate insulin doses, eating at short intervals complicates diabetes control. The high-dose insulin/excess carbohydrate intake behavior leads to weight gain. Insulin is the cornerstone of diabetes treatment, but diet is the key to successful glucose control. It is of most importance not to skip meals and to eat meals most of the time at similar times every day. In addition, gluten-free diets should be avoided, in the absence of celiac disease, and practices promoted as "alternative medicine", such as very low-carbohydrate or ketogenic diets, should be avoided too. Furthermore, adolescents should avoid behavior, such as not eating and not taking insulin to lose weight or using unhealthy dietary supplements with unclear ingredients, such as unlabelled protein powders for body-building.

5. Living a life without exercise: Inactivity and prolonged screen time reduce insulin sensitivity, negatively affect fat metabolism, cause weight gain, and decrease vitality and happiness. It is healthy for everyone, particularly children, to spend time in nature, walking, playing games, spending time with friends, reading books, and helping others. It is crucial to keep in mind that life is a gift, and we should welcome every sunny day and every beautiful cloud with joy.

6. Hiding diabetes: If you have diabetes, it is better to be open about it and explain it to others. Answering people's questions briefly by saying "I have type 1 diabetes and I use insulin" will help you lead a more comfortable life. Trying to hide your condition can be exhausting and make you feel sad. In the long run, it can lead to behaviors such as denial and avoidance of your diabetes and its requirements, which can negatively impact your glycemic control.

7. Ignoring diabetes: When diabetes is ignored, it reminds itself with hypoglycemia or hyperglycemia, reduces the quality of life, makes the person feel bad, and these negative emotions can push the person further away from their diabetes. To break this cycle, it is important to accept the condition, take necessary measures, and move forward. This process can be described as befriending diabetes. Neglecting and forgetting diabetes supplies can lead to difficult situations in life. It is crucial for individuals with diabetes to always have access to the necessary materials for measuring blood glucose, insulin, and hypoglycemia treatment.

8. Not collaborating for diabetes management: Managing diabetes is a continuous and challenging process, and having the support and shared responsibility with loved ones can help the person with T1D feel more comfortable and achieve successful diabetes management. On the other hand, blocking the efforts of parents and siblings by saying "stay out of my way", refusing the people who offer to help, hiding information from relatives, and pushing them out of diabetes management can make life difficult and lead to negative outcomes. In addition, such behaviors can upset, disempower and depress parents whose hearts are always beating for their children. Life is bigger than diabetes, and the way to make more time for oneself and one's life is to reduce the burden of one's diabetes through cooperation. Making diabetes management as easy and enjoyable as possible depends on this shared behavior. Adolescents (and their parents), who are gradually achieving self-control in many areas of life, need to understand that their parents are needed as "diabetes coaches" during the teenage years. Try to distinguish between diabetes parenting and diabetes coaching.

9. Refusing to embrace certain technologies by stating "I prefer not to incorporate anything into my body": Today, sensors and AID systems are of great importance in reducing the burden and improving the quality of life for people dealing with T1D. Sensors provide a wealth of information to understand the course of glucose and master diabetes management. Taking the right steps towards a diabetes management goal, such as maintaining normal glucose levels, creates a "positive feedback loop" and diabetes management becomes easier. Diabetes technologies, especially sensors are of value for creating a positive cycle and turning point for individuals living with diabetes. Instead of dismissing these technologies outright,

it is important to give them a chance and try them out for a while. Eliminating the question marks and concerns about the sensors (showering, swimming, sports activities, social concerns) will accelerate the acceptance process. Starting a sensor at diabetes onset will facilitate the use of the device.

10. Scaring children with complications, blaming them for blood glucose levels that are not in the targeted range: Parents should avoid using scare tactics such as threatening their children with kidney damage or blindness, no matter how concerned they might be. Such threats are not productive and can create a sense of hopelessness in children. Instead, parents should try to motivate their children to think carefully about their actions and decisions. Constantly blaming children for high or low glucose levels can lead to feelings of inadequacy and failure. It is not recommended to use the words "good glucose" and "bad glucose" as it can be emotionally distressing. Instead, it is better to use words such as "glucose on target", "high glucose", and "low glucose". It is essential to remember that almost every day, despite all the efforts, unexpected blood glucose levels can occur, and it may not be possible to prevent this completely. It is the long-term average glucose, TIR and HbA1c that count in the long run.

Conclusion

The primary focus should be on achieving good glycemic control from the diagnosis of diabetes, and maintaining HbA1c levels at or below 6.5% in all age groups. Providing written, clear, and up-to-date recommendations and targets can provide a basis for good metabolic control, and each center can tailor recommendations such as the "10 Essential Recommendations" used by the authors. In the long run, maintaining a normal glucose target range between 70-140 mg/dL should be stressed more, in our opinion. It should be noted that the use of appropriate technology significantly contributes to achieving this goal. More effort should be made to provide support and adherence to goals at school, keeping in mind that teamwork and mentoring/guidance programs make a difference. Emphasizing and creating opportunities to learn from each other, other diabetes teams, other children/young people living with T1D, and their families are important and beneficial. We suggest that an open national registry should be set up with clear visibility of data from all centers. Finally, efforts should be made to ensure that fathers are involved from the beginning.

Ethics

Authorship Contributions

Surgical and Medical Practices: Şükrü Hatun, Concept: Şükrü Hatun, Tuğba Gökçe, Ecem Can, Elif Eviz, Kağan Ege Karakuş, Carmel Smart, Ragnar Hanas, Gül Yeşiltepe Mutlu, Design: Şükrü Hatun, Tuğba Gökçe, Ecem Can, Elif Eviz, Kağan Ege Karakuş, Carmel Smart, Ragnar Hanas, Gül Yeşiltepe Mutlu, Data Collection or Processing: Şükrü Hatun, Tuğba Gökçe, Ecem Can, Elif Eviz, Kağan Ege Karakuş, Gül Yeşiltepe Mutlu, Analysis or Interpretation: Şükrü Hatun, Gül Yeşiltepe Mutlu, Literature Search: Şükrü Hatun, Gül Yeşiltepe Mutlu, Writing: Şükrü Hatun, Tuğba Gökçe, Ecem Can, Elif Eviz, Kağan Ege Karakuş, Carmel Smart, Ragnar Hanas, Gül Yeşiltepe Mutlu.

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Adherence to Growth Hormone Treatment in Children During the COVID-19 Pandemic

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What is already known on this topic?

Treatment adherence is crucial for successful treatment with growth hormone (GH) therapy. Non-adherence affects linear growth. Non-adherence rates vary widely, from 5% to 80%. Older age and prolonged duration of treatment with growth hormone increase non-adherence.

What this study adds?

The median age at diagnosis was lower than KIGS data. Poor adherence was 15% of patients. Poor adherence rate was higher when compared to previous Turkish studies. The Coronavirus disease-2019 pandemic may have affected the non-adherence rate.

Abstract

Objective: Treatment adherence is crucial for the success of growth hormone (GH) therapy. Reported non-adherence rates in GH treatment have varied widely. Several factors may have an impact on adherence. Apart from these factors, the global impact of the Coronavirus disease-2019 (COVID-19) pandemic, including problems with hospital admission and routine follow-up of patients using GH treatment, may have additionally affected the adherence rate. The primary objective of this study was to investigate adherence to treatment in patients receiving GH. In addition, potential problems with GH treatment during the pandemic were investigated.

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Methods: This was a multicenter survey study that was sent to pediatric endocrinologists during the pandemic period (June-December 2021). Patient data, diagnosis, history of pituitary surgery, current GH doses, duration of GH therapy, the person administering therapy (either parent/patient), duration of missed doses, reasons for missed doses, as well as problems associated with GH therapy, missed dose data and the causes in the recent year (after the onset of the pandemic) were questioned. Treatment adherence was categorized based on missed dose rates over the past month (0 to 5%, full adherence; 5.1 to 10% moderate adherence; >10% non-adherence).
Results: The study cohort consisted of 427 cases (56.2% male) from thirteen centers. Median age of diagnosis was 8.13 (0.13-16) years. Treatment indications were isolated GH deficiency (61.4%), multiple pituitary hormone deficiency (14%), Turner syndrome (7.5%), idiopathic GH deficiency (7.5%), small for gestational age (2.8%), and "others" (6.8%). GH therapy was administered by parents in 70% and by patients in 30%. Mean daily dose was 32.3 µg/kg, the annual growth rate was 1.15 standard deviation score (minimum -2.74, maximum 9.3). Overall GH adherence rate was good in 70.3%, moderate in 14.7%, and poor in 15% of the patients. The reasons for non-adherence were mainly due to forgetfulness, being tired, inability to access medication, and/or pen problems. It was noteworthy that there was a negative effect on adherence during the COVID-19 pandemic reported by 22% of patients and the main reasons given were problems obtaining an appointment, taking the medication, and anxiety about going to hospital. There was no difference between genders in the adherence rate. Non-adherence to GH treatment decreased significantly when the patient: administered the treatment; was older; had longer duration of treatment; and during the pandemic. There was a non-significant decrease in annual growth rate as non-adherence rate increased.
Conclusion: During the COVID-19 pandemic, the poor adherence rate was 15%, and duration of GH therapy and older age were important factors. There was a negative effect on adherence during the pandemic period.
Keywords: Children, growth hormone, adherence, COVID-19, pandemic

Introduction

Treatment adherence is crucial for successful treatment with growth hormone (GH) therapy. Patient motivation and adherence to treatment may decrease over time because of several factors, including daily injections and prolonged duration of the therapy (1). Non-adherence is the leading cause of insufficient height gain in patients on GH therapy (2,3). Reported medication non-adherence rates vary widely, from 5% to 80% depending on the method (4). A more recent systematic review reported that medication non-adherence rates varied from 7 to 71% across the included studies (1). Treatment adherence may be influenced by many factors including patient unwillingness (fear, reasons associated with injections), forgetfulness, treatment duration, low socioeconomic status, type of injector, lack of satisfaction with treatment effect, and inability to perceive the consequences of missing a dose (3,4). There is no standardized method to ensure adherence to GH therapy. Medication adherence has been investigated through GH prescription reviews, GH patient family questionnaires, serum insulin-like growth factor 1 (IGF-1) monitoring and urinary GH measurements. Despite having lower sensitivity, questionnaires are the simplest method for these types of investigations.

There was widespread disruption of routine hospital visits and monitoring of patients on GH therapy, dating from the start of the Coronavirus disease-2019 (COVID-19) pandemic, with the first case reported in Turkey on March 11, 2020. The global effect of the pandemic included widespread disruption of routine health services and interruption to patients' treatments.

The objective of this study was to investigate treatment adherence in patients on GH therapy during pandemic period through a questionnaire. This study was also designed to investigate potential therapeutic problems that might be experienced during the pandemic.

Methods

The survey was conducted by the Turkish Society for Pediatric Endocrinology and Diabetes. The authors prepared the questionnaire via online meetings. The centers tested the draft questionnaire before sending it. The study questionnaire included separate items for physicians and families (Supplementary Questionnaire 1). An email was sent to each member, asking them to provide the study questionnaire to all their patients on GH.

Patient data, date of diagnosis, age at diagnosis, age at the onset of treatment, age at last examination, parental educational attainment, monthly household income, diagnosis [isolated GH deficiency, multiple pituitary hormone deficiencies, Turner syndrome, skeletal dysplasia, small for gestational age (SGA), chronic kidney insufficiency, Prader-Willi syndrome], history of pituitary surgery, current GH doses, duration of GH therapy, person administering GH therapy (mother and/or father or patient), duration of missed doses, reasons for missed doses, problems associated with GH therapy, and missed dose data in the preceding year (during the pandemic) and effects of the COVID-19 pandemic were queried. Treatment adherence was categorized based on reported missed dose rates over the month preceding questionnaire completion, as follows: 0 to 5% (0-1 missed doses per month) was designated full

adherence; 5.1 to 10% (2 missed doses per month) was moderate adherence; and >10% (≥3 missed doses per month) was non-adherence. The growth velocity standard deviation (SD) score (SDS) calculation was made using the Baumgartner method (5).

This study was approved on June 2, 2021 (approval no. 2021-7/22) by the Ethics Committee of the Medical School of Bursa Uludağ University.

Statistical Analysis

The IBM Statistical Package for the Social Sciences, version 23 (IBM Inc., Armonk, NY, USA) were used to analyze study data. Descriptive statistics are presented as numbers and percentages for categorical variables and mean ± SD or median (range or interquartile range) for numerical data. Visual analytics (histograms and probability graphs and analytic methods (Kolmogorov-Smirnov or Shapiro-Wilk tests) were used to investigate normality of data set distribution. The chi-square test was used for two- or multiple-group comparison of categorical variables, as appropriate. For non-parametric data the Mann-Whitney U test was used for two-group comparisons and the Kruskal-Wallis test was used for multiple-group comparison. Spearman's correlation coefficient test was used for analysis of correlation between non-normally distributed numerical data. A p-value less than 0.05 was considered statistically significant.

Results

This study included questionnaire responses about 427 patients (56.2% males) from 13 sites. The median age at diagnosis, at the onset of the GH therapy and at study entry were 8.13 (0.13-16 years), 8.71 (0.3-16.1 years) and 12.03 (1.08-18 years) years, respectively. Treatment duration was 0 to 6 months in 8.2% (n=35), 6 to 12 months in 12.6% (n=54), 1 to 3 years in 39.6% (n=169) and more than 3 years in 39.6% (n=169) of patients. More than three quarters (77.8%) of patients were on daily GH replacement therapy and 22.7% (n=97) reported that they returned empty vials for the purpose of adherence monitoring. The monthly family income was less than the minimum wage in 22.2%, up to minimum wage x2 in 44%, from minimum wage x2 to minimum wage x4 in 23% and more than minimum wage x4 in 10.3% of the families. The training for GH injections was provided by a company nurse (70.3%; n=300), a hospital nurse (25.1%; n=107), or a physician (4.7%; n=20). GH replacement therapy was administered by parents (299 patients; 70%), or by the patients themselves (128 patients; 30%).

Indications for GH replacement therapy included isolated GH deficiency (61.4%), congenital or acquired multiple pituitary hormone deficiency (14%), Turner syndrome (7.5%), idiopathic GH deficiency (7.5%), SGA (2.8%), and others (6.8%), the latter grouping including Noonan syndrome, skeletal dysplasia, Prader-Willi syndrome, chronic kidney insufficiency, congenital adrenal hyperplasia, Silver Russell syndrome, cystic fibrosis, distal renal tubular acidosis and hypophosphatemic rickets (Table 1). The mean daily GH dose was 32.69 (13.8-67) µg/kg. GH dose by diagnosis is also shown in Table 1. Overall annual growth rate was 1.15±1.37 SDS on treatment during the pandemic. The growth rate increase by diagnosis is shown in Table 1.

The analysis of the adherence to GH therapy indicated full adherence in 70.3%, moderate adherence in 14.7%, and poor adherence in 15% of patients. The reasons for missing a dose (n=193) included forgetfulness (51.8%), treatment fatigue (13.5%), running out of medication (13.5%), overnight stays (3.5%), pen cartridge problems (2.8%), infections (1.6%) and "others" (1.6%). When asked if the COVID-19 pandemic had a negative effect on adherence, 22% (n=94) of the patients/families responded that it had. In those who responded positively, inability to get an appointment, inability to access the medication, hospital visit anxiety, having COVID infection, and treatment discontinuation were the specific mechanisms by which the COVID-19 pandemic impacted their adherence (Table 2).

When the data were analyzed by good, moderate, and poor adherence grouping, there was no significant intergroup differences in terms of sex, age at diagnosis, parental educational attainment, daily dose or annual growth rate. However, prolonged treatment duration, older age, and self-injection had a significant impact on the number of missed doses during the COVID-19 pandemic. Although patients who missed more doses tended to have a poorer annual growth rate, this association was not significant and there was no correlation. However, there was a significant negative correlation between the decrease in the annual growth rate SDS and longer treatment duration ($r=-0.202$, $p<0.01$). Furthermore, higher rates of missing doses correlated with duration of GH treatment duration ($r=0.129$, $p<0.01$, Figure 1).

Missed dose rate was higher in the groups with acquired multiple pituitary hormone deficiency and chronic kidney insufficiency (Figure 2).

When the present study compared to a previous Turkish study (19), it was noted that non-adherence rate was higher (15% vs. 7.4%, Figure 3).

Table 1. The diagnosis age, growth hormone dose and annual growth rate by growth hormone treatment indication

Growth hormone treatment indication	n	Diagnosis age (median) (min-max)	Age of onset of the treatment (median) (min-max)	Growth hormone doses (μg/kg/day) (mean ± SD)	Annual growth rate SDS (median) (min-max)
Isolated GH deficiency	262	9.4 (0.5-16)	10.15 (0.6-16)	31.65 ± 6.75	1.16 (-2.55-9.4)
Multiple pituitary hormone deficiencies (congenital)	50	4.75 (0.16-15)	6.35 (0.3-15.2)	30.65 ± 6.77	1.19 (-2.74-5.8)
Multiple pituitary hormone deficiencies (acquired)	10	8.7 (3-15.9)	10.95 (3.7-16.1)	28.5 ± 4.17	0.92 (-0.11-3.24)
Turner syndrome	32	7.05 (0.13-13.8)	7.35 (1.2-14.1)	44.75 ± 6.52	0.91 (-1.43-3.4)
Idiopathic GH deficiency	32	8.7 (0.58-12.9)	9.35 (0.83-14)	32.83 ± 5.23	0.65 (-1.41-4.33)
SGA	12	7.2 (3-12.8)	7.2 (3-13)	42.5 ± 13.12	1.04 (-1.22-5.22)
Noonan syndrome	9	7.7 (3.5-14.5)	7.7 (4.8-14.5)	35.74 ± 5.88	0.84 (-0.81-2.03)
Skeletal dysplasia	7	6 (3.7-9.5)	7 (3.7-10)	32.19 ± 9.17	0.17 (-0.65-2.81)
Prader-Willi syndrome	4	0.92 (0.3-3)	1.05 (0.3-3.3)	22.25 ± 8.96	-0.14 (-2.08-2.7)
Chronic kidney insufficiency	3	7.1 (2.2-9.5)	8.08 (7-9.5)	25 ± 8.66	0.22 (-0.63-0.64)
Congenital adrenal hyperplasia	2	7.85 (5-10.7)	7.85 (5-10.7)	23.4 ± 2.26	0.63 (0.57-0.69)
Silver Russel syndrome	1	1	1.3	28	2.1
Cystic fibrosis	1	11	11	37	0.68
Distal renal tubular acidosis	1	7.08	7.08	22	1.89
Hypophosphatemic rickets	1	9	9	25	-0.04

SGA: small for gestational age, GH: growth hormone, SDS: standard deviation (SD) score, min-max: minimum-maximum

Table 2. Adherence rate, non-adherence reason, and pandemic effect on adherence

Adherence rate	Good (70.3%), moderate (14.7%), poor (15%)
Reason of non-adherence (n = 193)	Forgetfulness (51.8%) Treatment fatigue (13.5%) Inability to access medication (13.8%) Pen problems (2.8%) Infection (1.6%) Others (1.6%)
Pandemic effect on adherence (n = 94)	Appointment problems (58.5%) Taking medication problems (17%) Anxiety about going to the hospital (11.7%) COVID-19 infection in patient or relatives (8.5%) Cessation of GH treatment by patient (4.3%)

COVID-19: Coronavirus disease-2019, GH: growth hormone

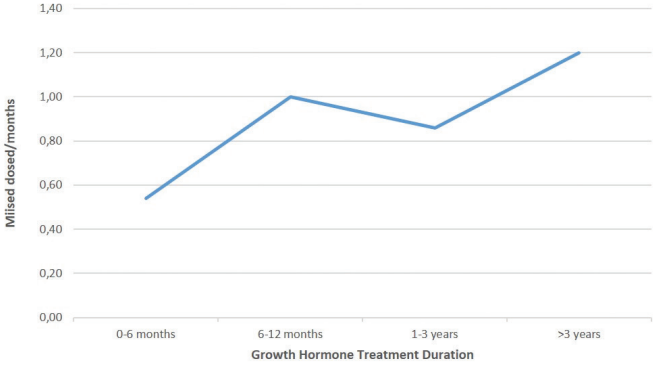

Figure 1. The missed dose rate by duration of growth hormone treatment

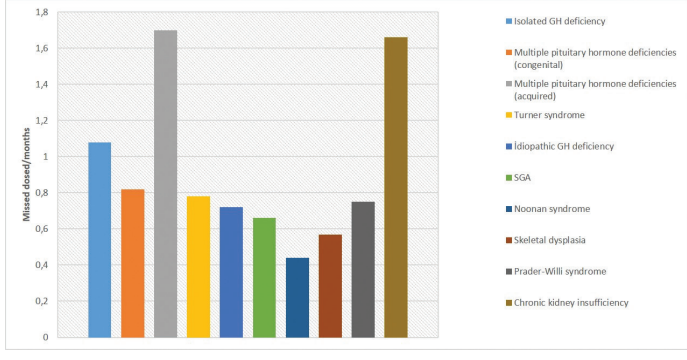

Figure 2. Missed dose per month rate according to the etiology of growth hormone treatment indication

SGA: small for gestational age, GH: growth hormone

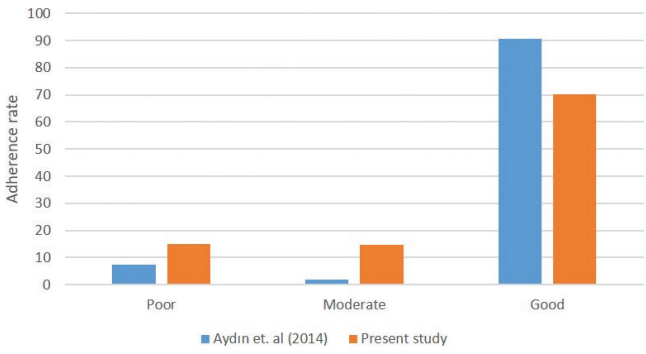

Figure 3. Comparison of GH adherence rate by category (good, moderate, poor) in the present study and in a previous Turkish study which was performed before the COVID-19 pandemic (17)

COVID-19: Coronavirus disease-2019, GH: growth hormone

Discussion

This multi-center, retrospective, questionnaire-based study provided data about adherence to GH replacement therapy in a Turkish pediatric population. However, as this study was conducted during the pandemic, study data may also be interpreted in the light of the effect of the Pandemic on GH treatment adherence.

The median age at diagnosis was lower than KIGS data. At the onset of GH therapy, the mean age in KIGS data was 10.7 years versus 8.7 years in the present study. The same trend was seen in GH indication subgroupings with KIGS reporting diagnosis ages of 9.1 years in IGHD, 6.2 years in congenital MPHD, 9.7 years in acquired MPHD, 9.7 years in ISS, 6.9 years in SGA, and 9.7 years in Turner syndrome (6,7,8). Lower median values in all subgroups might indicate earlier diagnosis in our cohort but the data from KIGS comes from many countries and settings and is therefore extremely heterogeneous. GH replacement doses were in line with those reported in the literature (9).

Results from adherence studies show wide variations due to methodological differences. Treatment adherence may be evaluated based on the number of missed injections since the last visit, or the number of missed injections per week or per month. In the present study poor treatment adherence was defined as ≥3 missed doses per month. Treatment adherence is a major factor in the efficacy of GH replacement therapy and poor adherence will also impact treatment costs. Previous studies indicated that non-adherence might result in medication waste of up to 15% (10). Early discontinuation rates have been reported in as much as 52% of patients on GH therapy (11) but an improvement of 10% in the adherence to GH therapy has been shown to result in an increase of 1.1 cm in the annual growth rate (1). The national survey of adherence to GH therapy in New Zealand concluded that a missed dose rate of more than one per week may lead to a significant decrease in linear growth. The height velocity (HV) SDS significantly decreased in 66% of children who missed more than one dose per week (12).

In a trial conducted in Israel between 2004 and 2015, adherence to GH treatment was evaluated based on proportion of days covered (PDC) defined as the days covered by filled medication/GH therapy days prescribed by physician, in 2,379 patients monitored through the healthcare system. A PDC of ≥80% was defined as good adherence. The rates of good adherence gradually decreased, being 78.2% in the first year, 75.6% in the second year and 68.1% in the third year (13). In a study using data from Easypod in 1,190 patients, treatment adherence was 93.7% in the first year and 70.2% in the fifth year (14). In keeping with these earlier reports, in the present study adherence rates decreased as the duration of GH therapy increased.

In a systematic review of 11 eligible studies conducted in 2022, reported 12-month adherence rates varied between 73.3% and 95.3% with a mean of 79.3% (15). In an earlier study from Turkey, Aycan et al. (16) reported an adherence rate of 92% in a series of 689 patients. A Turkish multicenter study evaluated 1-year adherence rate in a series of 216 patients (17). A missed dose rate higher than 10% was classified as poor adherence. The rate of poor adherence was reported to be 2.8% in the third month, 5.1% in the sixth month and 7.4% in the twelfth month. HV SDS was found to be increased with adherence and IGF-1 levels correlated with HV and HV SDS. Adherence rates were better in male patients. No differences were found in adherence rates between the subgroups when categorized by age, socioeconomic level and conditions underlying GH treatment requirement. Treatment adherence correlated with IGF-1. In the present study, the rate of poor adherence to GH therapy was 15%, and in keeping with earlier reports, increased non-adherence rates were associated with decreased growth SDS with statistical insignificance.

The missed dose rate was higher in the groups with acquired multiple pituitary hormone deficiency and chronic kidney insufficiency in the present study. This may be related to the characteristics of the diagnosis. There are many factors that will affect the GH response in both acquired multiple pituitary hormone deficiency and kidney insufficiency, such as excessive medication use, repeated surgery, interventions, and frequent hospitalizations.

Access to medication, patient and family motivation, and receipt of training may influence adherence rates (2). The response to GH therapy is influenced by several factors, mainly individual differences in response, age at diagnosis, current age, and medication dose (18). A study in 110 patients evaluated treatment adherence in the first two years. The rate of treatment adherence was 90% and there was a negative correlation between adherence and age, pretreatment growth rate and treatment duration, whereas a positive correlation was identified between the parental educational attainment and treatment adherence (19). Another factor that has been shown to negatively impact treatment adherence was a reluctance to undergo injections in adolescents, as these are largely self-administered. Treatment adherence rates were low and family support was shown to be important for adolescents requiring GH injections (20). Treatment fatigue is another reason for treatment discontinuation among patients or may lead to reductions in doses and dose frequency. Treatment fatigue is more likely to occur in older patients and patients who have longer durations of therapy (21).

Children may refuse to do the injections themselves while other factors that may influence treatment adherence include being in adolescence, treatment duration, low socioeconomic status, type of the injector used, reluctance to undergo injections, unsatisfactory treatment effect, and inability to perceive the consequences of missing a dose (22). Furthermore, needle visibility and painful injections (due to ingredients) have been reported as other issues associated with GH therapy (23). In the present study, adherence to GH therapy decreased as patient age and treatment duration increased. These findings are in keeping with earlier reports and suggest that there is still a need for novel strategies to counter these negative influences on GH treatment adherence.

Regional differences may also impact treatment adherence. A study conducted in Iran evaluated 169 patients and reported that high costs, inability to access medication, being anxious about long-term complications, treatment fatigue, unsatisfactory treatment outcome, and painful injections were the most prominent reasons for non-adherence (24). Problems associated with treatment adherence were reported in highly religious communities, based on data from a study of 2,263 patients assessed through the health system records in Israel. Thus report showed ultra-religious population had higher risk for non-adherence. Besides, a low adherence rate in the subgroup of patients starting GH replacement therapy before the age of eight years was found. Furthermore, treatment adherence got worse with increasing treatment duration (25).

In the present study, the reasons for missing a dose were mainly forgetfulness, treatment fatigue, running out of medication, overnight stays, pen cartridge problems, and infections. The last three reasons may have had a greater effect during the pandemic. The announcement of the Turkish Medicines and Medical Devices Agency of the Ministry of Health on "Access to Chronic Disease Medication without Prescription" on March 16, 2020 allowed access to medicines in our country. In studies conducted before the pandemic, the rate of non-adherence was found to be between 8% and 10%, considering methodological differences between these studies (16,17). The COVID-19 pandemic may have played a role in the increased rate of non-adherence in the present study. Non-adherence rates were higher during the COVID-19 pandemic, with 15% being classified as poor adherence and a further 14.7% being classified as moderate adherence.

There are limited studies on the impact of COVID-19 on adherence to GH therapy. In a study conducted in Italy, the mean good, moderate and low adherence rates were found to be 82.2%, 13.1% and 4.7% based on Morisky Medication Adherence Scale scores from 107 patients with a mean age of 11.3 years. The low adherence rate in adolescents was 5-fold higher than the rest of patients but this was consistent with pre-pandemic data (26). Another study conducted in Italy reported that treatment adherence was not negatively affected by changes in behavior mandated because of the pandemic (27). Treatment adherence was evaluated before and after the pandemic in a larger series from 18 countries using data recorded by the Easypod system. Adherence was evaluated by restrictions, school closures, and stay at home orders during the pandemic in 9,562 patients before the pandemic and 7,782 patients after the pandemic in a population of patients aged 6 to 18 years. Surprisingly, treatment adherence increased by 3% compared to the rates before the pandemic (28). Moreover, a study conducted in Saudi Arabia reported an adherence rate of 92%, in 130 patients with a mean age of 12.5 years (29).

Study Limitations

The strengths of the study include multicenter design, standardized questionnaire and forms for physicians. Limitations include survey design with self-reporting of some data, differences between the centers in terms of diagnostic and therapeutic approaches to GH deficiency and a lack of standardization in completing the forms. Serum IGF-1 levels were requested in the questionnaire. However, as the IGF-1 norms and measurement methods of each center were not standardized, they were not evaluated in the results section.

Conclusion

The results of this study showed the age at diagnosis to be lower than previously reported. GH replacement therapy was administered to patients at appropriate doses. However, the rate of non-adherence to GH therapy was higher than previously reported in Turkish studies. In keeping with earlier reports, older age and prolonged duration of treatment with GH contributed to increased non-adherence rates while the effects of the pandemic may have contributed to overall worse adherence in this study.

Ethics

Ethics Committee Approval: This study was approved on June 2, 2021 (approval no. 2021-7/22) by the Ethics Committee of the Medical School of Bursa Uludağ University.

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices - Concept - Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: Erdal Eren, Semra Çetinkaya, Yasemin Denkboy Öngen, Ummahan Tercan, Şükran Darcan, Hande Turan, Murat Aydın, Fatma Yavuzyılmaz, Fatih Kilci, Beray Selver Eklioğlu, Nihal Hatipoğlu, Kübra Yüksek Acinikli, Zerrin Orbak, Emine Çamtosun, Şenay Savaş Erdeve, Emrullah Arslan, Oya Ercan, Feyza Darendeliler.

Conflict of Interest: One author of this article, Feyza Darendeliler, is a member of the Editorial Board of the Journal of Clinical Research in Pediatric Endocrinology. However, she did not take part in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from different institutions. The other authors declared no conflict of interest.

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ORIGINAL ARTICLE

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Reversibility of Hyperglycemic States in Children with Obesity - Diagnostic Pitfalls in the Assessment of Glucose Metabolism in Children and Adolescents with Obesity

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What is already known on this topic?

The current rate of weight gain in the child and adolescent population is worrying. As the prevalence of obesity increases, so does the occurrence of associated comorbidities, one of which is disordered glucose metabolism. Unfortunately, the data regarding glucose metabolic alterations in children and adolescents with obesity is both limited and varies greatly from study to study.

What this study adds?

Therefore, in this study we established high prevalence of prediabetes in pediatric patients with obesity as well as high reversibility of this condition. Moreover, our study showed that every change in body mass index Z-score had a significant impact on changes in carbohydrate metabolism parameters and low density lipoprotein cholesterol.

Abstract

Objective: Disorders of glucose metabolism in children with obesity are less common than in adults. There is also evidence that they may be transient. The aims of this study were to determine the prevalences of impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and type 2 diabetes mellitus (DM2) and its reversibility in pediatric patients with obesity and to define the factors determining the reversibility of prediabetes or progression to diabetes.

Methods: Retrospective analysis included of young patients with obesity. Patients presented and were treated between 2000-2022 at a single center.

Results: The study included 573 (316 girls; 55.15%) Caucasian patients with median body mass index (BMI) Z-score of 3.95 (range 2.0-9.9) and median age 13.9 (2.9-17.1) years old. OGTT results were normal in 90.8% (n=520) and signs of prediabetes occurred in 9.2% (n=53); IFG 17%, IGT 88.7%, DM 0%. Among those who underwent OGTT twice (n=53), impaired glucose regulation was present in 9.3% (n=5) (IFG 40%, IGT 80%, DM 0%) at baseline and in 14.8% subject (n=8) (IFG 25%, IGT 50%, DM 25%) at follow-up after lifestyle modification only. After 12-36 months of follow up, in those with a history of IGT, 60% reverted to normal glucose tolerance, while IFG and IGT persisted in 20% and 20%, respectively, and none progressed to DM. The risk factors for progression of glucose metabolism disorders were increase of BMI Z-score, higher insulin levels and elevated homeostatic model assessment-insulin resistance.

Conclusion: IFG and IGT are common in pediatric patients with obesity, while the progression to DM2 is rare. Disorders of glucose metabolism have reversible character.

Keywords: Childhood obesity, glucose metabolism, type 2 diabetes

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Introduction

According to World Health Organization data, up to 35.5% of boys and 23.1% of girls at school-age in Poland are affected by overweight or obesity (1). Moreover, the current rate of weight gain in Polish children and adolescents is the greatest in Europe (2). As the prevalence of obesity increases, so does the occurrence of associated comorbidities. The most important impact on the risk for morbidity and premature death during adulthood is associated with a number of metabolic changes, including hypertension, dyslipidemia, atherosclerosis, steatohepatitis and glucose metabolism disorders (3). These glucose metabolism disorders are defined as impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). The progression from normal glucose tolerance (NGT) to type 2 diabetes mellitus (DM2) through IFG and IGT, also termed prediabetes, is well described in obese adult population (4). Although the underlying pathophysiology of this development is multifactorial, one of the most important factor is the balance between insulin sensitivity and insulin secretion (5). The data regarding the natural history of glucose metabolic alterations in children and adolescents with obesity is limited (6). It seems that, and in contrast to adults, both impaired fasting plasma glucose and 120 minute and later postload glucose measurements, as well as increased insulin resistance (IR), may be reversible in the pediatric population.

Aim

The aim of this study was to determine the prevalence of hyperglycemic states, including IFG, IGT and DM2 and its reversibility in Polish pediatric patients with obesity. In addition, the factors determining the reversibility of prediabetes or progression to diabetes would be investigated.

Methods

This was a retrospective study performed in the Department of Pediatric and Adolescent Endocrinology, Children's University Hospital in Kraków. Bioethics Committee of Jagiellonian University in Kraków was provided (protocol no: KBET/169/B/2014, date: 12.06.2014). The study population consisted of young Caucasians diagnosed and treated from 2000 to 2022. The inclusion criteria for the study were: body mass index (BMI) greater than or equal 95th percentile for age and sex; age below 18 years at the first visit; and undergoing a two hour oral glucose tolerance test (OGTT) (7). Of the patients, a proportion underwent a second OGTT within 12-36 months. A week prior to the OGTTs, patients received a normo-caloric, mixed diet. The OGTTs were performed after an overnight fast, at 08:00 a.m. after admission to the hospital. Two baseline samples were obtained for measurements of plasma glucose, insulin, total cholesterol, low density lipoprotein (LDL) and high density lipoprotein (HDL) fractions of cholesterol, triglycerides, uric acid and liver enzymes, alanine aminotransferase and aspartate aminotransferase. Thereafter, flavored glucose at a dose of 1.75 g per kilogram of body weight (up to a maximum of 75 g) was given orally and blood samples were obtained after 120 minutes for the measurement of plasma glucose and insulin. Glucose concentration was measured by the dry chemistry method with a Vitros 5.1.FF machine (Ortho-Clinical Diagnostics). Insulin levels were measured by an immunoluminometric assay (ADVIA Centaur, Siemens). Homeostatic model assessment-IR (HOMA-IR) was calculated using the standard formula: fasting insulin level (mU/L) × fasting glucose level (mmol/L)/22.5. Each time weight, height, BMI, pubertal stage, systolic and diastolic blood pressure (mean of three measurements) were recorded and standardized in all patients. Weight and height were measured using a standardized calibrated scale and blood pressure was measured using an automatic sphygmomanometer. The presence of hypertension was assessed by 'Paediatric office blood pressure calculator' by HyperChildNET COST Action (8). The diagnostic criteria for IFG was a fasting blood glucose of 100-125 mg/dL (5.6-6.9 mmol/L), IGT was defined as a 2-hour plasma glucose level between 140 and 199 mg/dL (7.8-11.0 mmol/L) and DM as a fasting glucose level of 126 mg/dL (7.0 mmol/L) or higher (two abnormal readings required) or a two-hour plasma glucose level of more than 200 mg/dL (11.1 mmol/L) (one abnormal reading required) (9). In the duration of follow up, the interventions consisted of dietary and psychological guidance in order to achieve sustainable, long-term lifestyle changes. The patients were not put on medications affecting glucose metabolism.

Statistical Analysis

To assess the differences between groups, an ANOVA or Kruskal-Wallis tests were used. Calculations were performed using the Statistica 13.0 software (10). A p value of < 0.05 was assumed to indicate statistical significance.

Results

The study population consisted of 573 patients, of whom 316 (55.15%) were girls. All patients were Caucasian and the median age was 13.9 (2.9-17.11) years. The median BMI Z-score of the study group was 3.95 (range 2.0-9.9). Most patients (n = 520, 90.8%) had normal results on OGTT. Prediabetes, including IFG and IGT was present in 9.2% subjects (n = 53). In this group, including 34 (64.15%)

girls, with a median age 14.5 years old (range 3.5-17.11) the median BMI Z-score was 5.1 (range 2.1-9.6) The mean fasting glucose level was 4.5 mmol/L (standard deviation: 0.7). IFG was detected in 1.6% cases (n = 9). The mean post-load glucose level was 5.9 mmol/L (standard deviation: 1.0). IGT was detected in 8.2% of participants (n = 47). Three exhibited both IFG and IGT. No case of DM was detected at baseline.

Of the participants, 54 (29 girls; 53.7%) with a median age of 12.5 years old (range 3.7-16.1), including 13 children with a positive family history for DM2, underwent OGTT twice within 12-36 month of baseline measurements (Table 1). Of these 54, 49 (91%) had normal OGTT results at the baseline and 46 (85%) had normal results at follow up. At the baseline, prediabetes was diagnosed in 9.3% subjects (n = 5). The mean value of fasting glucose level was 4.7 mmol/L (standard deviation: 0.4). IFG was detected in two cases (40%). The mean post-load glucose level was 5.8 mmol/L (standard deviation: 1.4). Prediabetes was detected in four participants (2 girls) (80%) - one patient presented with both IFG and IGT. In the repeated test, prediabetes was diagnosed in 14.8% subject (n = 8). The mean value of fasting glucose level was 4.8 mmol/L (standard deviation: 0.4). IFG was detected in two cases (25%). The mean post-load glucose level was 6.1 mmol/L (standard deviation: 1.8). IGT was detected in four participants (2 girls) (50%). Two cases (25%) of DM were diagnosed. These were a 17.6 year-old boy with BMI Z-score 4.4 (BMI 33.4) who had baseline plasma insulin level of 28 mIU/L (168.0 pmol/L) and a two-hour insulin of 207 mIU/L (1242.0 pmol/L) together with positive autoantibodies to islet cell and protein tyrosine phosphatase (IA2). The second patient was a 13.7 year-old boy with BMI Z- score 3.4 (BMI 29.6) who had undergone treatment for acute lymphocytic leukemia. After 12-36 months of follow up, in patients with baseline IGT (3 girls and 2 boys at median age 12.9 years old (range 11.2-14.7) and median BMI Z-score 3.4 (range 2.3-6.0), one child with a positive family history for DM2), 60% (n = 3) reverted to NGT (1 girl and 2 boys, median age 12.9 years old (range 11.5-14.7), median BMI Z-score 3.1 (range 2.3-6.0), 20% persisted as IFG (one girl with a positive family history of DM2, aged 11.2 years old, BMI Z-score 3.4, BMI 27.3) and 20% had IGT (one girl, age 14.3 years old, BMI Z-score 3.9, BMI 29.6); none progressed to DM. In 11.1% of all subjects (n = 6) a new disorder, not present at baseline, developed (Figure 1). One who developed IGT had a positive family

Table 1. Clinical and metabolic characteristics of those who underwent oral glucose tolerance test twice

	Clinical and metabolic characteristics of the study group	
Parameter (units) **Median and range or mean and (standard deviation) or % and number of patients**	**At the first assessment (n = 54)**	**At the second assessment (n = 54)**
Age (years)	12.5 (3.7-16.1)	14.1 (6.6-17.11)
Sex - Female	54% (n = 29)	54% (n = 29)
Tanner stage - Prepubertal	35% (n = 19)	14% (n = 8)
BMI Z-score	3.7 (2.1-10.0)	4.2 (0.5-8.1)
Hypertension - Isolated systolic hypertension - Hypertension 1 grade - Hypertension 2 grade	 14% (n = 8) 16% (n = 9) 0%	 5% (n = 3) 12.5% (n = 7) 2.5% (n = 1)
Glucose at time zero (mmol/L)	4.7 (0.4)	4.8 (0.4)
Glucose at 2-hours (mmol/L)	5.8 (1.4)	6.1 (1.8)
Insulin at time zero (mIU/L) **Insulin at time zero (pmol/L)**	24.0 (12.0) 134.5 (71.8)	20.1 (12.0) 127.2 (72.0)
Insulin at 2-hours (mIU/L) **Insulin at 2-hours (pmol/L)**	111.2 (73.4) 645.7 (440.6)	107.2 (78.4) 609.2 (473.7)
Total cholesterol (mmol/L)	4.2 (0.8)	4.3 (0.9)
LDL cholesterol (mmol/L)	2.6 (0.7)	2.7 (0.7)
HDL cholesterol (mmol/L)	1.1 (0.3)	1.1 (0.2)
Triglycerides (mmol/L)	1.6 (1.0)	1.6 (0.8)
Uric acid (µmol/L)	329.4 (69.4)	357.6 (72.6)
ALT (U/L)	34.9 (16.1)	32.6 (19.2)
AST (U/L)	29.0 (9.8)	28.6 (10.9)

HDL: high density lipoprotein, LDL: low density lipoprotein, BMI: body mass index, ALT: alanine aminotransferase, AST: aspartate aminotransferase

history for DM2. All the metabolic changes within these groups are presented in Table 2.

There was no significant correlation between the incidence of disorders of glucose metabolism at the first or second OGTT and age, BMI Z-Score, blood pressure, total cholesterol, LDL, HDL, triglycerides, uric acid, and liver transaminase levels. However, in children progressing from NGT to IGT, the increase of BMI Z-score (mean 3.1 vs. 4.0, median 3.1 vs. 4.1), increase of the insulin levels both at time zero [mean 17.3 mIU/L (103.8 pmol/L) vs. 29.7 mIU/L (178.2 pmol/L)

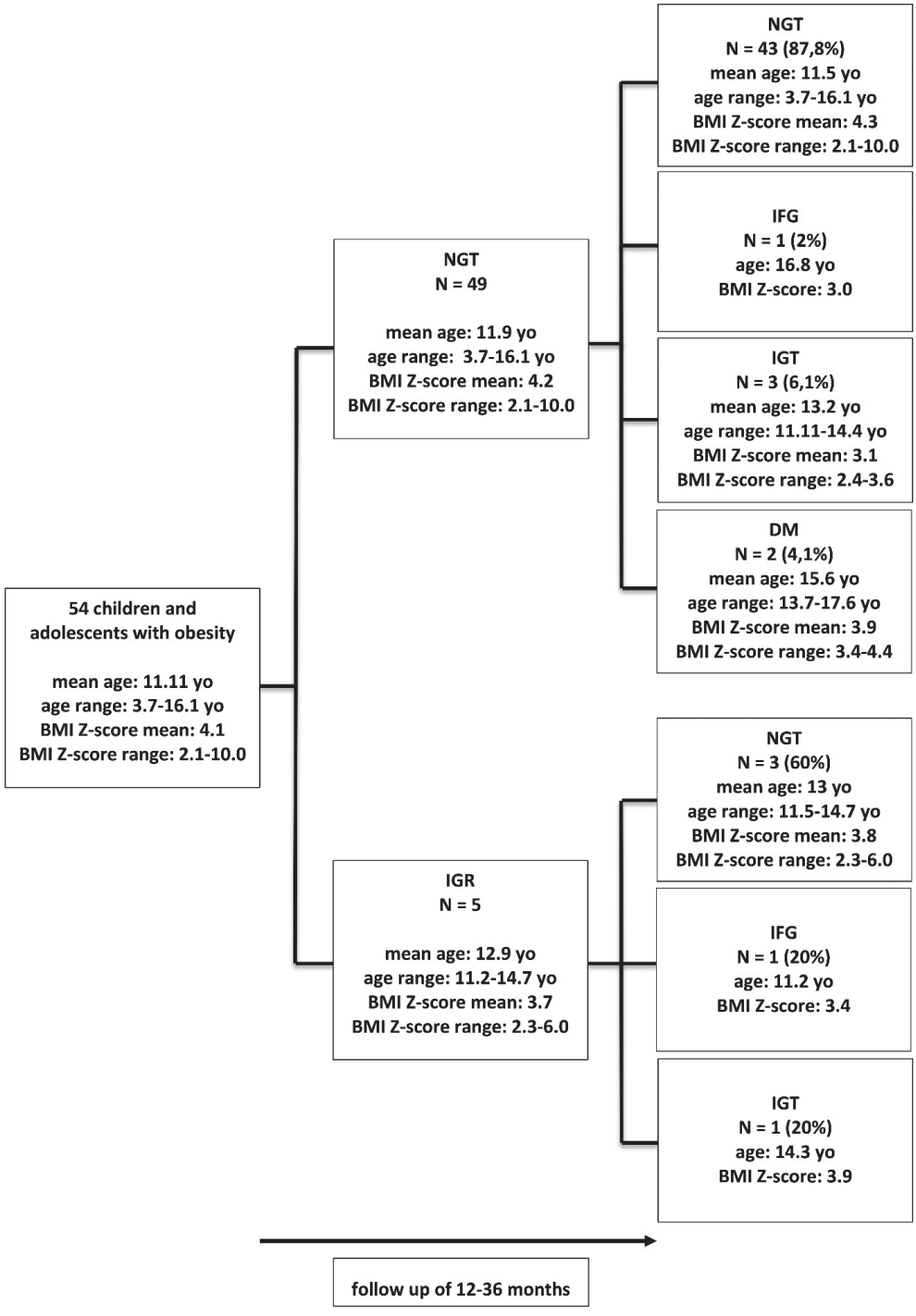

Figure 1. Trajectories of changes in carbohydrate metabolism disorders in the study group

NGT: normal glucose tolerance, IGR: impaired glucose regulation, IFG: impaired fasting glucose, BMI: body mass index, IGT: impaired glucose tolerance, DM: diabetes mellitus, yo: years old

Table 2. Detailed analysis of changes in a range of parameters between the groups with different glucose metabolic alterations

Parameter (units)	NGT -> IGR	IGR -> IGR	IGR -> NGT
Δ BMI Z-score	1.0	0.4	-0.3
Δ Glucose at time zero (mmol/L)	0.2	-0.5	-0.1
Δ Glucose at 2-hours (mmol/L)	3.5	0	-2.8
Δ Insulin at time zero (mIU/L)	12.4	-20.4	-3.9
Δ Insulin at time zero (pmol/L)	74.4	-81.6	-23.4
Δ Insulin at 2-hours (mIU/L)	114.6	9.9	-135.6
Δ Insulin at 2-hours (pmol/L)	687.6	59.4	-813.6
Δ HOMA-IR	3.0	-6.6	-0.9
Δ Total cholesterol (mmol/L)	0.2	-0.9	-0.1
Δ LDL cholesterol (mmol/L)	0.4	-0.4	0.1
Δ HDL cholesterol (mmol/L)	-0.1	-0.1	-0.1
Δ Triglycerides (mmol/L)	-0.3	-0.4	0.6
Δ Uric acid (μmol/L)	31.7	10.0	118.0
Δ ALT (U/L)	5.9	-34.7	2.0
Δ AST (U/L)	13.0	-20.0	-8.6

NGT: normal glucose tolerance, IGR: impaired glucose regulation, HDL: high density lipoprotein, LDL: low density lipoprotein, BMI: body mass index, HOMA-IR: homeostatic model assessment-insulin resistance, ALT: alanine aminotransferase, AST: aspartate aminotransferase

and at 2-hours (mean 92.5 mIU/L), (555.0 pmol/L) vs. 207.1 mIU/L (1242.6 pmol/L)] and decrease of insulin sensitivity measured by HOMA IR (3.8 vs. 6.8) were observed. In patients who had persistent prediabetes, the increase of BMI Z-score between the two OGTTs were evident but smaller than in previous group (mean 3.6 vs. 4.0), the insulin levels at time zero [mean 51 mIU/L (306.0 pmol/L) vs. 37 mIU/L (222.0 pmol/L)] and at 2-hours [mean 131.1 mIU/L (786.6 pmol/L) vs. 141 mIU/L (846.0 pmol/L)] were comparable. In those who reverted from IGT to NGT, the patients maintained comparable BMI Z-score (mean 2.7 vs. 2.8, median 3.1 vs. 2.8), there was a decrease in insulin levels both at time zero [mean 21.2 mIU/L (127.2 pmol/L) vs. 17.3 mIU/L (103.8 pmol/L)] and at 2-hours [mean 213.4 mIU/L (1280.4 pmol/L) vs. 77.7 mIU/L (466.2 pmol/L)] and an increase in insulin sensitivity between the assessments (mean HOMA IR 4.5 vs. 3.5). The 2-hour insulin level at presentation was particularly high in this group. Changes in values did not seem to be dependent on age or sexual development. In addition, there were significant correlations between the BMI Z-score changes between the first and the second assessment and changes in glucose level at time zero ($R = 0.3$, $p < 0.05$), insulin levels at time zero and 2-hours ($R = 0.3$ and $R = 0.4$, both $p < 0.05$), HOMA-IR ($R = 0.3$, $p < 0.05$) and LDL levels ($R = 0.3$, $p < 0.05$).

Discussion

As reported in the literature, the prevalence of IFG in children and adolescents with obesity ranges from 3.7% to 6.5%, whereas the prevalence of IGT ranges from 2.1% to 31% (11,12,13). In these studies, depending on ethnicity, BMI Z-score at baseline, the increase in the oral disposition index or the amount of gained weight during the follow up, the reversibility of prediabetes in pediatric patients with obesity varies from 45% to 65% (lifestyle modification alone) or even up to 84.2% (lifestyle modification and metformin) (13,14,15). In adults, these types of intervention achieve a reversal rate of 24% (16). Better results may also be attained but through second-line treatments, including glucagonlike peptide-1 analogues or bariatric surgery. These differences are explained by age-related changes leading to diminished insulin secretion (16,17). Obese youth usually exhibit higher insulin levels during OGTT but rates of progression from IGT to DM2 are lower in pediatric population than in adults. On the other hand, mean transition time from prediabetes to DM2 in children with obesity is more rapid due to faster deterioration of the beta cells. In addition, and in contrast to adults, because of reduction in insulin sensitivity particularly expressed at mid-puberty, some obese youth may improve their results on OGTT upon repeated testing at later pubertal stage (6). Glucose levels depend on many hormonal, neural and metabolic factors. This interplay is governed by insulin and glucagon secreted from beta and alpha cells respectively. The reduced insulin sensitivity in children with obesity is mostly associated with increased visceral, intra-hepatic and intramyocellular lipid deposition. To compensate for IR, both enhanced insulin secretion and reduced insulin clearance by the liver are activated. This continuous stress on the beta cells leads to firstly, their deterioration, and secondly, increasing glucose levels needed to stimulate them to secrete adequate amounts of insulin. It explains why children with obesity, and with IGT are comparable insulin resistant while those with NGT

may range from highly sensitive to markedly resistant. It has been shown that the ability of the child with obesity to compensate is limited by genetic and epigenetic factors in which ethnic background is the main clinical modifier. Genetic factors may affect both individual insulin sensitivity and beta cell response (6,13). The results of the present study showed the prevalence of prediabetes amounting to 9.2% (including 1.6% of IFG and 8.2% of IGT) in obese Polish youth and a low prevalence of DM2 (none at the first and two cases at the second assessment). Those two patients had additional risk factors for DM development, one with two autoantibodies and the other having treatment for acute lymphocytic leukemia. Furthermore, high reversibility of prediabetes has been shown, with 60% of the patients reverting from IGT to NGT. No one progressed from IGT to DM2. Although the study did not identify significant factors determining the reversibility of prediabetes or progression to diabetes, the results suggested that not only weight loss but also maintenance of BMI Z-score increases a chance of regression from IGT to NGT in our pediatric population. We found a significant correlation between BMI Z-score changes between the two assessments and changes in LDL levels. In Galderisi et al.'s (12) prospective study (39% Non-Hispanic White, 31% Non-Hispanic Black and 30% Hispanic) after median follow-up of 2.9 years, 65% of youth with IGT at baseline reverted to NGT, 27% adolescents had persistent IGT and 8% progressed to DM2. Participants who reverted from IGT to NGT showed a four-fold increase in the oral disposition index. Among youth with persistent IGT and those who progressed to DM2, insulin secretion declined. Non-Hispanic White ethnic background conferred an odds ratio of reverting from IGT to NGT five times greater than Non-Hispanic Black (13). In Weiss et al.'s (13) initial cohort (47% Caucasians, 32% African Americans and 21% Hispanics), all with IGT at baseline, 45% reverted to NGT, 30% remained IGT and 24.2% developed DM2. Those who exhibited improvements in glucose tolerance had lower BMI Z-scores at baseline and gained much less weight on follow-up than those who developed DM2. In youth who progressed to DM2, seven of eight subjects were African-American with a significantly higher BMI and BMI *Z*-score and they continued to gain excessive weight during the follow-up period. In Numbenjapon et al.'s (14) prospective study (100% Thai, all with IGT at baseline), apart from lifestyle modification, some patients received at least six months of treatment with metformin. In this group, 84.2% reverted to NGT. The patients who reverted also had lower LDL levels than patients who had persistent IGT, in both pre- and post-intervention periods (15). Conversely, in the adult population (55% Caucasian, 20% African-American, 16% Hispanic, 5% American Indian and 4% Asian-American, all with IFG and IGT at baseline) in the Diabetes Prevention Program, only 24% reverted to NGT over three years of follow up. Predictors for revertion to NGT were lower baseline fasting and 2-hour glucose, younger age and greater insulin secretion response to the oral glucose load. Intensive lifestyle modification and greater weight loss had significant and independent effects on revertion (16,18).

Study Limitations

A limitation of our study is that the classification of glucose tolerance relied on a single OGTT. Previous studies showed poor reproducibility of the OGTT in obese youth, in particular for the 2-hour plasma glucose (18). Another potential limitation is limited number of patients resulting in lack of some statistically significant correlations. The final limitation is the retrospective nature of the study.

Conclusion

IFG and IGT are common consequences of obesity in a Polish pediatric population, while the progression to DM2 is rare. Glucose metabolism disorders appear to be largely reversible in this pediatric population. Every change in BMI Z-score had a significant impact on changes in carbohydrate metabolism parameters and LDL cholesterol. However, these results should be validated by further, larger, prospective studies.

Ethics

Ethics Committee Approval: Bioethics Committee of Jagiellonian University in Kraków was provided (protocol no: KBET/169/B/2014, date: 12.06.2014).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: Anna Iwańska, Małgorzata Wójcik, Ewa Szczudlik, Anna Stępniewska, Concept: Anna Iwańska, Małgorzata Wójcik, Design: Anna Iwańska, Małgorzata Wójcik, Jerzy B. Starzyk, Data Collection or Processing: Anna Iwańska, Małgorzata Wójcik, Ewa Szczudlik, Anna Stępniewska, Analysis or Interpretation: Anna Iwańska, Małgorzata Wójcik, Literature Search: Anna Iwańska, Małgorzata Wójcik, Writing: Anna Iwańska, Małgorzata Wójcik, Jerzy B. Starzyk.

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Assessment of Thyroid Gland in Children with Point-of-Care Ultrasound (POCUS): Radiological Performance and Feasibility of Handheld Ultrasound in Clinical Practice

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What is already known on this topic?

Point-of-Care Ultrasound (POCUS) refers to the use of portable ultrasound machines to perform quick and focused ultrasound examinations at a patient's bedside or point-of-care. POCUS can be performed by all health workers with specific training to use POCUS.

What this study adds?

The radiological performance and feasibility of POCUS was investigated using a handheld ultrasound device (HHUSD) in children from the perspective of the thyroid gland. A pediatric endocrinologist, equipped with sufficient training in thyroid ultrasonography evaluation, incorporated the HHUSD as a routine tool for clinical examinations in outpatient settings, These can effectively assess normal thyroid tissue in pediatric patients. Moreover, the HHUSD proved to be useful in detecting thyroid pathologies.

Abstract

Objective: Point-of-Care Ultrasound (POCUS) refers to the use of portable ultrasound machines to perform quick and focused ultrasound examinations at a patient's bedside or point-of-care. POCUS can be performed by all health workers with specific training to use POCUS. The aim of this study was to investigate the radiological performance and feasibility of POCUS using a handheld ultrasound device (HHUSD) in children for examining the thyroid gland.

Methods: A pediatric endocrinologist performed thyroid imaging in children referred to our hospital with suspected thyroid disease using an HHUSD. The same children underwent ultrasonography (US) imaging using the same device by the first radiologist, and a second radiologist performed thyroid US using an advanced high-range ultrasound device (AHUSD) (defined as the gold-standard method) within two hours. The data obtained by the three researchers were compared with each other.

Results: This study included 105 patients [68.6% girls (n = 72)] with a mean age 12.8 ± 3.6 years. When the thyroid volume was evaluated, a strong correlation was found between the measurements of the three researchers (AA vs. MG: r = 0.963, AA vs. GT: r = 0.969, MG vs. GT: r = 0.963, $p < 0.001$). According to the Bland-Altman analysis for total thyroid volume, AA measured 0.43 cc [95% confidence interval (CI): -0.89-0.03] smaller than MG, and 0.11 cc (95% CI: -0.30-0.52) larger than GT, whereas MG measured 0.52 cc (95% CI: 0.09-0.94) larger than GT. When evaluated for the presence of goiter and nodules, a near-perfect agreement was found between the results of the three researchers (AA vs. GT; $\kappa = 0.863$, MG vs. GT; $\kappa = 0.887$, $p < 0.001$, and AA vs. GT; $\kappa = 1.000$, MG vs. GT; $\kappa = 0.972$, $p < 0.001$, respectively). When evaluated in terms of the longest axis of nodules, a high correlation was found between the measurements of the three researchers (AA vs. MG; r = 0.993, AA vs. GT; r = 0.996, MG vs. GT; r = 0.996, $p < 0.001$). When evaluated in terms of the final diagnosis, the evaluations of the three researchers showed excellent agreement with each other (AA vs. GT; $\kappa = 0.893$, MG vs. GT; $\kappa = 0.863$, $p < 0.001$, accuracy rate AA vs. GT: 93.3%; MG vs. GT: 91.4%).

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Conclusion: A pediatric endocrinologist, equipped with sufficient training in thyroid US evaluation, incorporated HHUSD examination as a routine clinical tool in an outpatient setting. It was shown that, they could effectively assess normal thyroid tissue in pediatric patients. Moreover, the HHUSD proved to be useful in detecting thyroid pathologies. However, it is important to note that for a more comprehensive evaluation of thyroid nodules, including detailed assessment and Thyroid Imaging Reporting and Data System (TIRADS) classification, patients should be referred to radiology departments equipped with AHUSD systems. These specialized devices, along with the expertise of radiologists, are essential for in-depth evaluations and accurate classification of thyroid nodules.
Keywords: Bedside ultrasound, handheld ultrasound device, hyperthyroidism, hypothyroidism, imaging, pediatric, point-of-care ultrasound

Introduction

Sonographic evaluation of the thyroid gland is routinely performed by radiologists to diagnose various thyroid diseases in children, including autoimmune thyroiditis, thyroid nodules, thyroid cancer, and goiter (1,2). Effective communication between clinicians and radiologists is central to accurate assessment and proper management of these conditions (3).

With advancements in ultrasonography (US) technology, the range of US products has expanded to include mobile devices that enable bedside examinations. These devices complement the traditional fixed US equipment found only in radiology departments. Commonly referred to as Point-of-Care Ultrasound (POCUS) in the literature, these devices are categorized into three types: laptop-associated devices, hand-carried systems, and handheld ultrasound devices (HHUSD). The introduction of these systems brings us closer to the realization of the "ultrasound stethoscope" concept (4).

The advances in POCUS technology are outpacing clinical studies conducted on the clinical performance of these technologies. Therefore, we believe that further research should be conducted to assess the clinical performance of POCUS. Such studies are crucial for advancing the development of this technology and realizing the concept of the ultrasound stethoscope. We propose that these studies should encompass various organ systems, different pathologies, and even different age groups. Clinical performance is influenced by both the device's capabilities and the proficiency of the user.

HHUSDs have gained significant interest and attention in recent years (5). Studies have shown that they have high diagnostic accuracy and can be used for various applications, such as abdominal, cardiac, and musculoskeletal imaging (6,7). However, there is no research specifically into the use of HHUSD for thyroid imaging in children. In the present study, we focused on evaluating the clinical performance of HHUSD in children with suspected thyroid disease. We conducted a comparison between one HHUSD and the gold standard advanced high-range ultrasound device (AHUSD) and aimed to assess the performance of pediatric endocrinologists who have received sufficient basic training in thyroid US. We evaluated the performance of a pediatric endocrinologist and two radiologists who had substantial expertise in conducting thyroid US. By considering both user expertise and device performance, we aimed to gain comprehensive insights into the clinical application of HHUSD for thyroid imaging in children.

Methods

Study Subjects

The university hospital where the study was conducted is a tertiary healthcare center located in a city with a population of over 1,000,000. It is the only pediatric endocrinology center in the city and provides services to all types of pediatric endocrinology patients. This study included pediatric patients aged 5-18 years who were referred to our hospital with suspected thyroid disease, including neck swelling, symptoms of hypothyroidism or hyperthyroidism, family history of thyroid diseases, and abnormalities in thyroid function tests.

Height was measured using a Harpenden stadiometer with a precision of 0.1 cm, while weight was measured using a scale with a precision of 0.1 kg (SECA, Hamburg, Germany). Subjects were weighed with all clothing removed, except for undergarments. Body mass index (BMI) was calculated by dividing weight (kg) by the square of height in meters (m^2). A BMI at or above the 95th percentile, according to data from healthy Turkish children was defined as obesity (8). Serum thyroid hormones, anti-thyroid peroxidase (TPO), and anti-thyroglobulin (TG) antibody levels were measured using standard methods on blood samples obtained from all patients under appropriate conditions.

Ultrasonography

After obtaining informed consent from the patients and their parents, a pediatric endocrinologist (AA) with 13 years of clinical experience in pediatric endocrinology and one year of thyroid US experience performed thyroid US imaging at the out-patient clinic using a Sonostar C5PL

HHUSD (Sonostar Technologies Co Ltd, Guangzhou, China). Within two hours, the same patients underwent thyroid US imaging using the same device (Sonostar C5PL HHUSD) by a radiologist (MG) with 15 years of experience and lastly, a detailed thyroid US imaging, using AHUSD Samsung RS80 (Gyeonggi-do, Republic of Korea) with LA2-9A linear probe by another experienced radiologist (GT) with 16 years of experience. The US data obtained by the pediatric endocrinologist and the two radiologists were noted in detail. The three dimensions of the thyroid gland (anterior-posterior "AP", medio-lateral "ML" and longitudinal "Long"), volume, parenchymal echogenicity, size of any nodules, composition (solid, semisolid, cystic), and echogenicity of the dominant nodule, and final sonographic diagnosis were recorded. The calculation of the volume for each lobe was done individually using the formula for an ovoid (depth x length x width x pi/6) (9). The total thyroid volume was then determined by adding the volume of both lobes together. Thyroid volume standard deviation score (SDS) was calculated using the normal range for Turkish children (10). Those with a total thyroid volume >2 SDS were considered to have a goiter. To isolate the operator from device performance, we separately compared the pediatric endocrinologist who used HHUSD to the radiologist using the same device (AA vs. MG) and we also compared the radiologist who used HHUSD to the radiologist who used AHUSD (MG vs. GT). Lastly, we compared the pediatric endocrinologist who used HHUSD to the radiologist who used AHUSD (AA vs. GT).

Institutional Ethics Committee of Aydın Adnan Menderes University was provided (protocol no: 2022/142, date: 25.08.2022).

Definitions used for Final Diagnosis (11)

Normal: Patients with euthyroidism, negative anti-TPO and anti-TG antibodies, and normal US findings.

Hashimoto's thyroiditis: Patients with euthyroidism/biochemical hypothyroidism, positive anti-TPO and anti-TG, and ultrasound findings consistent with thyroiditis.

Graves' disease: Patients with biochemical hyperthyroidism, positive anti-TPO and anti-TG, and ultrasound findings consistent with thyroiditis.

Obesity-related changes: Patients with euthyroidism, negative anti-TPO and anti-TG, and parenchymal heterogeneity on ultrasound.

Statistical Analysis

The statistical analysis for this study was performed using IBM Statistical Package for the Social Sciences statistics version 27.0 (IBM Corp., Armonk, NY, USA) and NCSS 11 (NCSS 11 Statistical Software, 2016, NCSS, LLC, Kaysville, Utah, USA, ncss.com/software/ncss). The normality of the data distribution was assessed through descriptive statistics, kurtosis and skewness coefficients, histograms, and the Shapiro-Wilk test. As the data were found to be non-normally distributed, Friedman's test was used to compare the three groups. Pearson and Spearman's correlation tests were employed for correlation analyses. Kappa and intraclass correlation coefficient (ICC) statistics were utilized to assess agreement. The agreement between the US measurements was evaluated using the Bland-Altman method. Type 1 error was determined as 5%.

Results

A total of 105 children [68.6% (n = 72) girls] were included. The mean age was 12.8 ± 3.6 years, with a median (range) of 13.0 (4.7-18.0) years. The reasons for referral were: 72.4% (n = 76) for abnormal thyroid function tests, 17.1% (n = 18) for neck swelling, 5.7% (n = 6) for symptoms of hyperthyroidism, and 4.8% (n = 5) for symptoms of hypothyroidism.The clinical and laboratory characteristics of the subjects are given in Tables 1 and 2.

There was a strong positive correlation between AA vs. MG, AA vs. GT, and MG vs. GT in terms of total thyroid volumes ($r = 0.963, 0.969, 0.963$, $p < 0.001$, respectively) (Table 3). The ICC for thyroid volumes was 0.963 [95% confidence

Table 1. The clinical characteristics of the subjects

	Mean ± SD or % (n)
Age (years)	12.8 ± 3.6
Gender (girl)	68.6 (72)
Height - SDS	0.1 ± 1.2
Weight - SDS	0.2 ± 1.5
Body mass index - SDS	0.2 ± 1.4
Complaint	
Abnormalities in thyroid tests*	72.4 (76)
Neck swelling	17.1 (18)
Symptoms of hyperthyroidism	5.7 (6)
Symptoms of hypothyroidism	4.8 (5)
Final diagnosis	
Normal	23.8 (25)
Hashimoto thyroiditis	40.0 (42)
Nodule + Hashimoto's thyroiditis	16.2 (17)
Graves' disease	7.5 (8)
Obesity related changes	6.7 (7)
Solitary nodule	5.6 (6)

*Patients were referred from another hospital due to abnormal thyroid tests (high or low TSH, high fT3) during screening.
SDS: standard deviation (SD) score, TSH: thyroid-stimulating hormone

interval (CI): 0.949-0.974]. In the Bland-Altman analysis performed in terms of the correlation of detailed US measurements (right thyroid volume, left thyroid volume and total thyroid volume), a strong correlation was found between the measurements. The difference between the measurements in terms of total thyroid volume was -0.43 [95% CI: (-0.89)-0.03] for AA vs. MG; 0.11 [95% CI: (-0.30)-0.52] for AA vs. GT; and 0.52 (95% CI: 0.09-0.94) for MG vs. GT (Figure 1, Table 3).

When evaluated in terms of the presence of goiter, the

Table 2. Laboratory characteristics of the subjects

	Median	Minimum	Maximum	25 P	75 P
Free T3 (pg/mL)	3.4	0.9	20.0	3.1	3.8
Free T4 (ng/dL)	1.0	0.5	3.8	0.9	1.1
TSH (uIU/mL)	1.7	0.0	49.0	1.0	4.1
Anti-TPO (IU/mL)	3.0	0.0	1000.0	3.0	579.0
Anti-TG (IU/mL)	3.0	0.0	1000.0	3.0	60.0

Anti-TPO: anti-thyroid peroxidase, Anti-TG: anti-thyroglobulin

Table 3. Comparison of thyroid volumes

		Right thyroid volume	Left thyroid volume	Total thyroid volume
	Mean of differences	-0.46	0.03	-0.43
	Upper limit of 95% CI	-0.80	-0.18	-0.89
AA vs. MG	Lower limit of 95% CI	-0.13	0.25	0.03
	r	**0.934**	**0.968**	**0.963**
	p	**< 0.001**	**< 0.001**	**< 0.001**
	Mean of differences	0.08	0.03	0.11
	Upper limit of 95% CI	-0.19	-0.19	-0.30
AA vs. GT	Lower limit of 95% CI	0.35	0.26	0.52
	r	**0.956**	**0.960**	**0.969**
	p	**< 0.001**	**< 0.001**	**< 0.001**
	Mean of differences	0.53	-0.02	0.52
	Upper limit of 95% CI	0.23	-0.28	0.09
MG vs. GT	Lower limit of 95% CI	0.84	0.24	0.94
	r	**0.938**	**0.955**	**0.963**
	p	**< 0.001**	**< 0.001**	**< 0.001**

CI: confidence interval

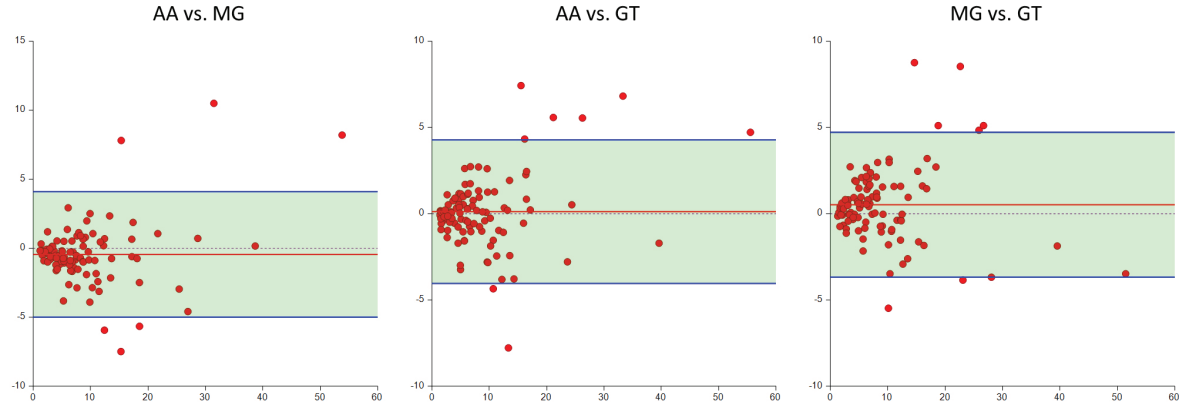

Figure 1. Bland-Altman analysis for total thyroid volumes. Red line (-0.43, 0.11, 0.52) is the bias (average of the differences between total thyroid volumes of AA vs. MG, AA vs. GT, MG vs. GT; respectively). Blue lines [(-5.00)-4.14, (-4.04)-4.26, (-3.67)-4.71] are the limits of agreement, respectively

measurements of all three researchers showed near-perfect agreement (AA vs. MG; $\kappa = 0.887$, AA vs. GT; $\kappa = 0.863$, MG vs. GT; $\kappa = 0.889$, $p < 0.001$). The measurements of all three researchers demonstrated substantial agreement when assessing parenchymal echogenicity (AA vs. MG; $\kappa = 0.685$, AA vs. GT; $\kappa = 0.771$, MG vs. GT; $\kappa = 0.730$, $p < 0.001$). A near-perfect agreement was again observed among all three researchers' evaluations when assessing the presence of nodules (AA vs. MG; $\kappa = 0.972$, AA vs. GT; $\kappa = 1.000$, MG vs. GT; $\kappa = 0.972$, $p < 0.001$) (Table 4).

When evaluated for the presence of nodules and considering AHUSD as the gold standard method, nodules were detected in a total of 23 patients (22%). Among these cases, 43.5% (n = 10) were identified as cystic nodules, 21.7% (n = 5) exhibited semisolid nodules, and 34.8% (n = 8) presented with solitary nodules (AA vs. MG; $\kappa = 0.864$, AA vs. GT; $\kappa = 0.864$, MG vs. GT; $\kappa = 0.858$, $p < 0.001$). The features that can predict malignancy in solid nodules, such as irregular margins and microcalcifications, could not be evaluated with HHUSD. When evaluated in terms of the last diagnosis according to the AHUSD 23.5% (n = 25) of the patients were diagnosed as normal, 40.0% (n = 42) had Hashimoto's thyroiditis, 16.2% (n = 17) had coexistence of nodules and thyroiditis, 7.5% (n = 8) had Graves' disease, 6.7% (n = 7) had obesity-related changes, and 5.6% (n = 6) had solitary nodules (Table 1). The final diagnoses of all three researchers showed near-perfect agreement (AA vs. MG; $\kappa = 0.871$, AA vs. GT; $\kappa = 0.910$, MG vs. GT; $\kappa = 0.884$, $p < 0.001$). The ICC for the long axis of the nodule was 0.995 (0.989-0.998). In the Bland-Altman analysis performed in terms of the correlation of nodule size, a strong correlation was found between the measurements. The difference between the measurements of the nodule size was 0.49 [95% CI: (-0.30)-1.27] for AA vs. MG; 0.26 [95% CI: (-0.32)-0.83] for AA vs. GT; and -0.25 [95% CI: (-0.87)-0.38] for MG vs. GT (Figure 2).

Table 4. Comparison of goiter, parenchymal heterogeneity, and nodule

		Goiter			Parenchymal heterogeneity			Nodule		
		Radiologist handheld US (MG)		Kappa p	Radiologist handheld US (MG)		Kappa p	Radiologist handheld US (MG)		Kappa p
		Positive	Negative		Positive	Negative		Positive	Negative	
Pediatric endocrinologist (AA)	Positive	71	4	0.887	66	8	0.685	82	0	0.972
	Negative	1	29	< 0.001	6	25	< 0.001	1	22	< 0.001
		Radiologist standard US (GT)			Radiologist standard US (GT)			Radiologist standard US (GT)		
Pediatric endocrinologist (AA)	Positive	71	4	0.863	69	5	0.771	82	0	1.000
	Negative	2	28	< 0.001	5	26	< 0.001	0	82	< 0.001
Radiologist handheld US (MG)	Positive	70	2	0.889	67	5	0.730	82	1	0.972
	Negative	3	30	< 0.001	7	26	< 0.001	0	22	< 0.001

US: ultrasonography

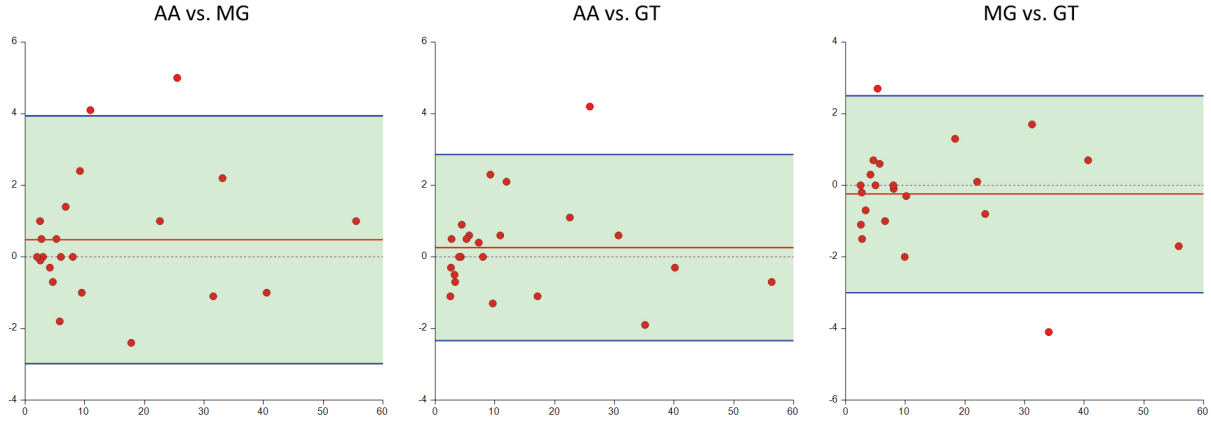

Figure 2. Bland-Altman analysis for nodule long axes. Red line (0.49, 0.26, -0.25) is the bias (average of the differences between nodule long axes of AA vs. MG, AA vs. GT, MG vs. GT; respectively). Blue lines [(-2.96)-3.94, (-2.34)-2.86, (-2.97)-2.53] are the limits of agreement, respectively

Discussion

The results of the present study showed a high correlation and near perfect agreement between the measurements and evaluations of the three researchers in terms of three-dimensional measurements of the thyroid, thyroid volume, presence of goiter, presence of nodules, the longitudinal plane of nodules, and final diagnosis. Additionally, the Bland-Altman analysis showed that the differences in measurements between the researchers were within acceptable limits. This study is the first clinical trial demonstrating the effectiveness of HHUSD performed by clinicians in the thyroid US examination in children.

Thyroid US is a gold standard imaging modality in the evaluation of thyroid nodules and other thyroid disorders (12,13). However, the accuracy of this imaging modality depends on several factors, including the experience and skill of the user, the ability to integrate US findings with the patient's clinical history and examination, and the quality of the US device. US is a highly accurate modality when performed by an experienced user (14). However, clinical findings are an important part of the accurate final diagnosis, so these findings need to be shared between the clinician and the radiologist. Several studies have shown that integrating clinical information with US findings can improve the diagnostic accuracy of thyroid US (15,16). Due to the significant outpatient workload in radiology departments, obtaining a US examination can pose challenges. If HHUSD were part of the clinical evaluation by the clinician, this would eliminate the unnecessary workload for the radiology departments (17,18).

POCUS systems have become an integral part of patient evaluation in departments, such as emergency services, anesthesia, intensive care, and general surgery, where triage or urgent assessment is required. They are now incorporated into the teaching curriculum and guidelines of these specialties (19). The widespread availability of HHUSD has made accessing POCUS systems easier, leading to increased use of these systems (20). At this point, the question arises as to whether POCUS systems should be included as part of the physical examination during routine outpatient services, not just for patients requiring urgent evaluation. If HHUSD becomes part of the examination, it would enable radiology departments to provide intensive outpatient services to minimize unnecessary patient burden and ensure triage for patients who require this service (21). Consequently, this could reduce the number of unnecessary diagnostic tests and decrease the unnecessary costs imposed on the healthcare system.

The results of the present study indicate that the effective use of POCUS systems relies on two essential components. The first component pertains to the appropriateness of the HHUSD used for the specific organ system, while the second component relates to the user's adequate knowledge and skill level for conducting sonographic examinations. Our study demonstrated that HHUSD when employed by a properly trained non-radiologist clinician exhibits a strong correlation with the gold standard, which involves the use of an AHUSD by an expert radiologist specialized in sonography. Our results showed that the HHUSD method proved to be effective in detecting thyroid nodules and distinguishing between cystic and solid nodules.However, limitations in the device's resolution capabilities hindered its ability to adequately address features indicative of malignancy in solid nodules, such as the presence of microcalcifications and irregular margins. Given the limited number of patients with solid nodules in the present study, making definitive conclusions would be unreliable and there is a need for more comprehensive research on the role of these devices in Thyroid Imaging Reporting and Data System (TIRADS) scoring. Our findings have highlighted the current limitations of HHUSD and underscore the necessity for further advances in their development. Furthermore, specialties seeking to integrate these systems into routine clinical practice must ensure that proper training is incorporated into their educational programs. In this regard, we propose the inclusion of radiology rotations during pediatric endocrinology training for these specialties and the implementation of certification programs that require periodic retraining in this area following the completion of residency.

Our study possesses several notable strengths. Firstly, we utilized two distinct US systems, providing a comparative analysis between them. Secondly, the participation of a pediatric endocrinology clinician along with two radiologists in the study ensured diverse perspectives and expertise in the evaluation process. Additionally, our patient group consisted of both individuals with normal thyroid function and those with various thyroid pathologies, enabling a comprehensive comparison of normal and pathological data. Furthermore, we implemented an internal evaluation process wherein measurements were independently assessed by the observers at different times, ensuring a blind evaluation unaffected by each other's observations. These strengths collectively enhance the robustness and validity of our study.

Study Limitations

Our study does have certain limitations that should be acknowledged. Firstly, it is important to note that there are various generations of HHUSD available in the market, but we

utilized a single standard device for our evaluation. Therefore, the findings may not directly generalize to other generations or models of HHUSD. Secondly, our study focused on evaluating the performance of the HHUSD specifically when used to evaluate thyroid tissue, which is a superficial tissue. It is worth mentioning that the performance of US devices may vary when imaging deeper tissues. Thus, our study's results may not fully reflect the performance of HHUSD in imaging deep tissues. Considering these limitations, future studies should explore the performance of different generations or models of HHUSD on various tissue types, including deeper structures, to provide a more comprehensive understanding of their capabilities and limitations.

Conclusion

The present study demonstrated that when a pediatric endocrinologist, equipped with sufficient training in thyroid US evaluation, incorporates the HHUSD as a routine tool for clinical examinations in outpatient settings, they can effectively assess normal thyroid tissue in pediatric patients. Moreover, the HHUSD proved to be useful in detecting thyroid pathologies. However, it is important to note that for a more comprehensive evaluation of thyroid nodules, including detailed assessment and TIRADS classification, patients should still be referred to radiology departments equipped with AHUSD. These specialized devices, along with the expertise of radiologists, are essential for in-depth evaluations and accurate classification of thyroid nodules.

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Ethics

Ethics Committee Approval: Institutional Ethics Committee of Aydın Adnan Menderes University was provided (protocol no: 2022/142, date: 25.08.2022).

Informed Consent: Informed consent was obtained from those included in the study.

Authorship Contributions

Surgical and Medical Practices: Ahmet Anık, Göksel Tuzcu, Concept: Ahmet Anık, Mustafa Gök, Design: Ahmet Anık, Mustafa Gök, Data Collection or Processing: Mustafa Gök, Göksel Tuzcu, Analysis or Interpretation: Ahmet Anık, Göksel Tuzcu, Literature Search: Mustafa Gök, Göksel Tuzcu, Writing: Ahmet Anık, Mustafa Gök, Göksel Tuzcu.

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ORIGINAL ARTICLE

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Estrogen Receptor 1 Gene Polymorphism and its Association with Idiopathic Short Stature in a North Indian Population

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What is already known on this topic?

In the hypothalamic-pituitary-gonadotrophin (HPG) axis, estrogen plays a key role in bone maturation regulation and growth plate closure. Estrogen is an important hormone in the HPG axis, and because of its association with bone maturation, modulates growth. Estrogen is involved in male and female reproduction but also affects other systems, including the neuroendocrine, vascular, skeletal, and immune systems. Single nucleotide polymorphisms in estrogen receptor 1 *(ESR1)* gene are known to be involved in growth and development.

What this study adds?

We investigated correlations between growth/height-related genetic polymorphisms in the *ESR1* gene and the phenotype of idiopathic short stature (ISS) in order to better understand the etiology of this enigmatic growth disorder that accounts for a significant portion of pediatric endocrine practice. Our study showed that the CC genotype at rs6557177 and also TT genotype of rs543650 of *ESR1* individually constituted risk factor for developing ISS in North Indian children. We have shown that rs543650 is in linkage disequilibrium (LD) with rs2234693 and rs9340799, rs2234693 and rs9340799 also showed strong LD (D': 0.89).

Abstract

Objective: In the hypothalamic-pituitary-gonadotrophin axis, estrogen plays a key role in the regulation of bone maturation and growth plate closure. This study was designed to explore the link between single nucleotide polymorphisms (SNPs) in the estrogen receptor 1 *(ESR1)* gene with idiopathic short stature (ISS) susceptibility in a North Indian population.

Methods: Four SNPs of *ESR1* (rs543650, rs6557177, rs2234693 and rs9340799) were genotyped by Sanger sequencing in ISS patients and controls. Linkage disequilibrium (LD) and haplotyping were done by SNPStat and SHEsisPlus software. The extent of LD was determined by calculating D' and R^2 values in SNP paired combinations.

Results: Fifty-two ISS patients were compared with 68 controls. A significant positive association was found between rs6557177 and rs543650 genotype and ISS susceptibility. The frequencies of the rs6557177 CC genotype [p = 0.030; odds ratio (OR) = 0.13; 95% confidence interval (CI): 0.01-1.10] and rs543650 genotype TT (p = 0.043; OR = 0.29; 95% CI: 0.09-0.92) were increased in the ISS

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group compared with controls. However, no significant correlation was observed between clinical parameters of patients and these SNPs. rs543650 showed strong LD with rs2234693 and rs9340799, similarly rs2234693 and rs9340799.
Conclusion: Our study showed that the CC genotype at rs6557177 and TT genotype at rs543650 of *ESR1* constituted a risk factor for developing ISS in North Indian children. These findings may lead to a better understanding of the SNPs associated with ISS susceptibility.
Keywords: Genotype, estrogen receptor 1 gene, haplotype, idiopathic short stature, linkage disequilibrium, single nucleotide polymorphism

Introduction

Height of less than two standard deviations (SD) from the corresponding mean for a given age, gender, and population is considered short stature (1). The worldwide prevalence of short stature is approximately 3-5% (2). Causes of short stature vary widely. Physiological short stature can be familial or constitutional, while pathological causes can be systemic or secondary to environmental factors. Once these factors are ruled out or excluded, the condition is diagnosed as idiopathic short stature (ISS). Looking further into the genetic factors, numerous monogenic and syndromic causes for short stature have been well established and genes concerned with growth regulators have emerged as major culprits. Among the growth regulators, growth hormone (GH) performs a major and vital role and variation in its sequence may result in GH deficiency which subsequently leads to short stature (3,4,5,6). There are many other genes, such as *IGF1*, *SHOX*, *GHRHR* and *PROP1*, in which sequence variations, chromosomal abnormalities, copy number changes and impaired genomic imprinting are known to contribute to short stature (5).

Two axes, namely the hypothalamic-pituitary-gonadal (HPG) axis and the GH-insulin like growth factor (GH-IGF) axis were thought to play key roles in the regulation of growth, according to previously published data (7). Any kind of abnormality in the latter GH-IGF axis is well known to result in short stature (8), especially with mutations in the genes *GHR* (9), *IGFALS* (10) and *IGF-1R* (11,12) but there is paucity of data on association of short stature risk with the HPG axis. However, recent findings have revealed that the GH-IGF-1 axis is just one of many regulatory systems that control chondrogenesis in the growth plate (13).

Estrogen is an important hormone in the HPG axis, that is associated with the regulation of bone maturation and growth. Estrogen is involved in male and female reproduction but also affects other systems, including the neuroendocrine, vascular, skeletal, and immune systems. The *ESR1* gene codes for the estrogen receptor (ER) and is located on the long arm of chromosome. The size of *ESR1* is 300Kb and it contains eight exons. It has shown strong association with stature via Genome-wide linkage analysis. The role of estrogen is already well established in skeletal development and growth in females. Recently, it has been recognised to affect body height in males as well (14).

Single nucleotide polymorphisms (SNPs) in growth-associated genes are considered an important cause of short stature. There is a possibility that other, more common variants of *ESR1* with smaller effects could affect body height in the general population, based on the powerful effects of rare sequence variations in *ESR1*. Therefore, we hypothesized that *ESR1* could be a factor that controls the tempo of growth and stature. A literature review was performed for similar earlier studies, which corroborated the hypothesis. El-Hefnawy et al. (15) studied the rs827421 SNP in *ESR1* and found that the GG genotype and the G allele were significantly dominant among children with constitutional delay of growth and puberty (CDGP). Another study by Quigley et al. (16) on the rs2234692 SNP in the same gene, showed similar results in children with ISS. Kang et al. (17) studied three SNPs in *ESR1*, namely rs3778609, rs12665044 and rs827421, and found positive results in SNP rs827421 only. We chose four rarely studied polymorphisms (rs543650, rs6557177, rs2234693 and rs9340799) (17) in the *ESR1* gene for our study. We also assessed patterns of linkage disequilibrium (LD) of these selected SNPs.

Methods

Subjects

This was a prospective study done in a tertiary care hospital from July 2021 to June 2023. This study was approved by Postgraduate Institute of Medical Education and Research Institutional Ethics Committee (ref: NK/7784/MD/527, date: 28.09.2021). The power of the study was calculated using Quanto (http://biostats.usc.edu/Quanto.html). Children with ISS i.e., with heights that are less than two SDs below the mean height for their age, gender, and population, with physiological, environmental, systemic and genetic causes ruled out, were enrolled. This included normal GH levels.

Control Group

The control group included children who had a height within +/- 2 SD of the mean height for normal. The control group was comprised of children who came to our hospital

in the same time frame for routine immunization or for outpatient management of transient viral illnesses and siblings of admitted children.

Inclusion Criteria for ISS

1. A height less than 2 SDs below the mean height of children of the same age and gender;

2. Normal routine investigation of blood (complete blood count), thyroid function, liver, and kidney function;

3. The weight and length at birth should fall within the normal range;

4. There shouldn't be any additional inherited metabolic diseases, congenital skeletal anomalies, chromosomal abnormalities, SHOX mutation, or chronic illnesses.

Exclusion Criteria

Short stature with an identified etiology.

Informed consent: For children fulfilling inclusion and exclusion criteria, parents/guardian was approached for enrolment in the study. Prior to enrolment in the study, written informed consent was obtained after providing a detailed information sheet. Assent was sought from children above eight years of age.

Evaluation

After taking fully informed consent, all individuals diagnosed with ISS were included in the study.

1. A detailed case review was performed, including review of family history, previous medical records and investigation reports;

2. A thorough anthropometric evaluation was performed, including measurements of the body mass index (BMI), height, arm span, upper-to-lower segment ratio, arm span to height, and sitting height to height;

3. Detailed physical examination was done to look for any deformity or dysmorphism, specifically for skeletal abnormalities and facial features.

Sample Collection

After written consent, 2-4 mL of EDTA blood was collected for DNA extraction, from the patients and controls.

Molecular Analysis

Genomic DNA was extracted from peripheral blood by QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany).

Genotyping

Sanger sequencing was done for genotyping and the primers used are available on request. Applied Biosystems' BigDye® Terminator Cycle Sequencing kit, v3.1, was used to perform direct DNA sequencing on purified amplicons using forward and/or reverse primers in a polymerase chain reaction. The results were analysed using ABI-3500xL Genetic Analyzer (Applied Biosystems, California).

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) for Windows version 14.0, was used for statistical analysis (Chicago, IL, USA). The frequencies of alleles and genotypes were calculated. For each polymorphism under consideration, the Hardy Weinberg equilibrium (HWE) was calculated. Fisher's exact test and ANOVA were used to compare gene polymorphisms within groups and between subgroups, respectively. Subgroups were further examined using the t-test and Wilcoxon Mann-Whitney analysis, depending on whether the data was parametric or not. Calculating odds ratios (ORs) and 95% confidence intervals (CI), a p value of 0.05 was set as cut-off for statistical significance. For genotype frequencies, logistic regression was used and for allele frequency, and cross-tabular calculation of OR and chi-square values were calculated, using SPSS, version 25. To rule out the effect of confounding factors on analysis, all the results were adjusted by the confounding factors, including age, gender, height, and BMI. Haplotype analysis was done using SNPStats online software (18) for all four SNPs. The extent of LD was determined in SNP combinations by calculating D' and R^2 values. The SHEsisPlus (http://shesisplus.bio-x.cn/SHEsis.html) and SNPStats (https://www.snpstats.net/start.htm) online tools were used to assess the LD between *ESR1* haplotypes (rs543650, rs6557177, rs2234693 and rs9340799).

Results

There were 52 patients with ISS and the control group included 68 children (Table 1). Among the ISS cases, 53.8% were male, while in the control group 64.7% of the participants were male. The median age of cases with ISS was 11 (8-13) years and controls was 8 (5.75-11) years. However, there were no significant differences in BMI in the control and patient group. Mean height SD score (SDS) in the ISS group was significantly less than that of the control group ($p < 0.001$), as expected (Table 1).

Genotyping

The genotypes were found to be in HWE, in cases and controls, for all four SNPs investigated.

Table 1. Descriptive clinical and laboratory data of studied cases and controls

Parameters	Group		p value
	Cases (n = 52)	Controls (n = 68)	
Gender			0.229
Male	28 (53.8%)	44 (64.7%)	
Female	24 (46.2%)	24 (35.3%)	
Median age (years)	11 (8-13)	8 (5.75-11)	NS
Weight (kg)	24.88 ± 10.58	28.41 ± 13.41	0.312
Weight for age (SDs)	-1.91 ± 1.24	-0.40 ± 1.16	**< 0.001**
Height (cm)	120.40 ± 18.71	127.35 ± 21.94	0.064
Height for age (SDs)	-2.78 ± 1.15	-0.42 ± 0.97	**< 0.001**
BMI (kg/m²)	16.36 ± 3.20	16.66 ± 3.34	0.721
Arm span (cm)	118.14 ± 19.15	127.93 ± 21.84	0.010
AS to height difference (cm)			**< 0.001**
Significant	12 (23.1%)	0 (0.0%)	
Not significant	40 (76.9%)	68 (100.0%)	
AS-to-height ratio	0.98 ± 0.03	1.00 ± 0.01	**< 0.001**
US-to-LS ratio	1.00 ± 0.08	1.09 ± 0.11	**< 0.001**
US-to-LS ratio for age			**< 0.001**
WNL	24 (46.2%)	68 (100.0%)	
Abnormal	28 (53.8%)	0 (0.0%)	
MPH (cm)	156.77 ± 6.69	167.18 ± 7.88	**< 0.001**
Region: U/L			1.000
Normal	52 (100.0%)	68 (100.0%)	
Abnormal	0 (0.0%)	0 (0.0%)	
Region: wrist and hands			0.433
Normal	51 (98.1%)	68 (100.0%)	
Abnormal	1 (1.9%)	0 (0.0%)	
Region: L/L and feet			1.000
Normal	52 (100.0%)	68 (100.0%)	
Abnormal	0 (0.0%)	0 (0.0%)	
Region: thoracolumbar spine			1.000
Normal	52 (100.0%)	68 (100.0%)	
Abnormal	0 (0.0%)	0 (0.0%)	
Growth hormone therapy (yes)	11 (21.2%)	0 (NaN%)	1.000
Parents affected (yes)	16 (30.8%)	0 (0.0%)	**< 0.001**
Sibling relative affected (yes)	10 (19.2%)	0 (0.0%)	**< 0.001**
Micrognathia	2 (3.8%)	0 (0.0%)	0.186
High-arched palate	6 (11.5%)	0 (0.0%)	0.006
Short arms and forearms	24 (46.2%)	0 (0.0%)	**< 0.001**
Cubitus valgus	2 (3.8%)	0 (0.0%)	0.186
Madlung deformity	2 (3.8%)	0 (0.0%)	0.186
Short leg and feet	20 (38.5%)	0 (0.0%)	**< 0.001**
Genu valgum/bowing of tibia	0 (0.0%)	0 (0.0%)	1.000
Muscular hypertrophy	0 (0.0%)	0 (0.0%)	1.000

AS: arm span, SD: standard deviation, US to LS: upper segment to lower segment, U/L: upper limb, L/L: lower limb, WNL: within normal limits, bold values are representing significant p values, MPH: mid-parental height, NS: not significant, BMI: body mass index

1. Genotypic and Allelic Frequencies of *ESR1* SNPs Among Cases and Controls

rs543650

TT, GT and GG genotypes of rs543650 were found in 12 (23.1%), 15 (28.8%) and 25 (48.1%) ISS cases and in 5 (7.4%), 27 (39.7%) and 36 (52.9%) controls, respectively. There was a significant association between the various cases and controls in terms of genotypic frequency of rs543650 (OR = 3.46; 95% CI: 1.08-11.04; p = 0.036) (Table 2).

rs6557177

The CC, CT and TT genotypes of SNP rs6557177 were found in 6 (11.5%), 7 (13.5%) and 39 (75%) cases and 1 (1.5%), 16 (23.5%) and 51 (75%) controls, respectively. There was a significant association between the various cases and controls in terms of genotypic frequency of rs6557177, when compared between the wild and homozygous mutant genotypes (OR = 0.073; 95% CI: 0.01-0.072; p = 0.025) (Table 2). The allelic frequencies were however similar in both groups.

rs2234693

The TT, CT and CC genotypes of rs2234693 were found in 21 (40.4%), 24 (46.2%) and 7 (13.5%) cases and 27 (39.7%), 23 (33.8%) and 18 (26.5%) controls, respectively. There was no significant association between the various cases and controls in terms of genotypic or allelic frequency of rs2234693 (Table 2).

rs9340799

The AA, AG and GG genotypes of rs9340799 were found in 24 (46.2%), 23 (44.2%) and 5 (9.6%) cases and 30 (44.1%), 28 (41.2%) and 10 (14.7%) controls, respectively. There was no significant association between the various cases and controls in terms of genotypic frequency of rs9340799 (Table 2).

Analyses of Baseline Variables by Genotype

The clinical characteristics of the participants, including age, height, body weight, BMI, and mid-parental height (MPH) were summarized (Table 1). Further significant SNPs (rs6557177 and rs543650) were compared for baseline

Table 2. Comparison of genotype and allele frequencies of the four SNPs investigated between ISS cases and controls

SNP	Cases n (%)	Controls n (%)	OR (95% CI)	p value
rs543650				
TT	12 (23.1%)	5 (7.4%)		
TG	15 (28.8%)	27 (39.7%)	**3.46 (1.08-11.04)**	**0.036**
GG	25 (48.1%)	36 (52.9%)	0.80 (0.36-1.80)	0.590
T	39 (37.5%)	37 (27.2%)	1.61 (0.93-2.78)	$\chi^2 = 2.886$ p value-0.089
G	65 (62.5%)	99 (72.8%)		
rs6557177				
TT	39 (75%)	51 (75%)		
TC	7 (13.5%)	16 (23.5%)	0.13 (0.02-1.10)	0.127
CC	6 (11.5%)	1 (1.5%)	**0.073 (0.01-0.72)**	**0.025**
T	85 (81.7%)	118 (86.8%)	0.68 (0.34-1.38)	$\chi^2 = 1.145$ p value-0.285
C	19 (18.3%)	18 (13.2%)		
rs2234693				
TT	21 (40.4%)	27 (39.7%)		
TC	24 (46.2%)	23 (33.8%)	2.00 (0.71-5.67)	0.193
CC	7 (13.5%)	18 (26.5%)	2.68 (0.95-7.62)	0.064
T	66 (63.5%)	77 (56.6%)	1.33 (0.79-2.25)	$\chi^2 = 1.146$ p value-0.284
C	38 (36.5%)	59 (43.4%)		
rs9340799				
AA	24 (46.2%)	30 (44.1%)		
AG	23 (44.2%)	28 (41.2%)	1.60 (0.48-5.31)	0.443
GG	5 (9.6%)	10 (14.7%)	1.64 (0.49-5.49)	0.420
A	71 (68.3%)	88 (64.7%)	1.17 (0.68-2.02)	$\chi^2 = 0.335$ p value-0.563
A	33 (31.7%)	48 (35.3%)		

SNPs: single nucleotide polymorphisms, ISS: idiopathic short stature, CI: confidence interval, OR: odds ratio

variables. The differences in age, height, body weight, BMI, and MPH of the wild type genotype compared to heterozygous and mutant genotypes at loci rs6557177 and rs543650 of ISS were not significant ($p > 0.05$) (Table 3).

Association between response to "GH therapy" and ESR1 SNPs (rs6557177 and rs543650) in patients: The association between the response to GH therapy and *ESR1* SNPs (rs6557177 and rs543650) was done using Fisher's exact test. However, no significant association was found (Table 4).

Linkage Disequilibrium

Measures of LD play a key role in a wide range of applications from disease association to demographic history estimation. To search for LD between pairs of SNPs, pooled genotyping data for patients and controls were analyzed. Both the global statistic R^2 and the statistic D', which takes into consideration the limitations on R imposed by the different allele frequencies of the marker pair, were developed. A stronger disequilibrium between the alleles is suggested when the D' and R^2 values are closer to 1, indicating a high likelihood of the alleles co-inheriting. The values are suggestive of allele independence if they are nearer or equal to zero. It was evident that the rs543650 was in LD with rs2234693 and rs9340799 (D' value 0.73 and 0.84, respectively). rs2234693 and rs9340799 also showed strong LD (D' 0.89). A matrix with each LD statistic selected is shown in Figure 1. Weak LD between rs543650-rs6557177; rs6557177-rs2234693 and rs6557177-rs9340799 of *ESR1* were observed, as suggested by low D' values (0.16, 0.18 and 0.3 respectively). Low R^2 values (0.01, 0.0 and 0.03

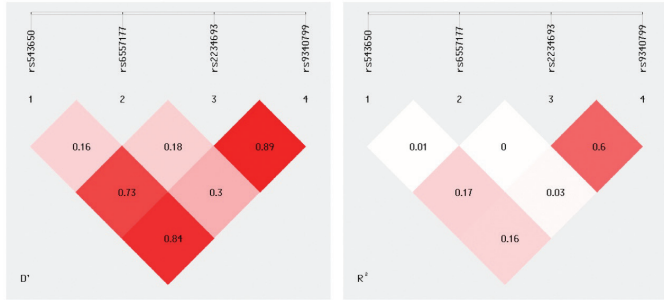

Figure 1. Plot showing linkage disequilibrium (D' and R^2 values) between the four SNPs. Increasing intensity of the red color indicates a stronger linkage disequilibrium

SNPs: single nucleotide polymorphisms

Table 3. Baseline data by genotypes of rs6557177 and rs543650

Parameters	rs6557177				rs543650			
	CC (n = 6)	TC (n = 7)	TT (n = 39)	p value	GG (n = 25)	GT (n = 15)	TT (n = 12)	p value
Age (years)	13.17 ± 2.14	11.00 ± 3.27	9.90 ± 3.72	0.097	10.68 ± 3.56	10.20 ± 3.69	10.17 ± 3.97	0.881
Gender				0.176				0.113
Male	5 (83.3%)	2 (28.6%)	21 (53.8%)		13 (52.0%)	11 (73.3%)	4 (33.3%)	
Female	1 (16.7%)	5 (71.4%)	18 (46.2%)		12 (48.0%)	4 (26.7%)	8 (66.7%)	
Weight for age (SDs)	-1.65 ± 0.61	-2.03 ± 1.66	-1.93 ± 1.25	0.660	-1.58 ± 1.15	-2.31 ± 1.15	-2.11 ± 1.43	0.129
Height for age (SDs)	-2.84 ± 0.50	-2.56 ± 1.11	-2.81 ± 1.24	0.660	-2.59 ± 0.80	-3.15 ± 1.19	-2.70 ± 1.64	0.199
BMI (kg/m²)	17.60 ± 2.32	15.89 ± 4.25	16.26 ± 3.14	0.239	17.08 ± 3.61	15.65 ± 2.35	15.77 ± 3.09	0.487
MPH (cm)	162.98 ± 7.75	154.27 ± 4.59	156.26 ± 6.43	0.071	156.03 ± 6.94	158.65 ± 5.98	155.96 ± 7.09	0.355

MPH: mid-parental height, SDs: standard deviations, BMI: body mass index

Table 4. ESR1 SNPs (rs543650 and rs6557177) and response to growth hormone therapy in cases

ESR1 SNPs	Response to growth hormone therapy		Fisher's exact test
	Yes	No	p value
rs543650			
GG	4 (36.4%)	21 (51.2%)	0.511
GT	3 (27.3%)	12 (29.3%)	
TT	4 (36.4%)	8 (19.5%)	
rs6557177			
CC	1 (9.1%)	5 (12.2%)	0.853
CT	2 (18.2%)	5 (12.2%)	
TT	8 (72.7%)	31 (75.6%)	

SNPs: single nucleotide polymorphisms

Table 5. Haplotype association of the four selected SNPs (adjusted by age and sex)

	rs543650	rs6557177	rs2234693	rs9340799	Freq	OR (95% CI)	p value
1	G	T	T	A	0.2925	1.00	---
2	T	T	T	A	0.2546	0.53 (0.25-1.13)	0.1
3	G	T	C	G	0.2249	0.90 (0.40-2.04)	0.81
4	G	T	C	A	0.0647	0.62 (0.19-2.06)	0.43
5	T	T	C	G	0.0399	0.73 (0.18-2.89)	0.65
6	G	C	C	G	0.0394	0.54 (0.13-2.32)	0.41
7	T	C	T	A	0.034	0.66 (0.12-3.60)	0.63
8	T	T	T	G	0.0232	0.65 (0.09-4.95)	0.68
9	G	C	T	A	0.016	0.44 (0.03-7.67)	0.57
10	T	C	C	G	0.006	0.00 (-Inf - Inf)	1

SNPs: single nucleotide polymorphisms, CI: confidence interval, OR: odds ratio

respectively) contradict the above alleles' coinheritance. The low allele frequencies may be the cause of the observed low R^2 values.

Haplotype

A haplotype analysis of all the SNPs was performed using haplotype frequencies predicted by SHEsisPlus. However, there were no significant differences between cases and controls (Table 5).

Discussion

We investigated correlations between growth/height-related genetic polymorphisms in the *ESR1* gene and the phenotype of ISS in order to better understand the etiology of this enigmatic growth disorder that accounts for a significant portion of pediatric endocrine practice. A study with animal model mice with knocked out *ESR1* gene have shown that *ESR1* plays a role in early growth plate fusion (19). Emons et al. (20) have shown that estradiol level stimulates the local vascular endothelial growth factor level at the growth plate during puberty, although the actual mechanism is not known and is under investigation.

In the present study, we investigated four SNPs of *ESR1* (rs2234693, rs9340799, rs543650 and rs6557177) and analyzed frequencies in an ISS population and a control population presenting in a North Indian tertiary care center. For the SNP rs543650, the wild allele (T) was found to be in lower frequency (0.3568) than the alternate allele (0.6432) in a South Asian population, as per dbSNP (21).

Of these four SNPs, two (rs6557177 and rs543650) had significant association with ISS in our cohort. *ESR1* SNP rs6557177 CC genotype and rs543650 genotype TG were found to be significantly associated with ISS, the former being a protective factor while the latter was a risk factor. This means that people with TG genotype of rs543650 were more likely to have ISS than people with other genotypes and the people with CC genotype of rs6557177 were less likely to be affected. The rs6557177 T > C SNP was increased in an ISS group in a study from a Chinese population (22). Moreover, rs543650T > G was reported to be associated with decreased bone marrow density by Scalco et al. (23).

Our findings that the "tall" (T) and (C) alleles at rs543650 and rs6557177, respectively, are significantly more common in our ISS cohort compared to controls provides independent evidence for an association between the ER and stature, indicating that variations in estrogen sensitivity may play a role in the impaired growth that characterizes ISS.

In addition, we looked for associations with response to GH treatment with selected *ESR1* SNPs. However, in our ISS cohort, we did not find a relationship between *ESR1* alleles and GH treatment response. Sowińska-Przepiera et al. (24) have shown an association between *ESR1* rs2234693 and rs9340799 with bone mass gain in lumbar spine after the onset of estrogen replacement in Turner syndrome patient in an adult cohort. A study by Harlid et al. (25) showed that women with SNP rs851987 in *ESR1* tend to have taller stature. Dahlgren et al. (26) reported that *ESR1* SNP rs2179922 was associated with height in a cohort of two Swedish populations. The *ESR1* rs2234693 polymorphism (PVUII intron 1) has been linked to height during puberty (10,27) and adulthood (28,29). However, we could not find any association between rs2234693 or rs9340799 SNPs with ISS in our cohort. Furthermore, two unrelated patients have been reported to have recessive germline *ESR1* mutations that result in estrogen resistance and significant pubertal growth delays (26,30). According to this study's findings, *ESR1* gene polymorphisms may contribute to the emergence of ISS. Further studies are needed to confirm these findings and to investigate the underlying mechanisms by which *ESR1* gene polymorphisms contribute to ISS. However, the findings regarding the association between

ESR1 gene polymorphisms and ISS are still inconclusive and inconsistent. The genetic basis of ISS is likely to be multifactorial, involving interactions between multiple genes and environmental factors. Therefore, it is important to consider that *ESR1* gene polymorphisms alone may not fully explain the development of ISS.

Further research with a larger sample size is needed to better understand the role of *ESR1* gene polymorphisms in ISS. Larger and more comprehensive studies, including diverse populations, are necessary to provide more definitive conclusions regarding the genetic factors contributing to this condition. It is also important to note that genetic factors are just one aspect of a complex interplay of factors involved in growth and development.

Study Limitations

Our study has several limitations, including a small sample size that limits our ability to detect associations, especially when genetic effects are small, and an even smaller sample size when we divide our sample by sex, which prevents us from conducting sex-specific analyses. A smaller sample size will make it more difficult to detect statistically significant results, and will also limit the generalizability of the results to the wider population.

Conclusion

Being a prospective study, children were subjected to assessment of phenotype and then enrolled for Sanger sequencing for *ESR1* gene SNPs. Hence, the risk of selecting the wrong population for sample analysis was very low. In addition, SNPs of *ESR1* gene are not widely studied as a cause of ISS in our part of the World and the study would be a stepping-stone in this direction. This study has provided valuable data that can be used to further investigate the role of *ESR1* SNPs in ISS. The phenotypic/genotypic correlation would also be an area of interest in the upcoming years. It will help to identify alternate etiologies in cases of ISS and will be helpful to give direction for new research in the field of treatment for ISS. We believe that this study has provided valuable initial data that can be used to design new studies to investigate the role of *ESR1* SNPs in ISS.

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Ethics

Ethics Committee Approval: This study was approved by Postgraduate Institute of Medical Education and Research Institutional Ethics Committee (ref: NK/7784/MD/527, date: 28.09.2021).

Informed Consent: Written informed consent was obtained from the families of the participants.

Authorship Contributions

Concept: Ravi Shankar Patel, Devi Dayal, Anupriya Kaur, Inusha Panigrahi, Harvinder Kaur, Priyanka Srivastava, Design: Ravi Shankar Patel, Priyanka Srivastava, Data Collection or Processing: Ravi Shankar Patel, Roshan Daniel, Chitra Bhardwaj, Anu Kumari, Pratibha Bawa, Ankita Tyagi, Devi Dayal, Anupriya Kaur, Inusha Panigrahi, Harvinder Kaur, Analysis or Interpretation: Ravi Shankar Patel, Roshan Daniel, Chitra Bhardwaj, Anu Kumari, Pratibha Bawa, Ankita Tyagi, Literature Search: Ravi Shankar Patel, Roshan Daniel, Chitra Bhardwaj, Anu Kumari, Pratibha Bawa, Writing: Roshan Daniel, Priyanka Srivastava.

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Comprehensive Insights Into Pediatric Craniopharyngioma: Endocrine and Metabolic Profiles, Treatment Challenges, and Long-term Outcomes from a Multicenter Study

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What is already known on this topic?

Craniopharyngiomas (CPG) are challenging to treat due to their proximity to vital structures, and the tendency for recurrence. The pituitary axis is frequently affected during the presentation of CPG.

What this study adds?

Recurrence of CPG was predominantly related to incomplete resection and the low rate of postoperative radiotherapy. The study revealed hesitancy among physicians regarding use of recombinant growth hormone, highlighting a need for further exploration and understanding.

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Abstract

Objective: Craniopharyngiomas (CPG) have complex treatment challenges due to their proximity to vital structures, surgical and radiotherapeutic complexities, and the tendency for recurrence. The aim of this study was to identify the prevalence of endocrine and metabolic comorbidities observed during initial diagnosis and long-term follow-up in a nationwide cohort of pediatric CPG patients. A further aim was to highlight the difficulties associated with CPG management.

Methods: Sixteen centers entered CPG patients into the ÇEDD NET data system. The clinical and laboratory characteristics at presentation, administered treatments, accompanying endocrine, metabolic, and other system involvements, and the patient's follow-up features were evaluated.

Results: Of the 152 evaluated patients, 64 (42.1%) were female. At presentation, the mean age was 9.1 ± 3.67, ranging from 1.46 to 16.92, years. The most common complaints at presentation were headache (68.4%), vision problems (42%), short stature (15%), and nausea and vomiting (7%). The surgical procedures were gross total resection (GTR) in 97 (63.8%) and subtotal resection in 55 (36.2%). Radiotherapy (RT) was initiated in 11.8% of the patients. Histopathological examination reported 92% were adamantinamatous type and 8% were papillary type. Postoperatively, hormone abnormalities consisted of thyroid-stimulating hormone (92.1%), adrenocorticotropic hormone (81%), antidiuretic hormone (79%), growth hormone (65.1%), and gonadotropin (43.4%) deficiencies. Recombinant growth hormone treatment (rhGH) was initiated in 27 (17.8%). The study showed hesitancy among physicians regarding rhGH. The median survival without relapse was 2.2 years. Median (range) time of relapse was 1.82 (0.13-10.35) years. Relapse was related to longer follow-ups and reduced GTR rates. The median follow-up time was 3.13 years. Among the last follow-up visits, the prevalence of obesity was 38%, but of these, 46.5% were already obese at diagnosis. However, 20% who were not obese at baseline became obese on follow-up. Permanent visual impairment was observed in 26 (17.1%), neurological deficits in 13 (8.5%) and diabetes mellitus in 5 (3.3%) patients.

Conclusion: Recurrence was predominantly due to incomplete resection and the low rate of postoperative RT. Challenges emerged for multidisciplinary regular follow ups. It is suggested that early interventions, such as dietary restrictions and increased exercise to prevent obesity, be implemented.

Keywords: Craniopharyngioma, pituitary, dysfunction

Introduction

One of the most difficult brain tumors to treat is a craniopharyngioma (CPG), which is characterized by benign histology but has the potential to cause serious functional and clinical problems because of where the CPG is usually located in the central nervous system, frequently involving the sellar and suprasellar regions, compressing nearby tissues (1,2,3,4,5). The complex nature of the surgical management of CPG, compounded by its complicated anatomical positioning, has encouraged the exploration of novel treatment strategies. In contrast to treatment techniques, follow-up data show no appreciable differences in long-term effects (1,6,7). CPG constitutes 1.2-10% of all pediatric brain tumors, with an incidence of 0.5-2.5 cases per 1,000,000 individuals, making it particularly prevalent in the pediatric population (1,2) and CPG is most commonly diagnosed in children. It exhibits a two-peak age distribution, with the highest occurrences observed in individuals aged 5 to 15 years during childhood and another peak in individuals aged 45 to 60 years in adulthood (2,3).

Endocrine disorders in patients with CPG significantly affect their quality of life (4,5,6,7,8,9,10,11,12). The pituitary axis is frequently affected during the presentation (2,3,13,14). Furthermore, radiotherapy (RT) impacts hypothalamic-pituitary function (3,6,15,16,17,18). Compared to the pituitary gland, the hypothalamus is more susceptible to damage by radiation (16,17). As hypothalamic function is often impaired, 40-50% of patients are affected by hypothalamic obesity (7,14,19,20,21). Moreover, the degree of hypothalamic involvement before surgical intervention is a significant factor in determining the persistent outcomes after the surgical procedure (21,22,23).

Interventions and treatments for endocrine and metabolic problems also present challenges. Although replacement therapy for pituitary hormones is stated not to cause a risk for CPG recurrence, it remains controversial (24,25,26,27). The effect of recombinant human growth hormone (rhGH) on the psychosocial status and quality of life has been investigated, and it has been shown that individuals who take rhGH during the growth period have better height growth; however, this did not lead to weight loss in adult patients, only in the pediatric group (27,28,29).

Managing pediatric CPG is challenging due to the complex diagnostic process, the distinctiveness of the anatomical position of the CPG, the extent of involvement in surrounding tissues, the secondary harm induced by treatments, the weight of endocrine and metabolic complications, and the adverse impacts on sustained quality of life. The aims of this research was twofold: first, to determine the prevalence of endocrine and metabolic comorbidities during the diagnostic phase and long-term follow-up in a national cohort of pediatric patients diagnosed with CPG; and second, to determine the problems involved in successfully treating the endocrine abnormalities in these patients.

Methods

Clinical and Laboratory Enrollment Criteria

This aim of this investigation was to comprehensively assess individuals under follow-up at pediatric endocrinology centers with experience in CPG management. The study involved the active participation of 16 different endocrinology departments. Patients' data entry into the ÇEDD NET database was uploaded through cooperative efforts of participating centers to enhance data gathering. The investigation of several aspects, such as the age at which the disease first manifested, clinical manifestation, length of symptoms, demographic traits, cranial magnetic resonance imaging (MRI) results at diagnosis, surgical and/or RT treatments used, and tumor histology, was covered by the study protocol.

Comprehensive datasets capturing concurrent endocrine, metabolic, ophthalmological, and neurological profiles were systematically collected. Physical evaluation included height standard deviation (SD) score (SDS), visual acuity (VA) assessment, body mass index (BMI), and Tanner's puberty stage. In addition, laboratory investigations, including thyroid function tests [thyroid-stimulating hormone (TSH), fT4], adrenal function (ACTH, cortisol), prolactin (PRL) levels, gonadotropins, testosterone/estradiol levels, and, in instances of growth hormone (GH) deficiency, insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding protein 3 (IGFBP3) levels, were assessed. The occurence of central diabetes insipidus (CDI) and treatments were also evaluated. Quantitative measures of fasting insulin levels, lipid profiles, aspartate aminotransferase and alanine transaminase levels, and uric acid concentrations were required to evaluate metabolic health.

The patients underwent a thorough ophthalmological and neurological examination and assessments of any associated social and psychiatric issues, cardiovascular symptoms, obstructive sleep apnea, and further symptomatic presentations. The complexities of post-diagnostic monitoring were investigated, including the typical follow-up time, the resulting MRI findings, and disparities observed in the endocrine, metabolic, ophthalmological and neurological systems. Reoperations that followed tumor recurrences were documented.

Inclusion criteria required participants to have received a definitive diagnosis before 18 years, substantiated by unequivocal histopathological confirmation of CPG. Conversely, candidates were excluded if their diagnosis rested solely on clinical and radiological grounds and lacked histopathological confirmation.

The study was approved by the Ankara University Faculty of Medicine of Human Research Ethics Committee (protocol number: I2-130-21, date: 16.02.2021).

Statistical Analysis

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 (Shapiro-Wilk test). Differences between dependent groups were analyzed using Student's t-test and independent groups were analyzed using the Mann-Whitney U test. A $p < 0.05$ was considered statistically significant.

Results

Study Participants and Clinical Presentation

One hundred fifty-two patients (64 females; 42.1%) were enrolled in the study. At the time of diagnosis, the mean age was 9.1 years. The height SDS was -1.09 ± 1.5, and the BMI SDS was 0.7 ± 1.6. The study included 110 prepubertal and 42 pubertal patients. Notably, 39 (25%) patients had short stature (< -2.0 SDS) at diagnosis. Distribution was as follows: 19 girls had a height SDS of -2.9 ± 0.14 and 20 boys had a height SDS of -3.1 ± 0.20. In addition, at the point of diagnosis, 35 (23%) patients were classified as obese, including 10 girls (BMI SD: 2.6 ± 0.16 SD) and 25 boys (BMI SD: 2.7 ± 0.15 SD) (Table 1).

Of the 152 patients, the foremost initial complaint was headache (68.4%). Sixty-five patients (42%) had vision problems including 52 patients with reduced VA, seven with restricted field of vision, two presented with diplopia, and two had nystagmus. Other presenting complaints included obesity in 38 patients (25%), impaired growth in 23 patients (15%), nausea and vomiting in 11 patients (7%), neurological symptoms (seizure, drowsiness, ataxia, tremor) in 14 patients (9%) and pubertal problems in 4 patients (3%) (Table 1).

In the first evaluation, hormone deficiencies in descending order of frequency were: TSH deficiency in 121 patients (79%); ACTH deficiency in 106 patients (69%); CDI in 95 patients (62.5%); GH deficiency in 83 patients (54.6%); and gonadotropin deficiency in 63 patients (41%). In total 137 patients had PRL measurements and of these, 48 had elevated PRL levels, 68 patients had normal and 23 patients had low PRL levels (Table 1).

Table 1. Demographic characteristics, clinical and laboratory evaluation of patients with craniopharyngioma at presentation

Demographic characteristics	
Patients (n)	152
Age at the time of diagnosis (year)	9.1 ± 3.6
Sex	
Female (n)	64 (42%)
Male (n)	88 (58%)
Height SDS	-1.9 ± 1.5
Short stature at diagnosis	
Female (n)	19 (height SD: -2.9 ± 0.14)
Male (n)	20 (height SD: -3.1 ± 0.20)
BMI SDS	0.7 ± 1.6
Obesity at diagnosis	
Female (n)	10 (BMI SD: 2.6 ± 0.16)
Male (n)	25 (BMI SD: 2.7 ± 0.15)
Pubertal status	
Prepubertal (n)	110 (72%)
Pubertal (n)	42 (28%)
Symptoms at diagnosis	
Headache	104 (68%)
Had vision problems	
Reduced visual acuity	52 (34%)
Restricted vision	7 (5%)
Diplopia	2 (1%)
Nystagmus	2 (1%)
Impaired growth	23 (15%)
Nausea and vomiting	11 (7%)
Neurological symptoms	14 (9%)
Pubertal problems	4 (3%)
Hormone deficiencies in patients	
TSH deficiency	121 (79%)
ACTH deficiency	106 (69%)
Central diabetes insipidus	95 (62.5%)
GH deficiency	83 (54.6%)
Gonadotropin deficiency	63 (41%)
Prolactin level (n = 137)	
- Elevated	48 (35%)
- Normal	68 (49.6%)
- Low	23 (16.7%)

BMI SDS: body mass index standard deviation (SD) score, ACTH: adrenocorticotropic hormone deficiency, TSH: thyroid-stimulating hormone, GH: growth hormone

Cranial Imaging and Extension Patterns

Cranial imaging showed that extension into a single area was observed in 64 patients, with 55 involving the suprasellar region, five the third ventricle, four the anterior fossa (one each for infundibulum, optic chiasm, and other parts). Extension into multiple areas was noted in 43 patients. Thirteen patients showed no extension, while 32 patients had unspecified extension patterns.

Treatment Approaches and Outcomes

Transnasal interventions were performed in 76 patients (50%), endoscopic procedures in 73 patients (48%), and gamma knife treatment in two patients (1.3%). The type of operation was not specified in one patient. Notably, 73 patients (48%) underwent triple-phase interventions, which consisted of the first stage of transient CDI, second stage of an antidiuretic phase, and the permanent CDI phase (30,31). RT was initiated in 11.8% of the patients.

Pathology and Tumor Characteristics

The histopathological evaluation was not reported in 46 (30.3%) of patients. Among the 106 patients with histopathological reports available, 92% were classified as the adamantinomatous type, while 8% exhibited the papillary type. The mean tumor diameter measured was 3.7 ± 1.5 cm.

Postoperative Complications and Findings

The mean follow-up duration was 4.5 ± 3.9 years. At the last follow-up, the mean age was 13.7 ± 4.9 years, with the height SDS documented as -1.1 ± 1.62. Among the patients, 36 (23.6%) had a height SDS below -2 SD (Table 2). Relapse was observed in 56 patients (38%), with a median (range) time to relapse (from the first surgery) of 1.82 (0.13-10.35) years. No significant differences in age, gender, or tumor size were observed between patients with and without relapse ($p > 0.05$). The follow-up duration was 5.4 ± 3.6 years for patients with relapse and 3.8 ± 3.6 years for those without. Complete resection was achieved in 57% of patients with relapse and 71% in those without ($p < 0.05$). The significant factors contributing to the development of relapses were the inability to achieve complete resection and the low rate of adding postoperative RT.

Hormone Deficiencies and Outcomes

Hormone deficiencies were prevalent at the final assessment. These included TSH deficiency in 140 patients (92.1%), ACTH deficiency in 123 patients (81%), permanent CDI in 120 patients (78.1%), GH deficiency in 99 patients (65.1%), and gonadotropin deficiency in 66 patients (43.4%) (Table 2).

Table 2. Postoperative follow-up and outcomes in patients with craniopharyngioma

Postoperative findings - follow-up	
Follow-up duration (years)	4.5 ± 3.9 [0.08; 20]
Age at last follow-up (years)	13.7 ± 4.9 [2.9; 29]
Height SDS	-1.1 ± 1.6 [-6.4; 2.6]
BMI SDS	1.4 ± 1.4 [-2.35; 5.08]
Relapse (n)	58 (38.2%)
Remission (n)	92 (60.5%)
Type of surgery	
GTR (n)	97 (63.8%)
Subtotal (n)	54 (35.5%)
Transnasal (n)	76 (50%)
Endoscopic (n)	73 (48%)
Gamma knife (n)	2 (1.3%)
Radiotherapy (n)	18 (11.8%)
Postoperative hormone deficiencies in patients	
Growth hormone deficiency	99 (65.1%)
Gonadotropin deficiency (total 109 pubertal status)	66 (43.4%)
TSH deficiency	140 (92.1%)
ACTH deficiency	123 (81%)
Central diabetes insipidus	120 (78.9%)

BMI SDS: body mass index standard deviation (SD) score, ACTH: adrenocorticotropic hormone deficiency, TSH: thyroid-stimulating hormone, GTR: gross total resection

Among the preoperative patients, 35 individuals (23%) were obese, and 58 individuals (38%) were obese in the postoperative period. However, 20% who were not obese at baseline became obese on follow-up. In the postoperative period, 31 newly obese patients were evident. However, eight of 35 patients, that were preoperatively obese, had normal BMI in the postoperative follow-up.

Among the patients, 12/42 (28%) had a metabolic status indicating prediabetes and 4/120 (3%) had diabetes mellitus. On follow-up, neurological deficits were observed in 8 (6%) patients. Among these, five patients had epilepsy, while three had motor deficits.

There was no apparent difference in survival between relapse and nonrelapse groups. However, due to the limited number of patients, statistical significance could not be demonstrated.

Discussion

CPG constitutes a significant portion of childhood central nervous system tumors (1,2). Our patients had a mean age at diagnosis of around 9.2 years. According to Beckhaus et al. (21), CPG patients with a younger age at diagnosis (less than 12 years old) had a worse event-free survival rate. Our contribution could modify this interpretation, particularly considering the long-term follow-up encompassing adult age. The literature suggests that the most common initial clinical manifestations include headaches, visual impairment, and endocrine issues, such as growth retardation, obesity, delayed puberty, and CDI (5,13,22,23,32,33,34). Approximately 60-75% of patients primarily report headaches and in keeping with this, headaches were the most common presenting complaint (68.4%) (32). During initial evaluations, 70-80% of patients may have visual impairments (32). A notable 42% of our patients experienced visual disturbances as their first symptom. However, this rate might differ, based on the location of the tumor in the chiasm (anterior or posterior) and asymmetric extension of the tumor in our study. Among patients afflicted by suprasellar CPGs, 50% exhibited decreased VA and visual field impairment at diagnosis. Interestingly, previously published studies indicated a prevalence of 38% during the diagnostic phase, which further diminished to 15% postoperatively (35).

During the diagnostic phase, hormonal imbalances were described in 93% of the patients. Studies highlighted that hormonal involvement was evident in 40% to 87% of patients during their diagnostic assessment (34). Our findings were at the upper end of the range. This may be attributed to the age at diagnosis and the specific region affected by the tumor.

In the literature, approximately 26% to 75% of CPG patients are reported to have GH deficiency at the time of diagnosis, which increases to 70% to 92% postoperatively. Our study is compatible with these findings, suggesting a correlation with the limited preoperative endocrinological assessment. Without a preoperative multidisciplinary assessment, clinicians involved in the postoperative process may have missed the opportunity for a comprehensive examination at the time of diagnosis. In a study by Müller et al. (36), preoperative GH deficiency was observed in 54% (n = 83) patients, while postoperative GH deficiency was noted in 65.1% (n = 99) patients. Among them, 28 patients (18%) were started on rhGH. Similar to other studies in the literature, the most frequently reported hormone deficiency in our study was TSH deficiency (4,32).

In the present study, the majority of patients displayed permanent hypopituitarism symptoms. In the literature, postoperative hypopituitarism was reported at a rate of 57-98%. Permanent CDI was reported in 64-80%. Endocrine disorders frequently observed during diagnosis and follow-up in CPG patients significantly contribute to reduced quality of life (4-12).

Sklar (34) reported GH deficiency in 75% of patients, gonadotropin deficiency in 40%, TSH in 25%, and ACTH in 25%. They also indicated CDI in the 9-17% (postoperative: 40-80%) (34). Caldarelli et al. (37) found GH deficiency in 82%, ACTH deficiency in 76%, TSH deficiency in 73%, and gonadotropin deficiency in 67% of patients. In our cohort, tumor extension was predominantly suprasellar, consistent with the literature (38). The impact of the tumor, surgical intervention, and RT are significant factors contributing to hypopituitarism.

Although rarely malignant, CPG presents difficulties in treatment due to its proximity to vital structures, incomplete resection, and tendency to recur. Furthermore, delayed diagnosis is common in children. If complete removal is not possible, subtotal resection with RT for residual tumors is necessary. There is no consensus on the ideal surgical approach (3,4,13,14,21,22,39). Aggressive gross total resection (GTR), previously more commonly practiced, is now associated with higher endocrine problems and decreased quality of life. Subtotal removal with preservation of the pituitary and cranial nerves, followed by radiation, has become more widely used. Long-term quality of life data after endoscopic endonasal surgery is limited (4,5,13,14,21,23,32,39,40). In our study, GTR was achievable in most patients (n = 97; 63.8%). Despite the benign nature of CPGs, there is a marked potential for relapse. There are no guidelines on managing pediatric CPGs regarding the ideal surgical approach (3,4,13,14,21,22,39). Surgical procedures might differ based on the use of a personalized approach. Transnasal intervention was the most frequently performed in our patient group. However, due to tumor size, vital organ proximity, and unsuitable location, complete resection was not feasible for all subjects. In the literature, the likelihood of recurrence is more common in pediatric patients than in adults due to the adamantinomatous variant. GTR might not eliminate recurrence risk (22,41,42). It has been suggested that GTR provided variable disease control, with recurrence rates reported at 36.4-40% after GTR (41,43). Despite the prevailing notion that GTR tends to prevent a recurrence, the likelihood of hypothalamic/pituitary damage affects quality of life, and some researchers advocate the benefits of conservative treatments (4).

Following surgery, the number of patients undergoing triphasic response of pituitary stalk injury leading to CDI development reached 48%. In the pediatric age group, a higher number of patients undergo triple-phase interventions compared to adults (2). This seems to be associated with tumor size and the damage inflicted on surrounding tissues by surgery.

Pathologically, most of the patients in the present study were diagnosed with adamantinomatous type CPG. It is the most common histological subtype of CPG and exhibits a bimodal age distribution, with the highest incidence occurring in children aged 5-15 years and adults aged 45-60 (2). Pediatric CPG, which predominately manifests as the adamantinomatous subtype, differs in pathology and genetic characteristics from adults. The underlying molecular and cellular mechanisms for the adamantinomatous type involve mutations in the *CTNNB1* gene, responsible for encoding β-catenin. On the other hand, papillary CPGs in adults are associated with *BRAF* V600E mutations. The pathological and genetic characteristics could also affect recurrence outcomes, hormonal imbalances, and survival rates (2,32). While the papillary type is observed at a low frequency, cases occurring during childhood have also been reported in the literature (44,45). This situation is elucidated more clearly through the identification of underlying molecular mechanisms. However, due to the retrospective nature of our study, molecular assessment could not be assessed.

Eighteen patients had received postoperative RT. There was no significant difference in survival compared to other patient groups. However, due to the limited number of patients, statistical analysis would not be reliable. A recent meta-analysis suggested that GTR and STR, along with RT, exhibit similar survival outcomes for CPG (45); due to the small sample size in our study, statistical confirmation was not performed.

On follow-up, neurological deficits were observed in 13 patients, accounting for 8.6% of the patients, including epilepsy and motor deficits. The reported rates of neurological complications in an earlier series ranged from 8% to 36%, aligning well with our findings (10).

In CPG, hypothalamic obesity is challenging, leading to metabolic problems and being unresponsive to lifestyle changes. Hypothalamic findings may be missed during the follow-up. It is known that preoperative hypothalamic engagement and hypothalamic damage during operation lead to preoperative and postoperative obesity (2,46). In individuals affected by hypothalamic damage, there is a decrease in energy expenditure, an increase in daytime sleepiness, and a disrupted response in signals related to leptin, ghrelin, and insulin. As a consequence, hyperinsulinemia develops (47). Obesity developed in 31 patients during follow-up. At diagnosis, 35 patients (23%) were obese. Among the postoperative patients, 58 patients (38%) were obese, of which 27 (18%) were already obese at the time of initial presentation. In the literature, the prevalence of obese patients at diagnosis ranges from

12% to 19%. Furthermore, it has been reported that the frequency of severe obesity after six months postoperatively is approximately 55% (10). Similar to the literature, half of the patients exhibited obesity (48). Notably, lifestyle modifications and other traditional treatments for obesity typically fail to control the condition (2,46). Medical treatment options such as triiodothyronine, octreotide, dextroamphetamine, methylphenidate, sibutramine, and GLP-1 receptor agonists lacked generally applicable scientific evidence and showed side effects (46). Obesity surgery can not achieve permanent weight loss and also causes malabsorption of oral hormone replacement medications (46). Unfortunately, there is no definitive treatment option for hypothalamic obesity. Treatment with rhGH replacement improves growth, weight, and neuropsychology in the pediatric population receiving rhGH treatment (24,25,26,27). In the present study, the use of rhGH appears to be less widespread than recommended to control metabolic parameters (25,46). The study revealed a hesitancy among physicians regarding rhGH therapy. The delay may be due to the risk of side effects. However, rhGH replacement therapy does not adversely affect disease-free survival (25,46,49,50). It is important that rhGH treatment should not be delayed in these patients with CPG.

Study Limitations

This study has some limitations attributed to the retrospective design of the study and the lack of homogeneity in data collection. Specifically, hypothalamic syndrome data could not be obtained from all participating centers. The onset of obesity could not be assessed for the initial year. Moreover, there is a deficiency in the detailed features of cranial imaging.

Conclusion

In conclusion, CPG is challenging when it occurs in the pediatric age group, requiring a comprehensive approach. Difficulties with regular multidisciplinary follow-ups have been identified by the present study, and it is suggested that early interventions involving calorie restriction and increased exercise for obesity should be considered. The components of hypothalamic syndrome, including eating disorders, circadian sleep changes, temperature variations, and heart rate variability, should be taken into consideration during patient follow-ups. Recurrence was predominantly due to incomplete resection and the low rate of postoperative RT.

Ethics

Ethics Committee Approval: The study was approved by the Ankara University Faculty of Medicine of Human Research Ethics Committee (protocol number: I2-130-21, date: 16.02.2021).

Informed Consent: Written informed consent was obtained from the patients and parents for publication of this study.

Authorship Contributions

Surgical and Medical Practices: Zeynep Şıklar, Elif Özsu, Sirmen Kızılcan Çetin, Samim Özen, Filiz Çizmecioğlu-Jones, Hanife Gül Balkı, Zehra Aycan, Damla Gökşen, Fatih Kilci, Sema Nilay Abseyi, Ummahan Tercan, Gözde Gürpınar, Şükran Poyrazoğlu, Feyza Darendeliler, Korcan Demir, Özge Besci, İlker Tolga Özgen, Semra Bahar Akın, Zümrüt Kocabey Sütçü, Emel Hatun Aykaç Kaplan, Emine Çamtosun, Elif Sağsak, Hüseyin Anıl Korkmaz, Ahmet Anık, Gül Yeşiltepe Mutlu, Bahar Özcabı, Ahmet Uçar, Aydilek Dağdeviren Çakır, Beray Selver Eklioğlu, Birgül Kırel, Merih Berberoğlu, Concept: Zeynep Şıklar, Merih Berberoğlu, Design: Zeynep Şıklar, Merih Berberoğlu, Data Collection or Processing: Zeynep Şıklar, Elif Özsu, Sirmen Kızılcan Çetin, Samim Özen, Filiz Çizmecioğlu-Jones, Hanife Gül Balkı, Zehra Aycan, Damla Gökşen, Fatih Kilci, Sema Nilay Abseyi, Ummahan Tercan, Gözde Gürpınar, Şükran Poyrazoğlu, Feyza Darendeliler, Korcan Demir, Özge Besci, İlker Tolga Özgen, Semra Bahar Akın, Zümrüt Kocabey Sütçü, Emel Hatun Aykaç Kaplan, Emine Çamtosun, Elif Sağsak, Hüseyin Anıl Korkmaz, Ahmet Anık, Gül Yeşiltepe Mutlu, Bahar Özcabı, Ahmet Uçar, Aydilek Dağdeviren Çakır, Beray Selver Eklioğlu, Birgül Kırel, Merih Berberoğlu, Analysis or Interpretation: Zeynep Şıklar, Sirmen Kızılcan Çetin, Elif Özsu, Merih Berberoğlu, Literature Search: Zeynep Şıklar, Sirmen Kızılcan Çetin, Elif Özsu, İsmail Dündar, Writing: Zeynep Şıklar, Sirmen Kızılcan Çetin, Elif Özsu, Merih Berberoğlu.

Conflict of Interest: Three authors of this article, Damla Gökşen, Korcan Demir, Samim Özen, are member of the Editorial Board of the Journal of Clinical Research in Pediatric Endocrinology. However, they did not take part in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from different institutions. The other authors declared no conflict of interest.

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ORIGINAL ARTICLE

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Clinical and Laboratory Characteristics of MODY Cases, Genetic Mutation Spectrum and Phenotype-genotype Relationship

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What is already known on this topic?

Maturity onset diabetes of the young (MODY) is the term used to describe a group of inherited, non-autoimmune forms of diabetes mellitus. MODY diagnosis is often made at a young age (under 25 years of age), pancreatic autoantibodies are not present, there is a low insulin requirement, often a family history of autosomal dominant diabetes, no history of obesity and no history of diabetic ketoacidosis (DKA).

What this study adds?

This large-series national study showed that the diagnostic criteria in the prediagnostic process of MODY should be reconsidered because some patients with genetically diagnosed MODY had a history of DKA, pancreatic antibody positivity and no family history of diabetes.

Abstract

Objective: Maturity onset diabetes of the young (MODY) occurs due to mutations in genes involved in pancreatic beta cell function and insulin secretion, has heterogeneous clinical and laboratory features, and account for 1-5% of all diabetes cases. The prevalence and distribution of MODY subtypes vary between countries. The aim of this study was to evaluate the clinical and laboratory characteristics, mutation distribution, and phenotype-genotype relationship in a large case series of pediatric Turkish patients genetically diagnosed with MODY.

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Methods: MODY cases from 14 different pediatric endocrinology departments were included. Diagnosis, treatment, follow-up data, and results of genetic analysis were evaluated.
Results: A total of 224 patients were included, of whom 101 (45%) were female, and the mean age at diagnosis was 9.4 ± 4.1 years. Gene variant distribution was: 146 (65%) *GCK*; 43 (19%) *HNF1A*; 8 (3.6%) *HNF4A,* 8 (3.6%) *KLF11* and 7 (3.1%) *HNF1B*. The remaining 12 variants were: *PDX* (n = 1), *NEUROD1* (n = 3), *CEL* (n = 1), *INS* (n = 3), *ABCC8* (n = 3) and *KJNC11* (n = 1). Of the cases, 197 (87.9%) were diagnosed with incidental hyperglycemia, 16 with ketosis (7%) and 7 (3%) with diabetic ketoacidosis (DKA), while 30% presented with classical symptoms of diabetes. Two-hundred (89%) had a family history of diabetes. Anti-GAD antibody was detected in 13 cases, anti-islet antibody in eight and anti-insulin antibody in four. Obesity was present in 16. Distribution of therapy was: 158 (71%) diet only; 23 (11%) intensive insulin treatment; 17 (7.6%) sulfonylureas; 10 (4.5%) metformin; and 6 (2.7%) insulin and oral anti-diabetic treatment.
Conclusion: This was the largest genetically diagnosed series from Turkey. The most common gene variants were *GCK* and *HNF1A* with much lower proportions for other MODY types. Hyperglycemia was the most common presenting symptom while 11% of patients had diabetes-associated autoantibodies and 7% were obese. The majority of patients received dietary management only.
Keywords: Childhood, MODY, diagnosis

Introduction

Maturity onset diabetes of the young (MODY) is the term used to describe a group of inherited, non-autoimmune forms of diabetes mellitus (DM). MODY was initially thought to be relatively rare but it has become apparent that it is more common than first thought. Despite increasing awareness of MODY, it has been estimated that up to 1-5% of MODY cases remain undiagnosed. Since the clinical spectrum is variable due to the range of genetic defects, there may be diagnostic delays, and some cases may not be diagnosed until adulthood. Furthermore, cases may sometimes be misdiagnosed as type 1 (T1DM) or type 2 DM (T2DM) (1,2,3).

Given the natural history of MODY, that there is cost-effective treatment, and the potential impact on more than one family member, it is essential to diagnose these cases correctly. The presence of family history, clinical and physical examination findings incompatible with T1DM or T2DM, and negative diabetes autoantibodies when present, strengthen the diagnosis of MODY (3). In addition, urinary c-peptide/creatinine ratio > 0.2 nmol/mmol has high specificity and sensitivity in differentiating MODY from T1DM (4).

Genetic testing is important for definitive diagnosis and identification of other family members with the same variant. Mutation analyses classify the type of MODY and this in turn informs about the expected clinical courses and guides treatment planning. In patients with *HNF4A* and *HNF1A* mutations, forms of MODY which are responsive to sulphonylurea therapy, it is important to change treatment regimens from insulin treatment, as low-dose sulphonylurea treatment may provide good glycemic control for years and, in patients with *GCK* mutations, will prevent unnecessary insulin treatment. Mutation screening is also important for asymptomatic relatives with a 50% risk of inheriting the mutation.

The distribution of MODY types differs between countries. Although *HNF1A* MODY is reported to be the most common type globally, there are countries where *GCK* MODY is observed more frequently (5). Therefore, knowing which of the MODY types is more common in one's own country is important. Determination of the MODY type will provide the most accurate approach for the pediatric endocrinologist to manage MODY patients. Moreover, managing clinicians should understand the phenotype-genotype relationship in MODY.

The aim of this study was to investigate the distribution of MODY types in a pediatric population from Turkey and to investigate the phenotype-genotype relationship.

Methods

The study included pediatric patients with genetically diagnosed MODY from 14 pediatric endocrinology and diabetes departments in Turkey. Ethical approval was obtained from Ankara University Faculty of Medicine (decision number: 12-112-20, date: 13.02.2020). The National Pediatric Endocrinology and Diabetes Association (ÇEDD) supported the study. An electronic case registration form was created in the ÇEDD-NET data system of the association, and centers were asked to record their data using this system. The data entry took one year. Retrospective medical history (at least three generations of family history), clinical characteristics (age at diagnosis, year of diagnosis, duration of diabetes, presence of classical diabetes symptoms), laboratory results (blood glucose, insulin, c-peptide, glycated hemoglobin (HbA1c) levels, lipid profile, presence of acidosis at diagnosis, presence of autoantibodies at diagnosis, and reserve insulin values) and genetic data were transferred from electronic case record forms to Microsoft Excel (Microsoft Inc., Redmond, USA) and Statistical Package for the

Social Sciences (SPSS), version 20.0 (IBM Inc., Armonk, NY, USA) files for statistical evaluation.

Informed consent was obtained from the parents/guardians of the patient from all centers.

Patients with a positive family history in which at least two consecutive generations were affected, early-onset hyperglycemia, negative diabetes autoantibodies, and no classical features of T1DM or T2DM (not insulin-dependent or not requiring insulin treatment even after three years of insulin treatment, or having insulin reserve with serum c-peptide level >0.60 ng/mL) were sought from the clinics. Genetic analysis was performed with a presumptive prediagnosis of MODY. Patients had already had genetic analyses and they were genetically diagnosed.

Body mass index (BMI) values of the cases were evaluated according to the Neyzi et al. (6) data. adjusted for age and sex, and cases with BMI >95th percentile were considered obese. Ketonemia was defined as a blood ketone level of 1 mg/dL, and diabetic ketoacidosis (DKA) was defined as a blood glucose level above 200 mg/dL with blood pH <7.3 or HCO_3 <15 mmol/L. Autoantibody status and reserve insulin values were recorded at diagnosis.

Mutation Interpretation

Genetic analyses of the cases were performed by next-generation sequencing and screening for 14 currently known mutations. Each patient was included in the study with the genetic diagnosis of MODY being made in the unit where they were followed.

MODY DNA sequence analysis panel was performed on each patient. All results were assessed by genetic specialist according to ACGM classification. Results, which were pathogenic or likely pathogenic were accepted as positive (7).

Statistical Analysis

Descriptive statistics were calculated for all patients taking part in the study using SPSS, version 21.0 (IBM Corp., Armonk, NY, USA). Data were expressed as the mean ± standard deviation (SD), median (minimum; maximum). The Kolmogorov-Smirnov test evaluated the normality of variables. Descriptive analyses are presented using means and SD for normally distributed variables. A p value <0.05 was assumed to indicate a statistically significant result in all analyses.

Results

General Features

A total of 224 cases were included in the study. Of these 101 (45%) were female, and the mean age at diagnosis was 9.4 ± 4.1 years and ranged widely from 0.2 to 17 years. On MODY genotyping, 146 (65%) cases were diagnosed as *GCK*, 43 (19%) cases were diagnosed as *HNF1A*, 8 (3.6%) cases were diagnosed as *HNF4A* and a further eight with *KLF11* while 7 (3.125%) patients had an *HNF1B* variant. In the remaining twelve cases there were three (1.3%) each with *NEUROD1*, *INS* and *ABCC8* mutations while the last three had PDX (n = 1), *CEL* (n = 1) and *KJNC11* (n = 1) mutations, respectively (Figure 1).

Most of the cases (n = 197, 87.9%) were diagnosed with incidental hyperglycemia, while 16 (7%) had ketosis and 7 (3%) had DKA; 30% had diabetes symptoms. There were 200 cases (89%) with a family history of DM; 93 (41%) had diabetes in two generations and 84 (37%) had diabetes in three generations. Relatives of 162 patients with diabetes were under the age of 40 years. In 24 patients, no family history was found. A proportion of the patients with genetically diagnosed MODY presented with T1DM-associated autoantibodies, including 13 (5.8%) with anti-GAD, 8 (3.6%) with anti-islet and 4 (1.8%) with anti-insulin antibodies. These cases were in HNF4A, GCK, HNF1A, KLF11, KJNC11 MODY (type 1, 2, 3, 7, 12 MODY) types. Sixteen of the cases were obese. Clinical and laboratory characteristics of MODY types are given in Table 1.

While 158 (71%) of the patients were followed up with diet alone, 23 (11%) received intensive insulin therapy, 17 (7.6%) received sulfonylurea, 10 (4.5%) received metformin, and 6 (2.7%) received insulin and oral

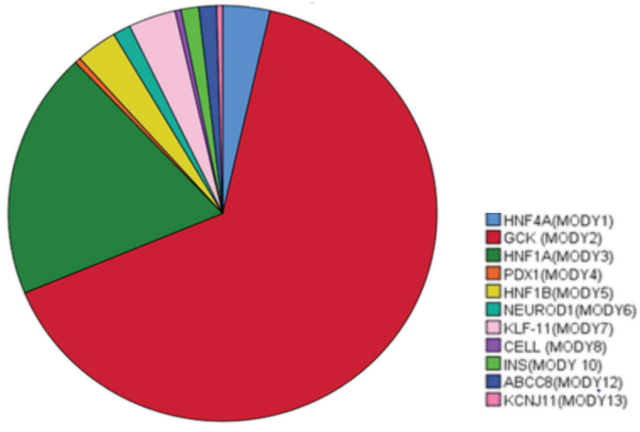

Figure 1. Distribution of gene variants found in the 224 MODY cases from a Turkish pediatric population

antidiabetic therapy (Table 2a, 2b). Seven patients presented with DKA or developed DKA during follow-up and these patients' genotypes were: *HNF4A* (n = 2, 0.89%), *HNF1A* (n = 2, 0.89%), *HNF1B* (n = 1, 0.44%), *NEUROD* (n = 1, 0.44%), and *ABCC8* (n = 1, 0.44%). The clinical and laboratory characteristics of the patients who presented with DKA are shown in Table 3. An *INS* gene mutation was found in one patient who developed DKA during follow-up.

The characteristics of patients receiving intensive insulin treatment are shown in Table 4. Insulin treatment was needed in 35 (15%) of the patients at diagnosis or follow-up. Of these patients, 13 (5%) had *HNF1A* and 6 (2%) had *HNF1B* MODY.

Genotype-phenotype Characteristics and Treatment According to MODY Types

GCK MODY

GCK mutation was found in 146 (65%) of 224 patients. The mean ± SD age was 8.8 ± 3.9 years. Although all of the GCK MODYs were diagnosed with incidental hyperglycemia, 23 of them had typical symptoms of T1DM. In 50% of the cases, there was a family history of diabetes in at least two generations. At the time of diagnosis, the mean blood glucose level was 125 ± 21 mg/dL, and c-peptide was 1.4 ± 1.3 mg/dL. HbA1c ranged from 5.4-10.2% with a mean of 6.3 ± 0.3%. Anti-GAD (n = 6), anti-insulin (n = 1) and islet cell antibodies (n = 5) were positive in some. Comorbidities included arachnoid cyst, short stature, asthma, cystic fibrosis, precocious puberty, connective tissue diseases, dyslipidaemia and undescended testis. While 135 patients were followed up with diet, six received oral anti-diabetic therapy, and five received intensive insulin treatment.

HNF1A MODY

Nearly a fifth (19.2%, n = 43) of the patients had this mutation. The mean ± SD age at diagnosis was 10.6 ± 4.3 years. Typical symptoms of T1DM were present in five patients, and 31 patients presented with incidental hyperglycemia. Thirty-five patients had a positive family

Table 1. Demographic characteristics of pediatric genetically confirmed MODY cases in a Turkish cohort (n = 224)

	HNF4A n = 8	*GCK* n = 146	*HNF1A* n = 43	*PDX1* n = 1	*HNF1B* n = 7	*NEUROD1* n = 3	*KLF-11* n = 8	*CEL* n = 1	*INS* n = 3	*ABCC8* n = 3	*KJNC11* n = 1	Mean
Age at diagnosis (years)	10.3 ± 4.6	8.8 ± 3.9	10.6 ± 4.3	3.9	12.4 ± 3	9 ± 7.8	11.1 ± 2	6	9.1 ± 2.3	10.5 ± 1.8	8.5	9.4 ± 4.1 (range 0.15-17)
Sex F/M	3/5	63/83	22/21	0/1	3/4	1/2	4/4	1/0	1/2	2/1	1	101/123 (45%/55%)
Symptom												30%
(+)	6	23	5	1	5	0	7	0	0	1	1	(49/224)
(-)	2	123	18	0	2	3	1	1	3	2	0	
Presentation (n)												
IH	5	145	31	0	5	2	3	1	3	3	1	197 (87%)
DK	1	0	9	0	1	0	3	0	1	1	0	16 (7%)
DKA	2	0	2	1	2	1	0	0	0	0	0	8 (3.5%)
Negative family history (n)	2	17	4	0	1	0	0	0	0	0	0	24/224 (11%)

F/M: female/male, IH: incidental hyperglycemia, DK: diabetic ketosis, DKA: diabetic ketoacidosis, MODY: maturity onset diabetes of the young

Table 2a. Treatment

	PDX1 n = 1	*HNF1B* n = 7	*NEUROD1* n = 3	*KLF-11* n = 8	*CEL* n = 1	*INS* n = 3	*ABCC8* n = 3	*KJNC11* n = 1
Literature	D OAD (metformin, DPP4) Insulin	Insulin	D OAD Insulin	OAD Insulin	OAD Insulin	D OAD Insulin	OAD	Diet OAD Insulin
Treatment	D: 1	D: 1 intensive insuline: 4 Basal ins + OAD: 1	D: 2 Intensive ins: 1	D: 1 M: 1 Intensive ins: 4 Basal ins + OAD: 2	D: 1	OAD: 1 Intensive ins: 1	OAD: 2	OAD: 1

OAD: oral anti-diabetic, M: metformin, DPP4: dipeptidyl peptidase-4 inhibitors, D: diet, ins: insuline

history. At diagnosis, mean blood glucose was 209 ± 162 mg/dL, and c-peptide was 1.5 ± 0.96 ng/mL. Mean HbA1c was 8.8 ± 3.1 %. Ten patients had autoantibody positivity. Comorbidities included exudative retinopathy (n = 2), asthma (n = 1), growth hormone deficiency (n = 1), celiac disease (n = 1), pelviectasis (n = 1), hearing loss (n = 2), and sensory neuropathy (n = 1). Thirteen of the patients were on diet therapy, 15 were on oral anti-diabetic therapy and nine were on insulin therapy.

Table 2b. Treatment

Treatment	*HNF4A* n = 8	*GCK* n = 146	*HNF1A* n = 43
Lecture	Diet SU GLP-1 RA Insulin	**Diet**	SU Meglitinide, GLP-1 RA, SGLT-2 Insulin
In our cases	D: 4 SU: 2 Intensive ins: 2	D: 135 SU: 3 OAD (M): 3 Insulin: 2 Intensive ins: 3	D: 13 OAD (S): 11 OAD (M): 4 ins: 7 Basal ins + OAD: 2

OAD: oral anti-diabetic, M: metformin, D: diet, SU: sulphonylureas, GLP-1 RA: glucagon-like peptide 1 receptor agonist, SGLT-2: sodium-glucose transport protein 2 inhibitors, ins: insuline

Table 3. Characteristics of patients presenting with acidosis or developing acidosis during follow-up

	HNF4A-MODY n = 2		*HNF1A*-MODY n = 2		*HNF1B*-MODY n = 1	*NEUROD1*-MODY n = 1	*INS*-MODY n = 1
Sex	M	M	M	F	M	F	F
Age of diagnosis, years	8.2	10	12	15	15	18	16.6
GAD	Negative	Negative	Negative	Negative	Negative	Negative	Negative
ICA	No data	Negative	Negative	**Positive**	Negative	Negative	Negative
Anti insulin	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Family history	No	No	Yes (grandfather)	Yes (brother, grandmother, grandfather)	Yes (father)	Yes (father uncle, aunt)	Yes Father
Glucose at Dx (mg/dL)	674	548	443	210	600	281	269
C-peptide (ng/mL)	0.60	0.53	0.1	0.1	0.1	0.1	3.47
HbA1c (%)	11.78	10.6	13.2	9	8	7.5	11.2
Treatment	Int insulin the same treatment continued	Int insulin after diet	Int insulin the same treatment continued	Int insulin the same treatment continued	Int insulin the same treatment continued	Int insulin the same treatment continued	Int insulin the same treatment continued

F: female, M: male, HbA1c: glycated hemoglobin, int: initially, MODY: maturity onset diabetes of the young

Table 4. Characteristics of patients on insulin

	HNF4A	*GCK*	*HNF1A*	*HNF1B*	*NEUROD1*	*KLF-11*	*INS*	*ABCC8*	Total
Number of patients	2	5	13	6	1	6	1	1	35
Features at presentation	K: 1 DKA: 1 DM symptom at diagnose: 2	K: 0 DKA: 0 Diabet symptom: 2	K: 5 DKA: 2 Diabet symptom: 5	K: 1 DKA: 2 Diabet symptom: 6	K: 1 DKA: 1 Diabet symptom: 1	K: 3 DKA: 0 Diabet symptom: 6	K: 0 DKA: 0 Diabet symptom: 0	K: 0 DKA: 0 Diabet symptom: 0	
HbA1c %	11.7, 12.9	5.8, 6, 6.4, 7.2, 7.4	6, 7, 7.4, 8, 8.4, 8.8, 9, 11.30 12.5, 13.6, 14.6, 20	6, 7.5, 7.7, 8, 9.5, 17.5	7.4	6.8, 9.8, 10, 11.9 14.7, 15.6	11.2	5.8	

K: ketosis, DKA: diabetic ketoacidosis, F: female, M: male, HbA1c: glycated hemoglobin, DM: diabetes mellitus

HNF4A MODY

This MODY subtype constituted 3.5% (n = 8) of the cases. The mean age at diagnosis was 10.3 ± 4.6 years. There was one case with a birth weight of over 4000 g. Five cases had incidental hyperglycemia, and six cases had typical symptoms of diabetes. Acidosis was present in two cases, and autoantibody positivity was observed in one case. At the time of diagnosis, mean blood glucose was 306 ± 216 mg/dL, and c-peptide was 1.9 ± 1.2 ng/mL. while HbA1c range was 4.7-11.7%. One patient was obese at diagnosis. The only comorbidity was spinocerebellar syndrome (n = 3). While four of the patients were being managed with the diet, two were being treated with oral anti-diabetic therapy and two were being treated with insulin.

HNF1B MODY

This mutation was detected in 3.1 % (n = 7) of the cases. The mean age at diagnosis was 12.4 ± 3 years. Five patients had incidental hyperglycemia, and five patients had symptoms of diabetes. Two patients presented with DKA. Six patients had a family history. At the time of diagnosis, blood glucose was 308 ± 177 mg/dL, and c-peptide was 1.18 ± 1.0 ng/mL. HbA1c range was 5.4-14.5%. No autoantibody positivity was found in any of the patients. Additional anomalies were chronic renal failure and elevated transaminases in one, focal segmental glomerulosclerosis in polycystic kidney in two, and renal cyst in one. Four of the patients were receiving insulin, one was on diet only and one was receiving oral anti-diabetic therapy and basal insulin.

KLF MODY

KLF MODY was present in 3.5% (n = 8) of the patients. The mean age at diagnosis was 11.2 ± 2.0 years. Three patients presented with incidental hyperglycemia, seven had symptoms of diabetes and three patients with diabetic ketosis. Family history was present in all cases. At diagnosis, blood glucose was 278 ± 175 mg/dL, and c-peptide was 2.2 ± 2.0 ng/mL. The range of HbA1c was 5.6-10.2%. Only one patient had autoantibodies (anti-GAD). One patient was on a diet, one on oral anti-diabetic therapy, two on oral anti-diabetic therapy and basal insulin, and four on intensive insulin treatment.

NEUROD1 MODY

NEUROD1 MODY was found in 1.3% (n = 3) of the cases. Their age was 1, 12 and 15 years old and one patient presented with DKA. No autoantibody positivity was detected. Additional anomalies included epilepsy in one patient and mental motor retardation (MMR) in one. At diagnosis, blood glucose was 164 ± 100 mg/dL and c-peptide was 0.8 ± 0.7 mg/dL. HbA1c was in the range of 5.5-8%. Family history was not found in any patient. Two patients were on a diet, and one received intensive insulin treatment.

INS MODY

This MODY subtype was found in 1.3% (n = 3) cases. Their ages were 7.5, 7.8 and 11 years old. Two patients had no symptoms of diabetes, and one patient presented with DKA. All three patients had a family history. Acidosis developed in one patient during follow-up. No autoantibody positivity was detected. At the time of diagnosis, blood glucose was 200 ± 64 mg/dL, and c-peptide was 8.5 ± 4.3 mg/dL. HbA1c ranged from 6.2 to 14%. MMR was associated with one patient. One patient was on a diet, and two were receiving intensive insulin.

ABCC8 MODY

This subtype of MODY was found in 1.3% (n = 3) of the cases. Their age was 8.5, 11 and 12 years and diabetes symptoms were absent in two. Family history was present in all patients, and one patient was positive for autoantibodies. At diagnosis, blood glucose was 140 ± 26 mg/dL, and c-peptide was 2.4 ± 0.58 mg/dL. Two of the patients were taking oral anti-diabetic therapy and the other was managed with diet.

PDX MODY

A 3.9-year-old patient presented with DKA. Family history was positive. No autoantibodies were detected. Admission HbA1c was 5.2%. Blood glucose was 135 mg/dL, and c-peptide was 3.4 mg/dL at the time of diagnosis. He was being managed with diet only.

CEL MODY

CEL variant associated MODY was detected in one patient, 6 years old, who presented with incidental hyperglycemia. There was no family history. Insulin was not started during the follow-up. Autoantibodies were negative and HbA1c was 5.6%. At diagnosis, blood glucose was 137 mg/dL, and c-peptide was 0.7 mg/dL. He was managed with diet only.

KJNC11 MODY

This form of MODY was detected in one case who was 8.5 years old. Symptoms of diabetes and autoantibody positivity were present at presentation. There was a family history of diabetes. HbA1c was 7.8%, and insulin was not started during follow-up. At the time of diagnosis, blood glucose was 191 mg/dL, and c-peptide was 2.1 mg/dL. The patient was followed up with oral anti-diabetic therapy.

Of the 224 patients evaluated, eight patients presented with acidosis. These cases were limited to the MODY

Table 5. Cases with autoantibody positivity

	HNF4A n = 8	*GCK* n = 146	*HNF1A* n = 43	*PDX1* n = 1	*HNF1B* n = 7	*NEUROD1* n = 3	*KLF-11* n = 8	*CELL* n = 1	*INS* n = 3	*ABCC8* n = 3	*KJNC11* n = 1	Total
Anti-GAD	Anti-GAD: 1	Anti-GAD: 6	Anti-GAD: 4	Anti-GAD: 0	Anti-GAD: 0	Anti-GAD: 0	Anti-GAD: 1	Anti-GAD: 0	Anti-GAD: 0	Anti-GAD: 1	Anti-GAD: 0	13
Anti-insulin	Anti-insulin: 0	Anti-insulin: 1	Anti-insulin: 3	Anti-insulin: 0	Anti-insulin: 0	Anti-insulin: 0	Anti-insulin: 0	Anti-insulin: 0	Anti-insulin: 0	Anti-insulin: 0	Anti-insulin: 0	4 8
Anti-islet	Anti-islet: 0	Anti-islet: 5	Anti-islet: 3	Anti-islet: 0	Anti-islet: 0	Anti-islet: 0	Anti-islet: 0	Anti-islet: 0	Anti-islet: 0	Anti-islet: 0	Anti-islet: 0	
	1	12	10	0	0	0	1	0	0	1	0	25

types 1, 3, 5, and 6 and their detailed characteristics are shown in Table 3. DKA developed in one patient with *INS* mutation during follow-up. Table 5 shows the characteristics of the patients with positive autoantibodies.

Discussion

In this study, which is the largest genetically diagnosed series from Turkey, the most common form of MODY was *GCK*-MODY (65%) followed by *HNF1A*-MODY (19%), while the other types were much rarer. Although the majority of cases were diagnosed because of incidental hyperglycemia, there were also cases with DKA and ketosis without a family history. Obesity was rarely associated with MODY, while comorbidities were seen in some MODY types.

MODY is reported with different frequencies depending on the ethnicity, selected patient population, number of genes analyzed and technical characteristics. The distribution of subgroups may also differ according to the method, environment, and technique. In the present study, which presents a representation of several centers from across Turkey, MODY2 (*GCK*-MODY) was the most common diagnosis, as reported previously (7,8). While *GCK* mutations were found incidentally, as expected, diabetes symptoms were observed in patients with *HNF1A* and *HNF4A* variants. Treatment was changed in 37 (16%) of our patients after genetic diagnosis or during follow-up (5,7,8,9,10,11).

In the largest series from Germany/Austria, MODY1 was found in 44, MODY2 in 609, MODY3 in 230, and MODY5 in 35 of 1047 MODY cases from 76,836 children under 20 years of age with diabetes (12). In the UK, MODY prevalence and subtype studies have been performed extensively and it has been reported that *HNF1A*-MODY is the most common type (52%) with *GCK*-MODY the second most common. While MODY due to *HNF1A* mutation is most common in European countries (13), *GCK*-MODY is the most common type in Japan, although there are few studies from Asian and Far Eastern countries (14).

Studies from Italy have shown that the selected patient population is important in determining the frequency of the MODY subtype. In the MODY subgroup in which patients with symptomatic hyperglycemia were evaluated, *HNF1A*- and *HNF1B*-MODY were most prevalent, while in the group with incidental hyperglycemia, *GCK*-MODY was most common. This is also a factor in the difference in frequency of MODY subtypes in adult and pediatric studies. For an accurate ranking, both age groups should be evaluated together (15).

Family history has been used as an essential criterion in MODY probability calculation models. This probability calculation reduces the probability of MODY in the absence of family history, but case reports and the present series show that there may be some exceptional cases. In a large series reported from Japan, 18% of the cases with genetically proven MODY did not have a family history (14). Furthermore, novel mutation was demonstrated in 7.3% of patients with the four most common MODY subtypes reported from Slovakia (16). In our series, the *de novo* mutation rate was 11%.

The use of anti-GAD positivity as a criterion for exclusion of MODY is a matter of debate. In a large series, anti-GAD was found to be positive in only 5 of 508 MODY cases, and this rate was similar to the normal population rate. However, in a Czech study, anti-GAD positivity was found in 7 of 28 MODY cases, and IAC2 positivity was found in one of 28 MODY cases. In these patients, the course of diabetes was worse, and HbA1c values were higher. Autoantibody negativity has been reported with appropriate therapeutic control of diabetes. Schober et al. (17) found a positive autoantibody rate of 17% in a German-Austrian series (18,19). In the present study, autoantibodies were positive in 25 patients. This may be due to a condition termed "double diabetes". Polygenic diabetes has been reported to affect 3-4% of MODY cases, and the coexistence of T2DM and MODY is more common than T1DM and MODY (20). In the present series, only three patients with *GCK* mutation had increased HbA1c values and insulin requirement during follow-up. In a

Polish study, increased HbA1c values were also reported in patients with *GCK* mutation. This was explained by T1DM or T2DM accompanying monogenic diabetes. Thus, monogenic DM does not preclude the existence of other types. In 8 of 285 cases with *GCK* mutation, HbA1c values above 7.5% were reported. Steele et al. (21) also found HbA1c values between 8.2-9.5% in 6 of 235 cases with *GCK* mutation.

Although obesity is used to differentiate cases with T2DM from MODY, obesity associated with rare types of MODY, such as those caused by variants in *IPF1/PDX1* (MODY 4), *NEUROD1* (MODY 6), *BLK* (MODY 11) and *ABCC8* (MODY 12), and even common types (*HNF1A-* and *HNF4A-*MODY) have been reported in recent years. Therefore, obesity in MODY may be more common than previously thought, probably due to the general increase in global obesity prevalence (14). In the present series, obesity was found in 16 (7%) cases at diagnosis. In a Japanese study, the rate of overweight was 8.2%, and insulin resistance was 22% (21).

While MODY due to variants in *HNF4A*, *HNF1A* and *HNF1B* may present with DKA, *INS*, *NEUROD1* and *PDX*, which are rare types, may also present with DKA (20). Consistent with the literature, our patients also had these mutations and presented with acidosis due to insulin deficiency. It seems that using acidosis as anexclusion criterion for MODY may exclude some cases.

The criteria of three generations affected with diabetes, autoantibodies being negative, and insulin reserve present do not always hold true. In the present series, autoantibodies were positive in 25 patients, seven patients presented with DKA, and no family history was found in 24 patients. Approximately 50% of positive MODY cases do not fulfill the classical MODY diagnostic criteria; some patients with genetically confirmed MODY may also have pancreatic antibodies, and some *de novo* mutations may have occurred (21).

Over time and with more extensive research, knowledge of the natural history of MODY has increased. The ability to partially or completely change the treatment of people diagnosed with MODY from insuline to oral hypoglycaemic agents has saved patients from injections and unnecessary insulin therapy. However, while *GCK* mutations are followed up only with diet, *PDX*, *INS*, *BLK* and *APPL1* variant-associated MODY cases require earlyinsulin, and, *HNF4A*, *HNF1A* and *PAX4* defects may also require oral antidiabetic or insulin. MODY due to *ABCC8* and *KCNJ11* mutations respond well to oral antidiabetics. In *HNF1B*, *NEUROD1*, *KLF11* and *CEL* mutations, the response to oral antidiabetics is variable and insulin requirement develops over time (22). In our series, treatment changes were made in 37 patients during follow-up. While 71% of the patients were followed up only by adjusting their diet, 10% were receiving intensive insulin treatment, and 7% were receiving sulfonylurea.

Strengths of the present study include the high number of cases compared to early studies from Turkey, the detailed information about clinical characteristics of the identified cases and that this was a nationally supported study.

Study Limitations

However, the limitations include the fact that not all centers participated, the MODY panels used varied from center to center and the interpretation of the MODY reports were performed by several geneticists.

Conclusion

This large-series national study showed that the diagnostic criteria in the prediagnostic process of MODY should be reconsidered in the presence of DKA, the presence of antibody positivity and the absence of family history in patients with genetically diagnosed MODY in the Turkish population. Therefore, it does not seems appropriate to use obesity and/or the absence of DKA as absolute criteria for suggesting that a patient does not have MODY, and this will need to be considered in the development of updated MODY screening guidelines.

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Ethics

Ethics Committee Approval: Ethical approval was obtained from Ankara University Faculty of Medicine (decision number: 12-112-20, date: 13.02.2020).

Informed Consent: Informed consent was obtained from the parents/guardians of the patient from all centers.

Authorship Contributions

Concept: Elif Özsu, Zehra Aycan, Merih Berberoğlu, Zeynep Şıklar, Design: Elif Özsu, Zehra Aycan, Zeynep Şıklar, Data Collection or Processing: Elif Özsu, Semra Çetinkaya, Semih Bolu, Nihal Hatipoğlu, Şenay Savaş Erdeve, Olcay Evliyaoğlu, Firdevs Baş, Atilla Çayır, İsmail Dündar, Emine Demet Akbaş, Seyid Ahmet Uçaktürk, Merih Berberoğlu, Zeynep Şıklar, Şervan Özalkak, Nursel Muratoğlu Şahin, Melikşah Keskin, Ülkü Gül Şiraz, Hande Turan, Ayşe Pınar

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ORIGINAL ARTICLE

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Triglyceride Glucose Index is Associated with Ultrasonographic Fatty Liver Indicator in Children and Adolescents with Non-alcoholic Fatty Liver Disease

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What is already known on this topic?

Triglyceride glucose (TyG) index has been shown to be a reliable surrogate marker for insulin resistance and non-alcoholic fatty liver disease (NAFLD) in adults. The usefulness of this index as a predictive marker for the development and severity of NAFLD in children and adolescents is unknown.

What this study adds?

The TyG index may be a useful tool for predicting severity of NAFLD and determining the need for a liver biopsy.

Abstract

Objective: Non-alcoholic fatty liver disease (NAFLD) is defined as chronic hepatic steatosis and is becoming prevalent, along with the increasing trend for obesity in children and adolescents. A non-invasive and reliable tool is needed to differentiate non-alcoholic steatohepatitis from simple steatosis. This study evaluated the association between the triglyceride glucose (TyG) index and the ultrasonographic fatty liver indicator (US-FLI), and the possibility of using the TyG index for prediction of severity of pediatric NAFLD.

Methods: One hundred and twenty one patients who were diagnosed with NAFLD by ultrasonography were included. They were categorized into three groups according to body mass index (BMI). Ninety-two were obese, and 19 and 10 were overweight and normal weight, respectively.

Results: The homeostatic model assessment for insulin resistance (HOMA-IR) was highest in the group with obesity ($p = 0.044$). The TyG index and US-FLI did not differ significantly among the three BMI groups ($p = 0.186$). Fourteen (11.6%) of the 121 patients had US-FLI ≥6, in whom the BMI-SDS and TyG index were higher ($p = 0.017$, $p = 0.004$), whereas HOMA-IR did not differ significantly from the group with US-FLI < 6 ($p = 0.366$). US-FLI was associated with BMI-SDS and the TyG index. TyG index was significantly associated with US-FLI after adjustment for BMI-SDS. The cut-off value for the TyG index for predicting US-FLI ≥6 was 8.91, with an area under the curve of 0.785.

Conclusion: TyG index was associated with the degree of hepatic steatosis, suggesting that it might be a useful tool for predicting the severity of pediatric NAFLD.

Keywords: Non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, triglyceride glucose index

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by excessive fat accumulation in the liver and can occur at various severities, from simple steatosis to fibrosis and liver cirrhosis. The incidence of NAFLD is increasing along with the worldwide increase in obesity in children (1). The prevalence of NAFLD increased from 8.2% in 2009 to 12.1% in 2018 in Korea (2,3). The prevalence of NAFLD diagnosed on ultrasonography was 11.2% in a study of Korean children and adolescents (4). Some studies reported that the prevalence of obesity in childhood and adolescence increased during the Coronavirus disease-2019 pandemic due to reduced physical activity and increased sedentary time (5,6,7). The prevalence of obesity increased from 11.5% in 2019 to 12.7% in 2020 in a nationwide study of Korean adolescents (8). Obesity in youth can be accompanied by metabolic alterations such as insulin resistance and metabolic syndrome (9,10). Insulin resistance and dyslipidemia are strongly associated with the pathogenesis of NAFLD (11), which can be divided into NAFLD and non-alcoholic steatohepatitis (NASH) based on histology (12). NASH is defined as when steatosis is accompanied by inflammation and hepatocyte damage proved by a histological examination, and it could progress toward cirrhosis, even in children (13,14). Thus, early discrimination of NASH from benign simple steatosis in obese children suspected to have NAFLD is important. Non-invasive and reliable tools to predict the severity of NAFLD in children are needed, given the increase of obesity and NAFLD in children, because liver biopsy is limited for young patients.

Biomarkers of hepatic inflammation, oxidative stress, hepatic apoptosis, and fibrosis have been suggested; however, they are not easily measurable for clinical use (15). Ultrasonography is a convenient, widely available, and non-invasive modality. A non-invasive, semi-quantitative ultrasonographic fatty liver indicator (US-FLI) was recently suggested as a method for predicting hepatitis in patients with NAFLD and was shown to correlate with histopathological severity in adults (16). However, screening asymptomatic individuals with ultrasonography is not recommended. Therefore, simple indices based on laboratory findings or anthropometric data have been proposed to detect NAFLD. The triglyceride glucose (TyG) index was suggested as hyperglycemia, hyperinsulinemia, and hypertriglyceridemia are linked with triglyceride (TG) accumulation in hepatocytes and development of NAFLD. The TyG index has been shown to be a reliable surrogate marker for insulin resistance and NAFLD in adults (17,18). Evaluation of the usefulness of this index as a predictive marker for the development and severity of NAFLD in children and adolescents is needed. Therefore, this study evaluated the association between the TyG index and clinical parameters, including the US-FLI, and the usefulness of the TyG index for detecting the severity of NAFLD in pediatric patients.

Methods

Patients

Subjects who were diagnosed with NAFLD were enrolled in this study after they visited the pediatric endocrinologic clinic between January 2021 and May 2022. NAFLD was suspected when alanine aminotransferase (ALT) was higher than 26 IU/L for males and 22 IU/L for females (19). Abdominal ultrasonography was performed by a single experienced radiologist, and the US-FLI score was determined. Subjects with a US-FLI score of at least 2 accompanied by elevated ALT were diagnosed with NAFLD. NAFLD was diagnosed in the absence of a known etiology of hepatitis, such as viral hepatitis, Wilson's disease, autoimmune hepatitis, or drug-induced hepatitis. Furthermore, subjects in this study with a US-FLI score of 6 or greater were suspected of having NASH (20).

Weight and height were obtained, and body mass index (BMI) was calculated as body weight (kg)/height (m^2). The enrolled subjects were divided into three groups (obesity, overweight, normal weight) according to BMI, with obesity defined as BMI ≥95th percentile on sex- and age-adjusted charts (21,22). Patients with BMI between the 85th and 95th percentiles were categorized as overweight. The rest of the subjects composed the normal weight group. Severe obesity was defined as BMI above 99th percentile. Sex maturation ratings (SMR) of patients were described based on Tanner classification.

Venous samples for biochemical testing were obtained after a fast of at least eight hours. Aspartate transaminase (AST), ALT, low-density lipoprotein cholesterol, high-density lipoprotein-cholesterol (HDL-C), and TG were measured using an automatic analyzer (Hitachi 7600, Hitachi, Tokyo, Japan). Serum insulin level was measured using a Wizard 1470 gamma counter (PerkinElmer, Massachusetts, USA). Non-HDL-C concentration was calculated as total cholesterol – HDL-C. The TyG index was calculated using the following formula: Ln[fasting TG (mg/dL) x fasting glucose (mg/dL)/2]. The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as the product of the fasting insulin level (μU/mL) and the fasting glucose level (mmol/L), divided by 22.5. The HbA1c level was measured using high-performance liquid chromatography. Prediabetes and diabetes were defined as an HbA1c level of 5.7% to 6.4% and ≥6.5%, respectively (23).

US-FLI was scored based on a published report as mild/moderate (score 2) or severe (score 3) by the intensity of liver/kidney contrast. Additional criteria included the presence (score 1 each) of posterior attenuation of the ultrasound beam, vessel blurring, difficult visualization of the gallbladder wall, difficult visualization of the diaphragm, and areas of focal sparing (16). The US-FLI was determined by summing all scores for a total range from 2 to 8 in cases of NAFLD (16).

This study was approved by the Institutional Review Board of Hallym University Kangdong Sacred Heart Hospital, Seoul, Korea (IRB no. 2021-12-007, date: 08.02.2024). Our study was exempt from the requirement of informed consent because of the retrospective nature of the study and the anonymity of the clinical data.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 26.0 (SPSS Inc., Chicago, IL, USA). Independent t-tests and one-way ANOVAs were performed to compare the means of clinical parameters according to US-FLI and BMI, respectively. Fisher's exact test was performed when analyzing categorical parameters such as sex. Associations between US-FLI and clinical variables were analyzed using simple and multiple regression analyses. A receiver operating characteristics (ROC) analysis was performed to obtain cut-off values and the area under the curve (AUC) for the variables. A p value < 0.05 was considered statistically significant.

Results

Baseline Characteristics

Ninety-two patients were obese, and 19 and 10 were overweight and normal weight, respectively. The clinical and biochemical characteristics of the study population according to BMI are shown in Table 1. Ninety-two of the 121 subjects with NAFLD were obese, and 19 and 10 subjects were overweight and normal weight, respectively. Eighty-three (68.6%) of the 121 subjects were male. AST and ALT were higher in the groups with overweight or obesity than in the normal weight group ($p = 0.009$, $p = 0.041$). Biochemical parameters, fasting glucose, insulin, and lipid profiles other than high sensitivity C-reactive protein (CRP) and uric acid did not differ significantly among the three groups. HOMA-IR was highest in the group with obesity pgroups ($p = 0.186$). Two (10.5%) of the 19 subjects with overweight and 12 (13.0%) of the 92 subjects with obesity had US-FLI ≥6. Among 92 patients with obesity, 62 (67.4%) had severe obesity. The patients with severe obesity had higher US-FLI

Table 1. Clinical and biochemical characteristics of the study population according to BMI

	Normal (n = 10)	Overweight (n = 19)	Obese (n = 92)	p
Sex (M/F)	10/0	13/6	60/32	0.067
Age, years	11.7 ± 0.8	11.1 ± 1.4	11.4 ± 2.4	0.372
BMI, kg/m^2	21.3 ± 1.4	23.3 ± 1.6	28.0 ± 3.9	< 0.001
BMI-SDS	0.7 ± 0.4	1.5 ± 0.2	2.8 ± 0.8	< 0.001
AST, IU/L	42.8 ± 8.1	58.2 ± 37.7	55.7 ± 31.6	0.009
ALT, IU/L	66.3 ± 23.9	104.9 ± 81.1	89.0 ± 52.7	0.041
Glucose, mg/dL	94.5 ± 11.1	98.1 ± 9.6	101.9 ± 29.2	0.363
Uric acid, mg/dL	5.6 ± 1.0	5.7 ± 0.8	6.4 ± 1.4	0.015
Insulin, µU/mL	10.0 ± 5.1	13.9 ± 6.3	17.3 ± 11.3	0.056
HbA1c, %	5.2 ± 0.3	5.4 ± 0.1	5.7 ± 1.0	0.055
TC, mg/dL	159.3 ± 31.2	180.3 ± 35.7	178.0 ± 31.5	0.215
HDL-C, mg/dL	57.2 ± 19.8	53.9 ± 15.8	47.1 ± 9.7	0.15
LDL-C, mg/dL	92.9 ± 27.2	107.7 ± 29.0	111.4 ± 22.9	0.218
TG, mg/dL	103.8 ± 61.4	115.6 ± 61.2	141.4 ± 79.7	0.19
hsCRP, mg/L	0.4 ± 0.1	2.0 ± 2.3	2.3 ± 2.7	< 0.001
Non-HDL-C, mg/dL	104.1 ± 33.2	128.7 ± 42.9	132.0 ± 29.8	0.113
HOMA-IR	2.4 ± 1.3	3.4 ± 1.7	4.4 ± 3.1	0.044
TyG index	8.3 ± 0.7	8.5 ± 0.7	8.7 ± 0.5	0.186
US-FLI	3.4 ± 1.2	3.8 ± 1.3	4.0 ± 1.2	0.269
US-FLI ≥6 (%)	0 (0)	2 (10.5)	12 (13.0)	0.704

BMI: body mass index, SDS: standard deviation score, AST: aspartate transaminase, ALT: alanine aminotransferase, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TG: triglycerides, hsCRP: high-sensitivity C-reactive protein, Non-HDL-C: non-high-density lipoprotein cholesterol, HOMA-IR: homeostatic model assessment for insulin resistance, TyG index: triglyceride glucose index, US-FLI: ultrasonographic fatty liver indicator, M/F: male/female

compared to the rest of patients with obesity (4.26±1.20 vs. 3.6±1.22, p = 0.008). Among 83 male patients, 65 presented with signs of puberty with SMR 2 stage or above. The 65 male patients with pubertal signs showed higher HOMA-IR (p < 0.001) and TyG index (p = 0.009) in spite of similar BMI-standard deviation score (SDS). US-FLI tended to be higher in patients with signs of puberty (4.08±1.30 vs. 3.56±1.10, p = 0.069). Among 38 female patients, 9 were prepubertal. The age of the 29 female patients with SMR 2 stage or above ranged from 8.3 to 18.6 years and 11 of 29 were under 10 years of age. Four patients had type 2 diabetes with the level of HbA1c ranging from 6.6-12.3%. Their BMI-SDS ranged from 2.43-4.74. All four of the subjects with diabetes had US-FLI ≥6. Sixteen patients had prediabetes; with HbA1c in the range of 5.7-6.2%, and one had US-FLI ≥6.

Clinical Characteristics of Subjects Suspected of NASH (according to US-FLI ≥6)

The enrolled subjects were divided into two groups, based on a US-FLI cut-off level of 6. Table 2 shows the clinical characteristics of the resulting groups. The median US-FLI level was 4, ranging from 2 and 5 in 107 patients with US-FLI 6. Among 14 patients with US-FLI ≥6, 12 patients had 6 of US-FLI levels and the rest 2 patients had 7 of US-FLI levels. Fourteen (11.6%) of the 121 patients with NAFLD had US-FLI ≥6, and they were older (p = 0.007) and had higher BMI-SDS than the subjects in the other group (p = 0.017). The levels of AST, ALT, and HbA1c tended to be higher in the group with US-FLI ≥6 than in the other group. Biochemical data, such as glucose, insulin, and lipid profile, did not differ significantly between the groups (Table 2). The TyG index was significantly higher in the group with US-FLI ≥6 (p = 0.004),

Table 2. Clinical and biochemical characteristics of the study population according to US-FLI

	US-FLI < 6 (n = 107)	US-FLI ≥6 (n = 14)	p
US-FLI	3.7 ± 1.0	6.1 ± 0.4	< 0.001
Sex (M/F)	72/35	11/3	0.545
Age, years	11.1 ± 2.0	13.4 ± 2.6	0.007
BMI, kg/m^2	26.2 ± 3.8	30.7 ± 5.3	0.008
BMI-SDS	2.3 ± 0.9	3.2 ± 1.2	0.017
AST, IU/L	53.0 ± 29.9	70.5 ± 39.2	0.128
ALT, IU/L	85.4 ± 54.7	121.6 ± 63.7	0.06
Glucose, mg/dL	96.9 ± 8.6	129.1 ± 67.4	0.111
Uric acid, mg/dL	6.1 ± 1.2	7.3 ± 1.5	0.011
Insulin, µU/mL	16.6 ± 11.2	15.0 ± 4.5	0.43
HbA1c, %	5.5 ± 0.3	7.0 ± 2.3	0.081
TC, mg/dL	176.6 ± 32.3	177.9 ± 33.6	0.888
HDL-C, mg/dL	49.5 ± 12.2	44.6 ± 10.4	0.141
LDL-C, mg/dL	109.1 ± 24.5	111.7 ± 24.6	0.727
TG, mg/dL	128.4 ± 72.7	180.3 ± 90.4	0.068
hsCRP	2.2 ± 2.7	1.3 ± 0.6	0.041
Non-HDL-C, mg/dL	128.4 ± 32.3	136.5 ± 36.8	0.449
HOMA-IR	4.0 ± 2.7	5.4 ± 4.3	0.366
TyG index	8.6 ± 0.5	9.2 ± 0.6	0.004

US-FLI: ultrasonographic fatty liver indicator, BMI: body mass index, SDS: standard deviation score, AST: aspartate transaminase, ALT: alanine aminotransferase, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TG: triglycerides; hsCRP:high-sensitivity C-reactive protein, Non-HDL-C: non-high-density lipoprotein cholesterol, HOMA-IR: homeostatic model assessment for insulin resistance, TyG index: triglyceride glucose index, M/F: male/female

Table 3. Associations between US-FLI and clinical and biochemical variables in a linear regression analysis

	β	95% CI	p	Adjusted β	95% CI	p
BMI-SDS	0.41	0.2-0.62	< 0.001	0.27	0-0.53	0.05
AST, IU/L	0.01	0-0.02	0.013	-0.01	-0.02-0.01	0.334
ALT, IU/L	0.01	0-0.01	< 0.001	0.01	0-0.01	0.127
HOMA-IR	0.09	0-0.18	0.048	0.02	-0.08-0.12	0.708
TyG index	0.65	0.25-1.05	0.002	0.54	0.04-1.04	0.037

US-FLI: ultrasonographic fatty liver indicator, BMI: body mass index, SDS: standard deviation scores, AST: aspartate transaminase, ALT: alanine aminotransferase, HOMA-IR: homeostatic model assessment for insulin resistance, TyG index: triglyceride glucose index, CI: confidence interval

Table 4. Cut-off values and areas under the ROC curves for predicting US-FLI ≥6

	Cut-off values	AUC	Sensitivity/specificity, %
BMI-SDS	3.21	0.712 (0.539-0.885)	64/85
AST, IU/L	59.5	0.632 (0.457-0.807)	57/76
ALT, IU/L	126.5	0.690 (0.531-0.849)	57/84
HOMA-IR	2.61	0.634 (0.469-0.798)	100/34
TyG index	8.91	0.785 (0.659-0.911)	85/72

ROC: receiver operating characteristics, US-FLI: ultrasonographic fatty liver indicator, AUC: area under the curve, BMI: body mass index, SDS: standard deviation score, AST: aspartate transaminase, ALT: alanine aminotransferase, HOMA-IR: homeostatic model assessment for insulin resistance, TyG index: triglyceride glucose index

but HOMA-IR did not differ significantly between the groups (p = 0.366). Simple linear regression analysis showed that US-FLI was associated with BMI-SDS, AST and ALT levels, HOMA-IR, and the TyG index (Table 3). However, the TyG index was the only variable that was significantly associated with US-FLI after adjustment for BMI-SDS.

Clinical Parameters for Predicting US-FLI ≥6

The cut-off values of five parameters (BMI-SDS, AST, ALT, HOMA-IR, TyG index) that could be used to predict US-FLI ≥6 are shown in Table 4. The cut-off value of HOMA-IR was 2.61 and had high sensitivity but low specificity. The cut-off values of BMI-SDS and the TyG index were 3.21 and 8.91, respectively. The ROC curves of the five parameters are depicted in Figure 1. The AUC for the five parameters is shown in Table 4. The TyG index had the highest AUC score.

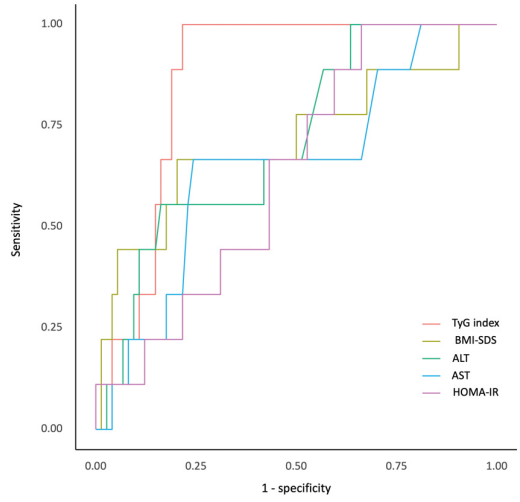

Figure 1. Ability of clinical variables to detect US-FLI ≥6, as shown by ROC analysis. The TyG index had the highest AUC score

US-FLI: ultrasonographic fatty liver indicator, ROC: receiver operating characteristics, TyG: triglyceride glucose, AUC: area under the curve, AST: Aspartate transaminase, ALT: alanine aminotransferase, HOMA-IR: homeostatic model assessment for insulin resistance

Discussion

This study evaluated the link between the TyG index and the degree of fatty infiltration in the liver in children and adolescents to enable prediction of the severity of NAFLD using the TyG index. Generally, screening for NAFLD should be considered for all children with obesity or overweight with risk factors, such as central adiposity, insulin resistance, prediabetes, dyslipidemia, or family history of NAFLD/NASH (12). Currently, ALT is widely used to screen for NAFLD. The normal cut-off value for ALT can differ depending on the studied cohort. The 95th percentile level for ALT was 24.1 U/L for male children and 17.7 U/L for female children in a study using KNHANES 2010-2015 data (24). However, serum ALT level can increase as a consequence of some acute diseases, and it does not exactly reflect the extent of fatty infiltration. On the other hand, previous study showed that 59.3% of NAFLD children had increased ALT levels (≥40 IU/L) (25). In this study, the AST and ALT levels correlated with the US-FLI in a simple linear regression analysis. However, the adjusted β values were not significantly associated with US-FLI. In addition, the TyG index was associated with the US-FLI in a multiple linear regression analysis conducted in the present study, suggesting that the TyG index could be used to predict the severity of NAFLD.

A liver biopsy is the gold standard for diagnosing the severity of NAFLD. However, liver biopsy is a painful and invasive procedure that can produce complications, such as infection or hemorrhage. Furthermore, a small biopsied sample of liver tissue might not represent the overall liver, and histologic findings of pediatric NASH can be different from those of adult NASH (26). The optimal timing of liver biopsies remains controversial, and no clear indication for liver biopsies has been established. Candidate criteria for immediate liver biopsy in pediatric NAFLD patients were suggested and include young age, highly increased serum AST or ALT, very severe insulin resistance, suspected comorbidity or other chronic liver disease, and a family history of NAFLD (27). The European Society for Paediatric Gastroenterology Hepatology and Nutrition panel recommended that liver biopsy be performed after considering differential diagnoses

and the risk of disease progression to liver cirrhosis (28). The North American Society for Pediatric Gastroenterology guideline also recommends liver biopsy in children with an increased risk of NASH or advanced fibrosis (12).

The US-FLI score was used to predict the severity of NAFLD in this study. Ultrasonography is non-invasive, widely available, and well tolerated as a first-line imaging study. However, inter-observer and intra-observer variability and lack of objective quantitative analyses are limitations. Generally, ultrasonographic findings are classified using a 4-grade scale (normal, mild, moderate, and severe) (29). Despite these limitations, ultrasonographically quantified fat is associated with metabolic disturbances, and the histologic extent of steatosis correlates with a NASH diagnosis, suggesting that ultrasonographic score could be used to predict the severity of NAFLD (30,31). The US-FLI, a semi-quantitative ultrasonographic score, reflects the severity of hepatosteatosis and correlates with liver histology, with the exception of fibrosis, so it can help clinicians when selecting patients for liver biopsy (16). In addition, the US-FLI score was associated with liver enzymes, the waist-to-height ratio, and uric acid, adiponectin, and cytokeratin 18 levels in a pediatric study (20). A US-FLI score >6 was suggested to indicate a relatively high risk for hepatitis, with a 71.4% positive predicted value (20).

We demonstrated an association between the TyG index and the degree of hepatic steatosis, indicating that the TyG index is a simple and cost-effective tool for predicting severe hepatic steatosis and considering liver biopsy in children and adolescents. Pediatric NAFLD can progress to clinically severe conditions, such as cirrhosis, and might present with an aggressive phenotype in the young population with obesity (32). In addition, severe phenotypes are expected to be more likely to progress to cirrhosis (33). All children and adolescents with obesity or overweight should receive lifestyle intervention counseling, and screening for NAFLD should be considered for early detection. If ALT is above the normal range, calculating the TyG index is helpful for identifying NAFLD and predicting the severity of steatosis, which could lead to more intensive lifestyle interventions. Modified TyG indices combine the TyG index with obesity-related parameters and have been reported to be superior to the TyG index for detecting NAFLD (4,34,35). Associations between other indices and the severity of hepatic steatosis should be investigated.

Study Limitations

This study has some limitations. First, we used ultrasonographic data to identify the patients suspected of having NASH. The data presented in this study was obtained retrospectively. Waist circumferences were not available in most patients although waist circumference better reflects abdominal obesity. Second, we used cross-sectional data from only Korean children and adolescents. Genetic predisposition could strongly affect the development of NAFLD. Third, this study included NAFLD patients with relatively low or moderate severity considering the ages and follow-up periods of enrolled patients. Thus, the number of subjects with US-FLI score ≥6 was small. Nevertheless, few studies have investigated the association between the semi-quantitative US-FLI score and the TyG index, and our results suggest the usefulness of the TyG index in children and adolescents. Given the increasing number of children and adolescents with NAFLD, further longitudinal investigations that use non-invasive tools to evaluate NAFLD severity and response to treatment are warranted.

Conclusion

Pediatric NAFLD presents asymptomatically but could progress to fibrosis and even cirrhosis. Thus, early recognition and proper intervention are required. No non-invasive modalities have been validated for assessing the severity of pediatric NAFLD, until now. The TyG index or its modifications may be a useful tool for predicting the severity of pediatric NAFLD and determining the need for a liver biopsy, as well as for detecting NAFLD in children and adolescents. Further research is needed to develop non-invasive indices or discover biomarkers that accurately reflect the progression or improvement of pediatric NAFLD.

Ethics

Ethics Committee Approval: This study was approved by the Institutional Review Board of Hallym University Kangdong Sacred Heart Hospital, Seoul, Korea (IRB no. 2021-12-007, date: 08.02.2024).

Informed Consent: Our study was exempt from the requirement of informed consent because of the retrospective nature of the study and the anonymity of the clinical data.

Authorship Contributions

Concept: Hye Young Jin, Il Tae Hwang, Data Collection or Processing: Bitgyeol Kim, Hye Young Jin, Jong Seo Yoon, Analysis or Interpretation: Hye Young Jin, Eu Seon Noh, Writing: Bitgyeol Kim, Hye Young Jin, Jong Seo Yoon, Eu Seon Noh, Il Tae Hwang.

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ORIGINAL ARTICLE

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Differentiated Thyroid Cancer in Children and Adolescents: 12-year Experience in a Single Center

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What is already known on this topic?

Differentiated thyroid cancer has a good prognosis in the pediatric population even in the presence of metastatic disease. For this reason, an individualized risk-based approach is recommended to select patients for additional therapy. Lymphovascular invasion is usually not considered, despite being a well-known risk factor in the adult population.

What this study adds?

Our study describes the outcomes of a cohort of patients diagnosed at pediatric age and suggests that lymphovascular invasion may be associated with a higher risk of persistence/recurrence and should therefore be considered for decision making.

Abstract

Objective: Differentiated thyroid cancer (DTC) is the most common pediatric endocrine cancer but studies are scarce. Latest recommendations advocate for an individualized risk-based approach to select patients for additional therapy. Lymphovascular invasion is not considered, despite being a well-known risk factor in the adult population. The aim of this study was to describe the outcomes of a cohort of DTC patients diagnosed at pediatric age and to evaluate the impact of lymphovascular invasion on the risk of persistence/recurrence.

Methods: A retrospective study of patients diagnosed with DTC at pediatric age from 2010 to 2022 at a single center was performed. All patients had total thyroidectomy. Radioactive iodine therapy (RAI) was used in selected patients. The response to therapy and occurrence of persistent/recurrent disease were evaluated.

Results: A total of 21 DTC were diagnosed, mostly papillary thyroid carcinoma (PTC) (81.0%, n=17). Six patients (28.6%) had nodal involvement and one (4.8%) had lung metastasis at the time of the diagnosis. Lymphovascular invasion was present in 11 patients (52.4%). After surgery, 13 patients (61.9%) underwent RAI. The mean follow-up time was 5.7±3.1 years. In total, 6 patients (31.6%) experienced persistent/recurrent disease during the follow-up time. Among PTC patients, persistent/recurrent disease was more frequent in the presence of lymphovascular invasion [55.6% (5/9) vs. 0.0% (0/6), p=0.031].

Conclusion: An individualized risk-based approach is recommended. Our study suggests that lymphovascular invasion may be associated with a higher risk of persistence/recurrence and should therefore be considered for decision making in children and adolescents with PTC.

Keywords: Differentiated thyroid cancer, papillary thyroid cancer, children and adolescents, pediatric, lymphovascular invasion, persistence, recurrence

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Introduction

Differentiated thyroid cancer (DTC) is the leading cause of pediatric endocrine cancer, accounting for over 6% of all pediatric cancers (1). Among 15- to 19-year-old adolescents, thyroid cancer is the eighth most frequently diagnosed cancer and the second most common among girls (1,2). However, pediatric papillary thyroid cancer (PTC) is still a rare disease.

The most common presentation of DTC in children and adolescents is a thyroid nodule. However, DTC also frequently presents as cervical adenopathy with or without a palpable thyroid lesion or as an incidental finding after imaging or surgery for an unrelated condition (3). The most common subtype of DTC is PTC, accounting for 90% or more of all childhood cases (4,5). Follicular thyroid carcinoma (FTC) and oncocytic follicular Hürthle cell carcinoma (OFTC) are less frequent (4,5).

When compared to adult patients, children and adolescents with DTC are more likely to have regional lymph node involvement, extrathyroidal extension and distant metastasis, thereby requiring the use of more aggressive treatment (3,4,5,6,7,8,9). However, the pediatric population with thyroid cancer is at very low risk of death and at higher risk for long-term harm from aggressive treatment. (3,4,5,6,7,8,9). Thus, more conservative strategies, including lobectomy and less radioactive iodine therapy (RAI) use, have been advocated.

Due to the lack of clinical trials, treatment options remain controversial. The latest American Thyroid Association (ATA) guidelines advocate for an individualized risk-based approach to identify patients who are likely to benefit from additional staging and therapy, after accurate preoperative staging for regional disease and appropriate surgery (10). These guidelines define three Pediatric risk levels: "Low risk", "Intermediate risk" and "High risk" (Table 1) with a more conservative strategy with less RAI use in low-risk patients. The assignment of a pediatric risk level results from the pathologic findings and postoperative clinical data. Nevertheless, only the extension of the primary tumor and the presence of nodal involvement and distant metastasis (TNM classification) are considered. Lymphovascular invasion is not considered in these recommendations, despite being a well-known risk factor for recurrence in the adult population with PTC (11,12,13,14,15). Most recently, European guidelines for the management of DTC have been published (16). However, although these include risk stratification based on some histological characteristics and subtypes, they again do not take lymphovascular invasion into account. Furthermore, specific recommendations on prophylactic RAI are not included due to the scarce evidence.

The criteria for diagnosing vascular invasion in thyroid carcinomas are poorly defined. Vascular invasion has been used by histopathologists to describe venous invasion exclusively, but also to describe lymphatic invasion sometimes (11,12,13,17). Moreover, distinguishing venous invasion from lymphatic invasion is not always straightforward, making it sometimes impossible to provide a clear classification (18). Therefore, specific information on the type of vascular invasion presented is often absent from histopathology reports.

The aim of this study was to describe the outcomes of a cohort of DTC patients diagnosed at pediatric age. A secondary aim was to evaluate the impact of lymphovascular invasion on the risk of PTC persistence/recurrence in this population.

Methods

Patients

A retrospective study of patients diagnosed with DTC at pediatric age was conducted, covering the period from January 2010 to July 2022 at the Pediatric Hospital of Coimbra, Portugal. Patients included in this study needed to meet the following criteria: (1) histopathological confirmation of DTC, including PTC, FTC or OFTC; and (2) under the age of 18 years at diagnosis.

All the patients participating in this study underwent total thyroidectomy by experienced thyroid surgeons. Therapeutic central and lateral neck lymph node dissection was performed in case of malignant cytology and clinical

Table 1. ATA pediatric thyroid cancer risk levels (11)

ATA pediatric risk level	Definition
Low risk	Disease grossly confined to the thyroid with N0/Nx disease or patients with incidental N1a disease (microscopic metastasis to a small number of central neck lymph nodes)
Intermediate risk	Extensive N1a or minimal N1b disease
High risk	Regionally extensive disease (extensive N1b) or locally invasive disease (T4 tumors), with or without distant metastasis

"Risk" is defined as the likelihood of having persistent cervical disease and/or distant metastases after initial total thyroidectomy - lymph node dissection by an experienced thyroid surgeon and is not the risk for mortality, which is extremely low in the pediatric population.
ATA: American Thyroid Association

evidence of gross extrathyroidal invasion and/or locoregional metastasis on preoperative staging or intraoperative findings. Prophylactic neck dissections were not performed in any of the patients.

The tumor stage was classified according to the American Joint Committee on Cancer TNM (AJCC/TNM) staging system, 7th Edition. Patients with PTC were also classified according to the ATA pediatric thyroid cancer risk levels as "Low risk", "Intermediate risk" or "High risk". The tumor was also evaluated for multifocality, lymphovascular invasion and extrathyroidal extension. The term lymphovascular invasion was used to describe venous and/or lymphatic invasion.

RAI therapy was used in selected patients after multidisciplinary discussion, followed by iodine whole-body scan. No patients received chemotherapy or kinase inhibitor therapy. Thyroid stimulating hormone suppression therapy was performed in all patients for at least five years.

Follow-up and Clinical Outcomes

Follow-up visits were performed every 3-6 months for at least three years and then annually. Upon turning 18 years-old, care was transitioned to adult endocrinology. During the follow-up period, laboratory tests, including serum thyroglobulin (Tg) and Tg antibody measurement, and ultrasound of the neck were performed. Fine-needle biopsy was used in the presence of suspicious lymph nodes or nodules in the neck area.

The response to therapy was evaluated at 12 months after surgery and at the last follow-up visit and classified as "no evidence of disease" or "persistent/recurrent disease". The persistent/recurrent disease was defined as any evidence of structural disease on imaging with or without abnormal biochemical findings after initial surgery. Persistent/recurrent disease at any time during follow-up was also evaluated and classified as locoregional or distant disease. To evaluate the outcomes, patients with a follow-up of less than one year were excluded.

The protocol was approved by the Ethics Committee of Centro Hospitalar Universitário de Coimbra (OBS.SF.135-2022, 03.11.2022). Patient consent was waived by the Ethics Committee due to the retrospective nature of the study and full data anonymization.

Statistical Analysis

Statistical analysis was conducted using Statistical Package for Social Sciences software version 27.0 (IBM Inc., Armonk, NY, USA). For continuous quantitative variables, distribution normality was tested through histogram observation and kurtosis and skewness analysis. The results are presented as mean ± standard deviation or median (interquartile range).

The goodness of fit χ^2-test was used to compare frequencies between the groups with and without persistent/recurrent disease. Student's t-test for independent variables and Mann-Whitney test were used to compare continuous variables with normal and non-normal distribution between the two groups, respectively. A two-sided p value < 0.05 was considered statistically significant.

Results

Sample Characteristics

During the study period, a total of 21 DTCs were diagnosed at pediatric age. The median age at initial diagnosis was 16 [14-16] years (minimum-maximum: 8-17 years). The majority of patients were female (85.7%, n = 18). The clinicopathologic characteristics of the patients are presented in Table 2.

Table 2. Clinicopathologic characteristics of the patients

	n = 21
Age (years)	16 [14-16]
Female gender (%, n)	85.7% (18/21)
History of any cancer in first degree relatives (%, n)	23.8% (5/21)
Reason for diagnosis (%, n)	
Indeterminate or suspicious thyroid nodule on fine needle aspiration cytology[a]	76.2% (16/21)
Atypia of undetermined significance	9.5% (2/21)
Follicular neoplasm	23.8% (5/21)
Malignant	42.9% (9/21)
Incidental diagnosis[b]	23.8% (5/21)
Thyroidectomy for benign multinodular goiter	14.3% (3/21)
Thyroidectomy for PTEN syndrome	4.8% (1/21)
Thyroidectomy for Grave's disease	4.8% (1/21)
Surgery (%, n)	
Total thyroidectomy	100% (21/21)
Central lymphadenectomy	19.0% (4/21)
Lateral lymphadenectomy	9.5% (2/21)

Table 2. Continued

	n = 21
Histopathology (%, n)	
PTC	81.0% (17/21)
Classic variant	61.9% (13/21)
Follicular variant	19.0% (4/21)
FTC (minimally invasive)	14.3% (3/21)
OFTC	4.8% (1/21)
Multifocality (%, n)	23.8% (5/21)
Largest size of the dominant tumor (mm)	16 [12-32]
Lymphovascular invasion (%, n)	52.4% (11/21)
Extrathyroidal extension (%, n)	33.3% (7/21)
Minimal	23.8% (5/21)
TNM classification[c] (%, n)	
T	
T1a	14.3% (3/21)
T1b	23.8% (5/21)
T2	28.6% (6/21)
T3	23.8% (5/21)
T4a	9.5% (2/21)
N	
Nx	52.4% (11/21)
N0	19.0% (4/21)
N1a	4.8% (1/21)
N1b	23.8% (5/21)
M	
Mx	38.1% (8/21)
M0	57.1% (12/21)
M1	4.8% (1/21)
ATA pediatric thyroid cancer risk levels[d] (%, n)	
Low risk	64.7% (11/17)
Intermediate risk	11.8% (2/17)
High risk	23.5% (4/17)
RAI after surgery (%, n)	61.9% (13/21)

Data are presented as median, 25th and 75th percentiles. [a]Fine needle aspiration cytology according to the criteria of the Bethesda System for Reporting Thyroid Cytopathology; [b]No identifiable nodules on thyroid ultrasound or previous benign cytology; [c]American Joint Committee on Cancer TNM (AJCC/TNM) stating system, 7th edition; [d]Applied to children and adolescents with PTC.

PTC: papillary thyroid carcinoma, FTC: follicular thyroid carcinoma, OFTC: oncocytic follicular Hürthle cell carcinoma, ATA: American Thyroid Association, RAI: radioactive iodine therapy

An indeterminate or suspicious thyroid nodule on fine needle aspiration cytology was the most frequent reason that led to diagnosis (76.2%, n = 16). All patients received total thyroidectomy. Therapeutic central and lateral neck lymph node dissection were performed in 4 (19.0%) and 2 (9.5%) patients, respectively.

On histopathology, the most frequent subtype of DTC was PTC (81.0%, n = 17), mostly classic variant (61.9%, n = 13). No high-risk variants were observed. Five patients (23.8%) had multifocal PTC. Three patients (14.3%) were diagnosed with minimally invasive FTC, and one (4.8%) with OFTC. The median size of the largest dominant tumor was 16 [12-32] mm. Four patients (19.0%) had microcarcinoma. Lymphovascular invasion was present in 11 (52.4%) and extrathyroidal extension in 7 (33.3%) patients. Six patients (28.6%) had nodal involvement at the time of the diagnosis. One patient (4.8%) had N1a disease and 5 patients (23.8%) N1b disease. Among patients with reported lymphovascular invasion, 6 (54.5%) had nodal involvement. The others were classified as Nx since it was not possible to evaluate regional lymph nodes on histopathology. According to the ATA pediatric thyroid cancer risk levels, the majority of the patients with PTC (64.7%, 11) had low risk of having persistent cervical disease and/or distant metastases after initial total thyroidectomy/lymph node dissection. After surgery, 13 patients (61.9%) underwent RAI. Iodine whole-body scan revealed cervical lymph node metastasis in 7 patients (33.3%) and lung metastasis (M1) in one (4.8%). RAI tended to be used more frequently in ATA intermediate and high-risk groups ["low risk" 45.5% (5/11), "intermediate risk" 100% (2/2), "High risk" 100% (4/4), p = 0.05].

Clinical Outcomes of the Cohort

Two patients had been recently diagnosed with PTC at the time of the study and were excluded from the follow-up analysis. The mean follow-up time in the remainder

was 5.7 ± 3.1 years, ranging from 13 months to 10 years. Twelve months after surgery, the majority (78.9%, 15) had no evidence of disease, while 4 (21.1%) had persistent/recurrent disease. At the last follow-up visit, only 3 (15.8%) had persistent/recurrent disease. Overall, 6 DTC patients (31.6%) experienced persistent/recurrent disease during the follow-up time, all but one of them with PTC. Their characteristics are summarized in Table 3. Four of them had persistent locoregional disease after initial surgery and RAI; one patient underwent supplementary neck dissection and additional RAI and was disease-free by the end of seven years of follow-up; another exhibited no evidence of disease after a single additional RAI. The latter patient had minimally invasive FTC and it was not clear if the persistent tissue was malignant or not. Two patients received additional RAI and achieved stable disease by the end of the follow-up, at 13 months and 10 years. One patient had persistent locoregional and pulmonary disease after initial surgery and RAI. Supplementary neck dissection and additional RAI were performed. By the end of the follow-up (5 years), the patient had no evidence of locoregional disease and stable pulmonary disease. Despite an undetectable level of Tg, one patient presented with a suspicious lymph node in the neck area two years after initial treatment. Supplementary neck dissection confirmed recurrent locoregional disease with a PTC. At the time of the study (9 years follow-up), there was no evidence of disease. In conclusion, at the last follow-up visit, 16 patients (84.2%) had no evidence of disease, 2 patients (10.5%) had stable locoregional disease and 1 patient (5.3%) had stable pulmonary disease, despite adequate treatment according to evidence-based guidelines. None of the patients had a persistent elevated Tg level without structural disease. The clinical outcomes of the follow-up are presented in Table 4.

Table 3. Clinical characteristics of the patients that experienced persistent/recurrent disease during the follow-up

Sex	Age at diagnosis	Tumor and stage[a]	Lymphadenectomy[b]	Lymphovascular invasion	Initial treatment	Persistence/ recurrence	Diagnosis	Additional treatments	Last-follow-up visit
Female	15	PTC T2Nx	No	Yes	Surgery + RAI	Persistent disease	Neck RAI avid disease after initial RAI	Surgery + RAI	No evidence of disease (7 years)
Female	8	FTC T1bNx	No	Yes	Surgery + RAI	Persistent disease	Neck RAI avid disease after initial RAI	RAI	No evidence of disease (11 years)
Female	16	PTC T2N1a	No	Yes	Surgery + RAI	Persistent disease	Neck RAI avid disease after initial RAI	RAI	Stable locoregional disease (1 year)
Female	12	PTC T2Nx	No	Yes	Surgery + RAI	Persistent disease	Neck RAI avid disease after initial RAI	RAI	Stable locoregional disease (10 years)
Female	9	PTC T4aN1b	Yes	Yes	Surgery + RAI	Persistent disease	Neck and pulmonary RAI avid disease after initial RAI	Surgery + RAI	No evidence of locoregional disease and stable pulmonary disease (5 years)
Male	13	PTC T3N1b	Yes	Yes	Surgery + RAI	Recurrent disease	Cytology diagnosis of locoregional disease after suspicious neck ultrasound, despite undetectable level of Tg, 2 years after surgery	Surgery	No evidence of disease (9 years)

[a]American Joint Committee on Cancer TNM (AJCC/TNM) stating system, 7th edition; [b]Central and/or lateral lymphadenectomy in addition to total thyroidectomy.
PTC: papillary thyroid carcinoma, FTC: follicular thyroid carcinoma, RAI: radioactive iodine therapy, Tg: thyroglobulin

Risk Factors of Persistent/Recurrent Disease in PTC

Among PTC patients, there were no significant differences of persistent/recurrent disease between ATA risk groups ["Low risk" 22.2% (2/9), "Intermediate risk" 50.0% (1/2), "High risk" 50.0% (2/4), p = 0.44]. Comparison between PTC patients with and without lymphovascular invasion showed that persistent/recurrent disease was more frequent in patients with lymphovascular invasion [55.6% (5/9) vs. 0.0% (0/6), p = 0.031] (Figure 1). Similarly, the comparison between PTC patients with and without persistent/recurrent disease showed that lymphovascular invasion was more frequent in the persistence/recurrence group ["Persistent/recurrent disease" 100% (5/5), "No evidence of disease" 40.0% (4/10), p = 0.031]. Additionally, this group tended to be younger at diagnosis, although the difference was not significant ["Persistent/recurrent disease" 13 (12-15) years, "No evidence of disease" 16 (15-17) years, p = 0.075]. There were no differences regarding gender, multifocality, size of the tumor, extrathyroidal extension, lymphadenectomy, nodal involvement, RAI use after surgery or follow-up time (Table 5). Due to the sample size, a logistic regression model was not possible to perform.

Table 4. Follow-up clinical outcomes of the patients with DTC

	n = 19
Follow-up time (years)	**5.7 ± 3.1**
Response to therapy (%, n)	
12 months after surgery	
No evidence of disease	78.9% (15/19)
Persistent/recurrent disease	21.1% (4/19)
At the last follow-up visit	
No evidence of disease	84.2% (16/19)
Persistent/recurrent disease	15.8% (3/19)
Persistent/recurrent disease at any time of the follow-up (%, n)	31.6% (6/19)
Type	
Persistent disease	26.3% (5/19)
Recurrent disease	5.3% (1/19)
Location	
Locoregional disease	26.3% (5/19)
Locoregional and distant disease (pulmonary)	5.3% (1/19)

Data are presented as mean ± standard deviation.
DTC: differentiated thyroid cancer

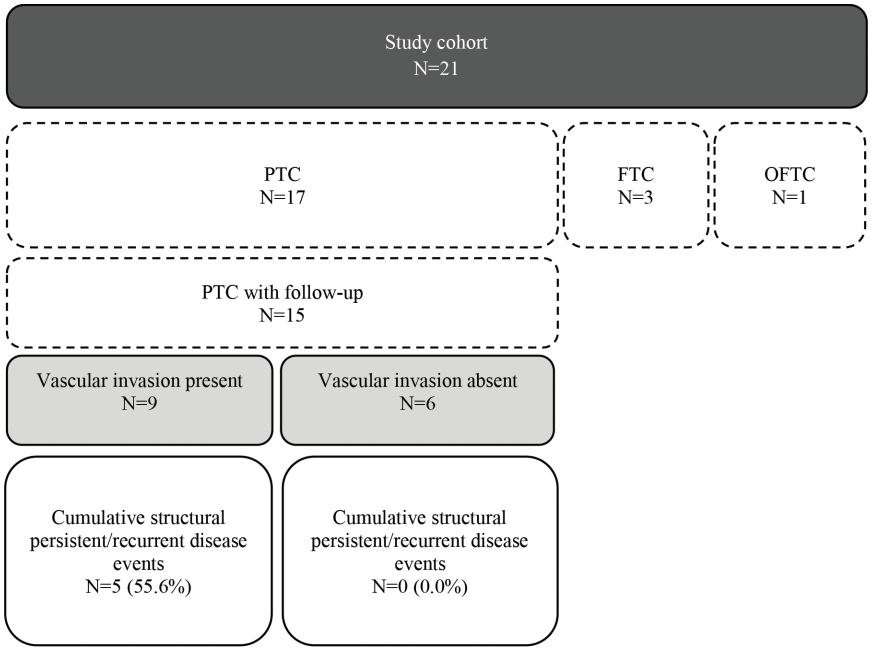

Figure 1. Comparison between patients with and without lymphovascular invasion

PTC: papillary thyroid carcinoma, FTC: follicular thyroid carcinoma, OFTC: oncocytic follicular Hürthle cell carcinoma

Table 5. Comparison of the clinical characteristics of PTC patients with and without persistent/recurrent disease during the follow-up time

	Persistent/recurrent disease	No evidence of disease	p
Age (years)	13 [12-15]	16 [15-17]	0.075
Female gender (%, n)	80.0% (4/5)	80.0% (8/10)	1.000
Multifocality (%, n)	40.0% (2/5)	10.0% (1/10)	0.242
Largest size of the dominant tumor (mm)	32 [26-35]	22 [8-33]	0.254
Lymphovascular invasion (%, n)	100.0% (5/5)	40.0% (4/10)	**0.031**
Extrathyroidal extension (%, n)	40.0% (2/5)	40.0% (4/10)	1.000
Lymphadenectomy (%, n)	40.0% (2/5)	20.0% (2/10)	0.560
Nodal involvement (%, n)	60.0% (3/5)	30.0% (3/10)	0.329
RAI after surgery (%, n)	100.0% (5/5)	60.0% (6/10)	0.231
Follow-up time (years)	6.4 ± 3.6	4.5 ± 2.7	0.270

Data are presented as mean ± standard deviation or as median, 25th and 75th percentiles.
RAI: radioactive iodine therapy, PTC: papillary thyroid carcinoma

Discussion

Since pediatric DTC is a rare disease, published data are scarce and from retrospective cohorts. To the best of our knowledge, there are no randomized controlled clinical trials for the treatment of children and adolescents with DTC. Nevertheless, retrospective studies of therapeutic options have led to reconsideration of the former concept that all children with DTC should be similarly treated (10). Although children and adolescents are more likely to have aggressive disease, pediatric thyroid cancer is associated with a very low risk of death (3,4,5,6,7,8,9,10). The challenge is to provide aggressive therapy when warranted and to limit overtreatment of those who are unlikely to benefit (3,4,5,6,7,8,9,10).

The latest ATA guidelines advocate for an individualized, risk-based approach combining histopathological findings and postoperative clinical data to identify patients who are likely to benefit from additional staging and therapy (10). Current ATA recommendations are founded on well-accepted approaches to therapy in adults, as well as personal experience in certain pediatric practices (10,19,20). However, the pediatric risk stratification considers only the extension of the primary tumor, the presence of nodal involvement and distant metastasis (TNM classification) (10). Other factors, such as lymphovascular invasion and minimal extrathyroidal extension are not considered, despite being well-known risk factors of recurrence in the adult population with PTC (11,12,13,14,15). Recently, European guidelines for the management of DTC in children and adolescents were published. However, they also do not take lymphovascular invasion into account for risk stratification (16).

Our retrospective study described the outcomes of a DTC cohort of children and adolescents diagnosed and treated in a pediatric reference center in Portugal, with a median follow-up of 6 years. In accordance with the literature, our cohort was essentially constituted by adolescents and female patients (3,4,5,6,7,8,9,10). As expected, PTC was the most frequent subtype (3,4,5,6,7,8,9,10). At the last follow-up visit, the majority had no evidence of disease and none had died from thyroid cancer, confirming the good prognosis of DTC in children and adolescents (3,4,5,6,7,8,9,10). Overall, six patients experienced persistent/recurrent disease during the follow-up. However, only three patients had active, but stable disease at the last follow-up visit.

All children and adolescents in our series underwent total thyroidectomy and the majority (62%) received RAI. However, central and lateral neck lymph node dissection were less commonly performed in our series (19%), compared to other studies, where lymphadenectomy was performed in more than 80% of the patients (21,22). Furthermore, only about a third of the patients in our series had nodal involvement at the time of the diagnosis, compared to 53-98% of the patients in other studies. (22,23,24,25,26,27). However, our cohort presented with a higher persistence/recurrence rate during follow-up in comparison with other studies (31.6% vs. 17-30%), suggesting that other factors must be considered for risk determination in addition to nodal involvement (10,22). Furthermore, according to the ATA pediatric risk groups, two of the PTC patients with persistent/recurrent disease in our series would have been classified as "Low risk", possibly delaying further staging and treatment. This highlights the importance of considering other factors for persistence/recurrence risk determination in this population.

In the present study, persistent/recurrent disease was more frequent in PTC patients with lymphovascular invasion (56% vs. 0%). This suggests that lymphovascular invasion may

be associated with a higher risk of persistence/recurrence and should therefore be considered for decision making in children and adolescents with PTC. Moreover, patients with persistent/recurrent disease tended to be younger, although the difference was not significant. Further research is needed to clarify whether younger age portends greater risk for extensive and recurrent disease, as suggested by other studies (4,8,28,29). In contrast to other studies, no significant differences were found regarding multifocality and the size of the tumor, probably due to the small size of our cohort (10).

In addition, our study contrasts with Redlich et al. (25), a German multicenter study, in which multivariate analysis revealed ATA high-risk level as a significant negative prognostic factor for event-free survival in pediatric patients with DTC. The small size of our cohort may explain this discrepancy. However, we have to consider the possibility that the risk of recurrence/persistence is underestimated in our cohort. The less aggressive strategy adopted in our sample, with lymph node dissection performed in a minority of cases, may have underestimated nodal involvement, and thus ATA risk prediction. Francis et al. (10) reported that "Low risk" patients may still be at risk for residual cervical disease, especially if the initial surgery did not include a central lymph node dissection, as seen in the "Low risk" patients that experience persistent disease in our cohort. The reduced rate of lymphadenectomy in our sample may explain the higher persistence/recurrence rate, compared to other studies. Therefore, other prognostic factors, such as lymphovascular invasion, can be particularly useful in the setting of a more conservative strategy, where nodal involvement may not be fully assessed.

Study Limitations

This study has some limitations. Unfortunately, due to the retrospective nature of the study, it was not possible to specify whether the lymphovascular invasion described in each case was venous, lymphatic or both. Furthermore, we did not have control over the preoperative and postoperative staging and management. Our series is based on a non-stratified approach in which all children underwent total thyroidectomy and variable extent of lymph node dissection and the majority received RAI. This contrasts with recent studies showing that more conservative strategies, including lobectomy and less RAI among children and adolescents, are safe and effective (10,22). Moreover, the small nature of our study limits the analysis, not allowing the performance of a logistic regression model to evaluate independent predictors of recurrent/persistent disease in PTC patients. Another limitation of the study is the relatively short follow-up time since pediatric patients may experience a recurrence 20-40 years after initial therapy (30,31).

Conclusion

In conclusion, our retrospective study describes the outcomes of a cohort of DTC patients diagnosed and treated at pediatric age in a reference center in Portugal. Children and adolescents with DTC were more likely to have more severe stages of disease. An individualized stratified risk-based approach is recommended to identify patients who are likely to benefit from additional staging and therapy. However, current recommendations consider only the extension of the primary tumor, the presence of nodal involvement and distant metastasis. Our study suggests that lymphovascular invasion may be associated with a higher risk of PTC persistence/recurrence in children and adolescents, especially in the setting of a more conservative strategy, where nodal involvement may not be fully assessed, and should therefore be considered for risk determination and decision making in this population. Further research is needed to confirm our results.

Ethics

Ethics Committee Approval: The protocol was approved by the Ethics Committee of Centro Hospitalar Universitário de Coimbra (OBS.SF.135-2022, 03.11.2022).

Informed Consent: Patient consent was waived by the Ethics Committee due to the retrospective nature of the study and full data anonymization.

Authorship Contributions

Surgical and Medical Practices: Joana Serra Caetano, Rita Cardoso, Isabel Dinis, Alice Mirante, Concept: Francisca Marques Puga, Laura Correia, Inês Vieira, Joana Serra Caetano, Rita Cardoso, Isabel Dinis, Alice Mirante, Design: Francisca Marques Puga, Laura Correia, Inês Vieira, Joana Serra Caetano, Rita Cardoso, Isabel Dinis, Alice Mirante, Data Collection or Processing: Francisca Marques Puga, Laura Correia, Inês Vieira, Analysis or Interpretation: Francisca Marques Puga, Literature Search: Francisca Marques Puga, Writing: Francisca Marques Puga.

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ORIGINAL ARTICLE

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Efficacy of Glucagon-like Peptide-1 Receptor Agonists in Overweight/Obese and/or T2DM Adolescents: A Meta-analysis Based on Randomized Controlled Trials

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What is already known on this topic?

In adolescents, previous meta-analyses of glucagon-like peptide-1 receptor agonists (GLP-1RAs) in patients with type 2 diabetes mellitus (T2DM) and obesity have demonstrated that GLP-1RAs were beneficial for glycemic control and weight loss. However, only nine randomized controlled trials were included. Meanwhile, limited sample size prevented further subgroup analyses.

What this study adds?

This study expanded the sample size included. Meanwhile, our study confirms that GLP-1RAs reduced glycosylated hemoglobin A1c, fasting plasma glucose, and weight loss in overweight/obese and/or T2DM adolescents. The GLP-1RAs have a no significant effect on lower blood sugar in adolescents with simple obesity. Based on subgroup analysis, liraglutide was more effective than exenatide in terms of glucose reduction. Nevertheless, in terms of weight control, exenatide was more effective than liraglutide.

Abstract

Objective: The aim of this meta-analysis was to investigate the effect of glucagon-like peptide-1 receptor agonists (GLP-1RAs) on blood glucose and weight in adolescents with overweight/obesity and/or type 2 diabetes mellitus (T2DM) aged < 18 years.

Methods: PubMed, Embase, Web of Science, and Cochrane Library were searched for all randomized controlled trials (RCTs) up to August 2023 comparing GLP-1RAs with placebo in overweight/obese and/or T2DM adolescents and extracted relevant data for meta-analysis.

Results: Fourteen RCTs were included in the meta-analysis with a total of 1,262 participants. Results revealed that the GLP-1RAs group had a more significant reduction in glycosylated hemoglobin A1c (HbA1c; risk difference (RD) = -0.34%, $p < 0.001$) than the control group. However, there was no difference in fasting plasma glucose [fasting plasma glucose (FPG); RD = -2.07 mg/dL, $p = 0.065$] between the two groups. Nonetheless, the experimental group that received exenatide showed no significant reduction in HbA1c ($p = 0.253$) and FPG ($p = 0.611$) between the two groups. The GLP-1RAs group had a more significant decline in body weight (RD = -4.28 kg, $p = 0.002$) and body mass index (BMI) (RD = -1.63 kg/m^2, $p = 0.002$) compared to the control group. The experimental group was given liraglutide (RD = -2.31 kg, $p = 0.038$) or exenatide (RD = -2.70 kg, $p < 0.001$). Compared to the control group, the experimental group had a more significant drop in body weight than the control group. However, for the experimental group that received liraglutide, the BMI had a no significant reduction between the two groups (RD = -0.81 kg/m^2, $p = 0.260$). For the experimental group using exenatide, BMI declined more significantly in the intervention group than in the control group (RD = -1.14 kg/m^2, $p < 0.001$).

Conclusion: This study showed that GLP-1RAs reduced HbA1c, FPG, and weight loss in overweight/obese and/or T2DM adolescents. Liraglutide was better than exenatide in terms of glucose reduction. Nevertheless, in terms of weight control, exenatide was more effective than liraglutide.

Keywords: Glucagon-like peptide-1 receptor agonists, overweight, obesity, type 2 diabetes, HbA1c, weight loss, FPG

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Introduction

Obesity is a global public health problem. More than two billion people worldwide suffer from obesity, and the number continues to increase (1). The global obese adolescent population was estimated to exceed 100 million (2). Adolescent obesity tends to persist and become adult obesity, which has been related to many chronic diseases, including type 2 diabetes mellitus (T2DM), cardiovascular disease, and cancer (3). Unfortunately, most treatments for childhood obesity are based only on prevention and lifestyle interventions. Until 2020, the European Medicines Agency (EMA) had not approved any pharmacological treatments for treating obesity in pediatric patients. In January 2021, the EMA authorized the use of a glucagon-like peptide (GLP)-1 analog, liraglutide, for treating adolescent (12-17 years) obesity (4). Morbidly obese adolescents could consider bariatric surgery, but both surgical complications and safety limited the promotion of surgery in adolescents (5).

The prevalence of T2DM was low in adolescents, but as rates of obesity have increased, T2DM has become increasingly prevalent in adolescents (2). T2DM in adolescence is manifested as severe progressive DM with frequent complications, such as diabetic retinopathy, cardiovascular disease, and nephropathy (6,7). Common clinical drugs used to treat T2DM include metformin, insulin and sodium-glucose cotransporter-2 (SGLT-2) inhibitors. Although insulin is used to treat diabetes, insulin resistance is often present in obese adolescents and thus its efficacy is limited (8).

Liraglutide is a GLP-1 receptor agonist (GLP-1RAs) recently approved for T2DM treatment in adolescents aged ten years and older (9). GLP-1RAs stimulate postprandial insulin secretion, reduced glucagon secretion, delayed gastric emptying, and reduced appetite, thereby improving blood glucose control (10). In adolescents, previous meta-analyses of GLP-1RAs in patients with T2DM and obesity have demonstrated that GLP-1RAs were beneficial for glycemic control and weight loss (11,12). However, only nine randomized controlled trials (RCTs) were included because of the limited number of RCTs. Therefore, conducting further subgroup analyses to explore the effect of therapeutic regimen, treatment duration, and subject participants on the efficacy of GLP-1RAs was unfeasible. Recently, as more and pertinent RCTs have been reported, an update of the earlier meta-analysis is possible. The aim of the present meta-analysis was to investigate the effectiveness of GLP-1RAs in managing overweight/obese and/or T2DM in adolescents under 18, along with exploring the factors influencing efficacy.

Methods

Search Strategy

This meta-analysis design and reporting followed the PRISMA 2020 updated guidelines (13) and was registered in PROSPERO 2023 (CRD42023467678). The aim of the present study was to investigate the effects of GLP-1RAs on blood glucose and weight in adolescents with overweight/obese and/or T2DM.

Two researchers independently searched four databases up to August 2023, including PubMed, Web of Science, Embase, and Cochrane Library. The search terms were: glucagon-like peptide-1 receptor agonist OR exenatide OR liraglutide OR dulaglutide OR lixisenatide OR semaglutide OR albiglutide OR taspoglutide OR loxenatide) AND (Children OR Adolescents OR Teens OR Teenagers OR Youths OR Adolescents, Female OR Adolescents, male. Moreover, reference lists in all retrieved articles were searched. The primary outcomes of the included articles involved glycosylated hemoglobin A1c (HbA1c), fasting plasma glucose (FPG), and body weight. Articles were filtered according to PICOS principles including when no consensus could be reached, a third person would be recruited for their opinion.

Inclusion and Exclusion Criteria

The included studies were based on the following PICOS principles: 1) overweight/obese and/or T2DM in adolescents aged < 18 years; 2) the intervention group received GLP-1RAs; 3) the control group received placebo; 4) the primary outcomes were HbA1c, FPG, and body weight; and 5) included studies were RCTs.

The exclusion criteria were: 1) full text not available; 2) participants included adults; 3) non-English articles; 4) unextracted data; and 5) updated RCTs. When updating published articles for the same study cohort, the most recent or largest population studies were selected.

Data Extraction and Quality Assessment

Two researchers extracted the data separately using pre-designed forms. Extracted data included: 1) the authors, publication year, country, and registration number of the study; 2) subject participants details, such as comorbidity, mean body mass index (BMI), age; 3) recruitment time, therapeutic regimen, treatment duration, sample sizes for experimental and control groups; 4) outcomes, including HbA1c, FPG, and body weight.

Following Cochrane guidelines, RCTs were assessed by two review authors. The labels "high risk," "low risk," and "unclear risk" were used to describe several bias types, including

random serial generation, allocation concealment, blinding of participants and staff, blinding of outcome assessment, insufficient outcome data, and selective reporting, among others. In the case of a disagreement, the two researchers solved the problem through discussion. When necessary, a third person was enlisted.

Statistical Analysis

This meta-analysis was explored using Stata Software 12.0 (Stata Corporation, College Station, TX, United States) and Review Manager 5.3 (RevMan version 5.3; Oxford, UK). The definition of risk difference (RD) is actually the mean difference. RD and 95% confidence intervals were used to assess the association of GLP-1RAs with HbA1c, FPG, and body weight. Heterogeneity between studies was assessed by the chi-square test with an inconsistency index (I^2): I^2 < 25% indicated low heterogeneity; I^2 = 25-50% indicated moderate heterogeneity; I^2 > 50% indicated significant heterogeneity (14). Due to potential heterogeneity in the participant population and experimental design, this study was analyzed using a unified random-effects model to increase our result credibility. All tests were two-sided and $p < 0.05$ was considered significant (15).

Results

Description of the Studies

In accordance with the search criteria, 3,120 records from four databases were thoroughly examined, and no more studies could be located in other sources. After duplicate articles were removed, 2,235 articles remained, while a further 2,111 irrelevant articles were removed by investigating article titles and abstracts. Through reading the full published texts, 110 more studies were eliminated, of which 45 were not RCTs, 42 included adults, 10 had no reported outcomes of interest, 5 were not in English, 5 were updated articles, and 3 had inaccessible data. Eventually, fourteen RCTs were included in the meta-analysis (Figure 1) (16-29).

The 14 RCTs were selected to research GLP-1RAs in adolescents who were overweight/obese and/or had T2DM. In these studies, most participants were aged 12-18 years, with an average BMI greater than 30 kg/m^2. All studies were in Western countries or predominantly Western multicenter studies with a treatment duration of 5-68 weeks. Six studies used liraglutide, five used exenatide, two used semaglutide, and one used dulaglutide. All participants included obesity, T2DM, and overweight combined with T2DM. In total, 754 adolescents were allocated to GLP-1RAs therapy, and 508 were treated with the placebo. Patients with T2DM had previously received metformin, insulin, or exercise therapy. Most trials combined lifestyle, diet, and exercise interventions. Table 1 and Supplementary Table 1 contain a list of the characteristics of the analyzed studies in this meta-analysis.

Quality Evaluation

Figures S1 and S2 depict the included studies assessments. We used the Cochrane Collaboration method to assess each RCTs quality. All included studies were assessed as low risk regarding random sequence generation and allocation concealment. Most studies were rated as low risk in blinding of participants and personnel and selective reporting, whereas a small number were rated as unclear. Most studies were classified as low risk, while only a small number were evaluated as high risk, and a few were at unknown risk concerning blinding of outcome assessment and incomplete outcome data. For other biases, the included studies were assessed as being of unclear risk.

Result Analysis

Figure 2 summarizes the effects of GLP-1RAs on HbA1c and FPG in the whole population. Nine studies reported HbA1c results, revealing that participants in the GLP-1RAs group had a more significant reduction in HbA1c compared to the control group [RD = -0.34%, $p < 0.001$, 95% confidence interval (CI) = -0.51, -0.18; Figure 2a]. However, the heterogeneity was 91.2%. Ten studies reported FPG findings, indicating that FPG had a greater decrease in the

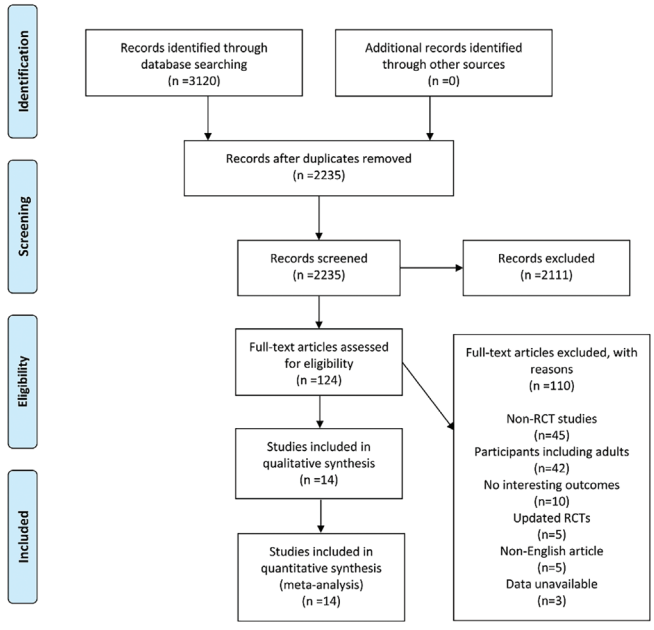

Figure 1. Flow diagram of selection

RCTs: randomized controlled trials

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

Author	Year	Participants	Mean age (years)	Experimental group (number)	Control group (number)	Intervention	Target dose	Treatment duration (weeks)
Weghuber et al. (17)	2022	Obesity	15.4	134	67	Semaglutide	2.4 mg weekly	68
Arslanian et al. (29)	2022	Overweight, T2DM	14.5	103	51	Dulaglutide	0.75 mg weekly, 1.50 mg weekly	26
Tamborlane et al. (18)	2022	T2DM	15	58	24	Exenatide	2.00 mg weekly	24
Diene et al. (27)	2022	Obesity	14.3	19	12	Liraglutide	3.00 mg daily	16, 52
Fox et al. (22)	2022	Obesity	16	33	33	Exenatide	2.00 mg weekly	52
Kelly et al. (25)	2023	Obesity	15.4	133	67	Semaglutide	2.40 mg weekly	75
Mastrandrea et al. (20)	2019	Obesity	9.9	16	8	Liraglutide	3.00 mg weekly	8
Kelly et al. (23)	2012	Obesity	12.7	5	6	Exenatide	0.02 mg daily	13
Klein et al. (21)	2014	Overweight, T2DM	14.8	14	7	Liraglutide	1.80 mg daily	5
Tamborlane et al. (19)	2019	Overweight, T2DM	14.6	66	68	Liraglutide	1.80 mg daily	26, 52
Danne et al. (28)	2017	Obesity	14.9	14	7	Liraglutide	3.00 mg daily	5
Kelly et al. (24)	2020	Obesity	14.5	125	126	Liraglutide	3.00 mg daily	56
Weghuber et al. (16)	2020	Obesity	14	22	22	Exenatide	2.00 mg weekly	24
Kelly et al. (22)	2013	Obesity	15.2	12	10	Exenatide	0.02 mg daily	13

T2DM: type 2 diabetes mellitus

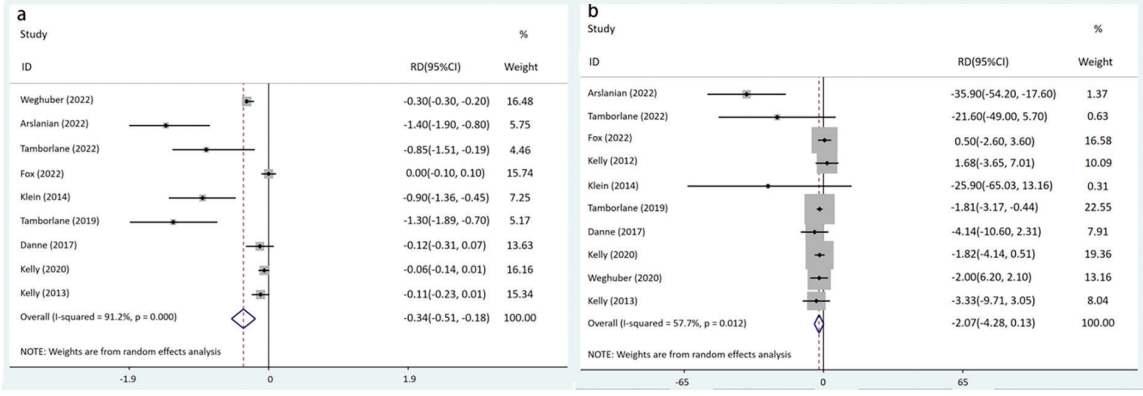

Figure 2. Forest plot of meta-analysis of the effect of GLP-1RAs on HbA1c and FPG in all participants. a) HbA1c, $p < 0.001$; b) FPG, $p = 0.065$

GLP-1RAs: glucagon-like peptide-1 receptor agonists, HbA1c: glycosylated hemoglobin A1c, FPG: fasting plasma glucose

intervention group than in the control group (RD = -2.07 mg/dL, 95% CI = -4.28, 0.13), but the difference was not significant ($p = 0.065$; Figure 2b). The heterogeneity was 57.7%.

For HbA1c, subgroup analysis was performed by participant type, showing that HbA1c exhibited no significant reduction between the two groups for obese participants (non-T2DM) ($p = 0.087$; Figure 3a). Notably, for T2DM patients, HbA1c showed a more significant decrease in the intervention group than in the control group (RD = -1.10%, $p < 0.001$, 95% CI = -1.38, -0.83; Figure 3b). Further subgroup analysis was conducted in terms of HbA1c in the whole population (Table 2-1). For the study of the participant number in the

Table 2-1. Subgroup analysis of HbA1c and fasting plasma glucose

	No. of studies	RD	95% CI	p	Heterogeneity
					I^2
HbA1c (%)					
Experimental group (number) <50	4	-0.17	-0.35, 0.02	0.079	80.4%
Experimental group (number) ≥50	5	-0.55	-0.82, -0.29	<0.001	93.4%
Liraglutide	4	-0.47	-0.84, -0.11	0.011	89.5%
Exenatide	3	-0.11	-0.30, 0.08	0.253	73.5%
Treatment duration <52 weeks	6	-0.66	-1.03, -0.29	<0.001	88.0%
Treatment duration ≥52 weeks	4	-0.23	-0.44, -0.02	0.034	94.8%
Fasting plasma glucose (mg/dL)					
Experimental group (number) <50	6	-0.83	-2.86, 1.20	0.421	0.9%
Experimental group (number) ≥50	4	-4.29	-8.93, 0.35	0.070	80.3%
Liraglutide	4	-1.91	-3.07, -0.75	0.001	0.0%
Exenatide	5	-0.62	-3.00, 1.76	0.611	12.8%
Treatment duration <52 weeks	8	-3.51	-7.10, 0.09	0.056	63.2%
Treatment duration ≥52 weeks	3	-1.52	-2.62, -0.42	0.007	0.0%

HbA1c: glycosylated hemoglobin A1c, RD: risk difference, CI: confidence interval

experimental group <50, HbA1c revealed a no significant decrease between the two groups (p = 0.079). For the study of the participant number in the experimental group ≥50, the GLP-1RAs group had a more significant reduction than the control group (RD = -0.55%, p < 0.001). For the experimental group that used liraglutide, HbA1c underwent a more significant decline in the intervention group than the control group (RD = -0.47%, p = 0.011). However, for the experimental group that used exenatide, HbA1c showed a no significant reduction between the two groups (p = 0.253). For treatment duration, both <52 (RD = -0.66%, p < 0.001) and ≥52 weeks (RD = -0.23%, p = 0.034), the experimental group had a more significant decrease in HbA1c than the control group.

The FPG was analyzed in subgroups, indicating that for adolescents with obesity, no significant differences were found in FPG reduction between the two groups (p = 0.119) (Figure 4a). For T2DM adolescents, FPG level exhibited a greater decrease in the intervention group than in the control group (RD = -19.48 mg/dL, 95% CI = -41.20, 2.24), but the difference did not reach statistical significance (p = 0.079; Figure 4b). Further subgroup analysis was performed in terms of FPG in the whole population (Table 2-1). For the study of participant number in the experimental group, both <50 (p = 0.421) and ≥50 (p = 0.070), FPG levels had a no significant reduction between the two groups. For the experimental group that used liraglutide, FPG exhibited a more significant decline in the intervention group than the control group (RD = -1.91 mg/dL, p = 0.001). For the experimental group that used exenatide, there was no statistically significant reduction in FPG between the two

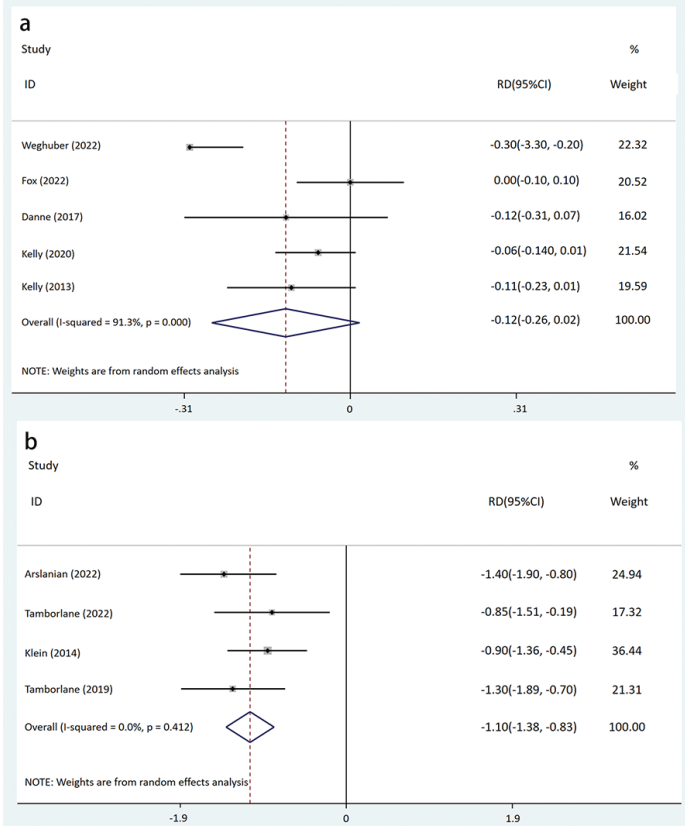

Figure 3. Forest plot of meta-analysis of the effect of GLP-1RAs on HbA1c. a) obesity, p = 0.087; b) T2DM, p < 0.001

GLP-1RAs: glucagon-like peptide-1 receptor agonists, HbA1c: glycosylated hemoglobin A1c, T2DM: type 2 diabetes mellitus

groups (p = 0.611). For treatment duration, both < 52 (RD = 3.51 mg/dL, p = 0.056) and ≥52 weeks (RD = -1.52 mg/dL, p = 0.007), FPG had a significantly greater decrease in the intervention group than in the control group, but the former difference was not statistically significant.

Figure 5 summarizes the effects of GLP-1RAs on body weight and BMI in the whole population. Nine studies reported results for body weight. Participants in the GLP-1RAs group had a more significant decline in body weight compared to the control group (RD = -4.28 kg, p = 0.002, 95% CI = -6.95, -1.60; Figure 5a). Eight studies reported BMI, and BMI decreased significantly more in the intervention group treated with GLP-1RAs compared with controls (RD = -1.63 kg/m^2, p = 0.002, 95% CI = -2.68, -0.57; Figure 5b).

Table 2-2 lists further subgroup analyses of body weight and BMI. For the study of the participant number in the experimental group < 50 (RD = -2.64 kg, p < 0.001) and ≥50 (RD = -7.64 kg, p = 0.070), body weight decreased more in the intervention group than in the control group, but the latter difference was not significant. The experimental group that used liraglutide had a mean weight reduction of -2.31kg (p = 0.038) while the exenatide group exhibited

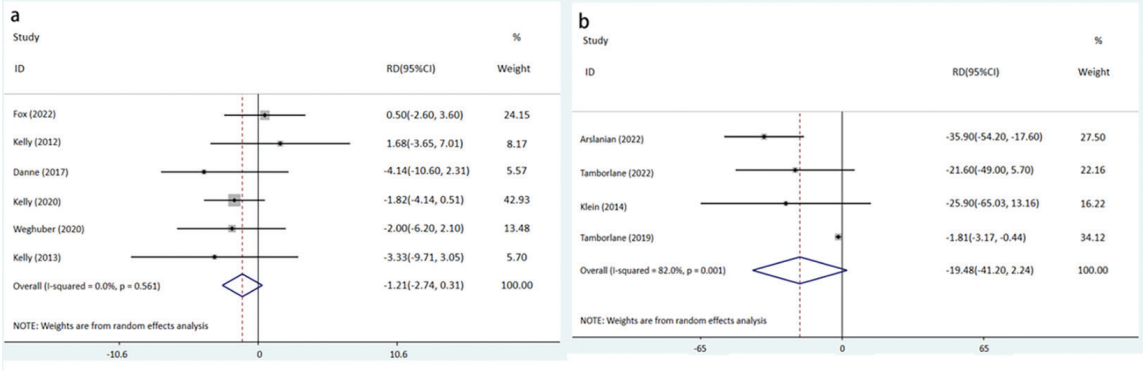

Figure 4. Forest plot of meta-analysis of the effect of GLP-1RAs on FPG. a) obesity, p = 0.119; b) T2DM, p = 0.079

GLP-1RAs: glucagon-like peptide-1 receptor agonists, FPG: fasting plasma glucose, T2DM: type 2 diabetes mellitus

Table 2-2. Subgroup analyses of body weight and BMI

	No. of studies	RD	95% CI	p	Heterogeneity
					I^2
Body weight (kg)					
Experimental group (number) < 50	6	-2.44	-3.64, -1.24	< 0.001	0.0%
Experimental group (number) ≥50	3	-7.64	-15.90, 0.61	0.070	95.8%
Liraglutide	3	-2.31	-4.50, -0.13	0.038	49.9%
Exenatide	5	-2.70	-4.05, -1.36	< 0.001	0.0%
Treatment duration < 52 weeks	6	-2.09	-3.18, -0.99	< 0.001	0.0%
Treatment duration ≥52weeks	3	-8.86	-17.52, -0.20	0.045	93.5%
BMI (kg/m^2)					
Experimental group (number) < 50	5	-0.88	-1.59, -0.17	0.015	60.0%
Experimental group (number) ≥50	3	-2.50	-5.38, 0.38	0.089	96.9%
Liraglutide	2	-0.81	-2.22, 0.60	0.260	87.2%
Exenatide	4	-1.14	-1.69, -0.59	< 0.001	0.0%
Treatment duration < 52 weeks	5	-0.56	-1.08, -0.04	0.034	70.7%
Treatment duration ≥52 weeks	4	-2.79	-5.44, -0.14	0.039	95.6%
BMI (%)					
Experimental group (number) < 50	4	-2.47	-4.96, 0.01	0.051	77.6%
Experimental group (number) ≥50	3	-13.24	-22.62, -3.87	0.006	96.2%
Exenatide	4	-2.47	-4.96, 0.01	0.051	77.6%
Treatment duration < 52 weeks	3	-2.15	-4.85, 0.55	0.119	81.2%
Treatment duration ≥52 weeks	4	-11.02	-18.71, -3.34	0.005	95.0%

RD: risk difference, BMI: body mass index, CI: confidence interval

a -2.70 kg weight reduction ($p < 0.001$). The experimental group had a more significant drop in body weight than the control group. For treatment duration < 52 (RD = -2.09 kg, $p < 0.001$) and ≥52 weeks (RD = -8.86 kg, $p = 0.045$), the experimental group had a more significant decrease in body weight than the control group.

For the analysis of the participant number in the experimental group < 50, the BMI decline in the experimental group was more significant than in the control group (RD = -0.88 kg/m^2, $p = 0.015$). When the participant number in the experimental group ≥50, there was no significant reduction in BMI between the two groups ($p = 0.089$). For the experimental group that used liraglutide, BMI showed a no significant reduction between the two groups ($p = 0.260$). However, for the experimental group that used exenatide, BMI showed a significant decline in the intervention group than in the control group (RD = -1.14 kg/m^2, $p < 0.001$). For treatment durations, both < 52 (RD = -0.56 kg/m^2, $p = 0.034$) and ≥52 weeks (RD = -2.79 kg/m^2, $p = 0.039$), BMI fell significantly in the intervention group compared to the control group. Furthermore, this study was further analyzed from a BMI perspective (%) (Table 2-2).

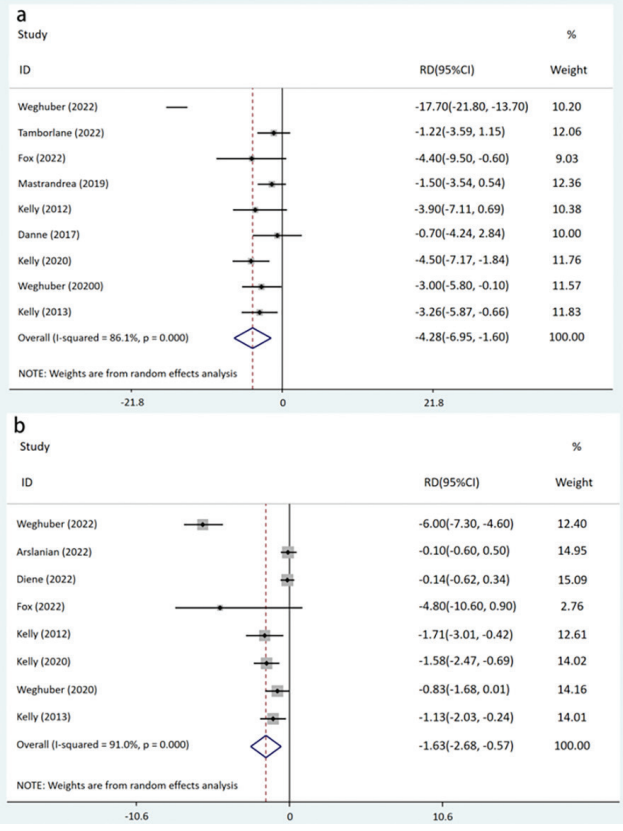

Figure 5. Forest plot of meta-analysis of the effect of GLP-1RAs on body weight and BMI in all participants. a) body weight, $p = 0.002$; b) BMI, $p = 0.002$

GLP-1RAs: glucagon-like peptide-1 receptor agonists, BMI: body mass index

Subgroup analysis was performed for participants with obesity regarding body weight (kg) and BMI (kg/m^2 and %). Body weight showed a more significant decrease in the intervention group than in the control group (RD = -4.72 kg, $p = 0.002$; Figure 6a). Furthermore, BMI also showed a significant drop in magnitude in the experimental group than in the control group (RD = -1.93 kg/m^2, $p = 0.003$; RD = -7.31 %, $p = 0.004$; Figures 6b, 6c).

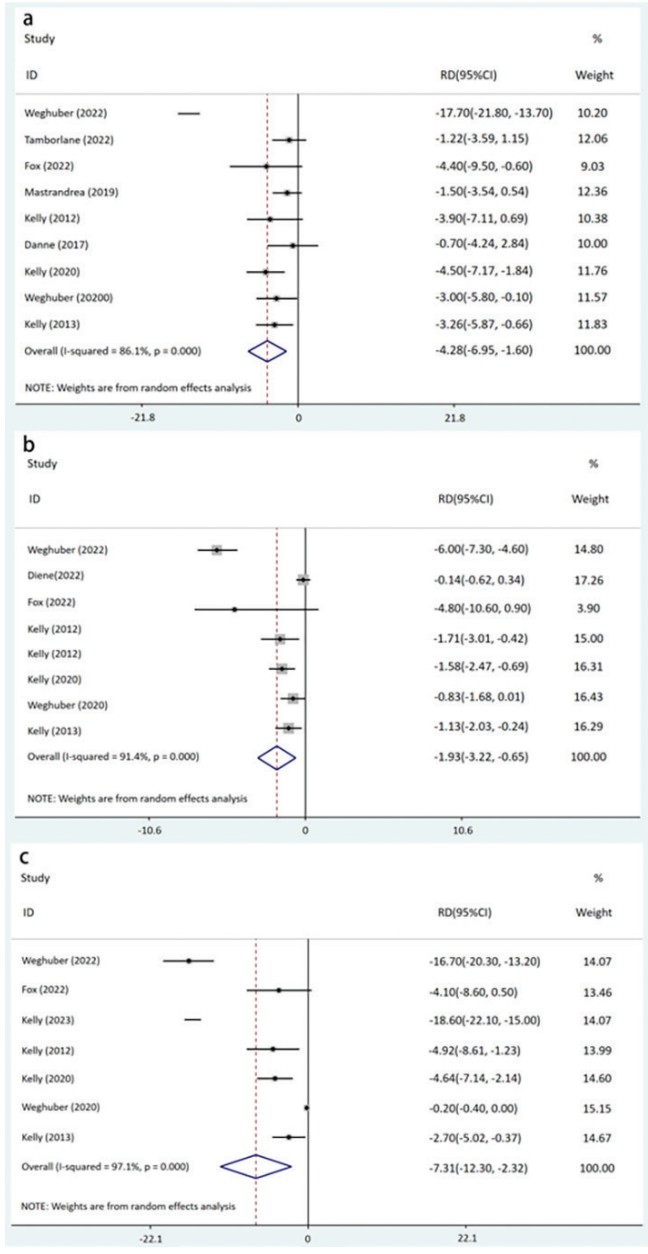

Figure 6. Forest plot of meta-analysis of the effect of GLP-1RAs on weight control in obesity. a) body weight, $p = 0.002$; b) BMI (kg/m2), $p = 0.003$; c) BMI (%), $p = 0.004$

GLP-1RAs: glucagon-like peptide-1 receptor agonists, BMI: body mass index

Discussion

This study indicated that GLP-1RAs, compared to placebo, decrease HbA1c, FPG, and body weight in adolescents with overweight/obesity and/or T2DM. Remarkably, GLP-1RAs had no significant effect on HbA1c and FPG in adolescents with non-T2DM obesity. In T2DM, liraglutide was more effective in adolescents than exenatide in lowering HbA1c and FPG. In contrast, exenatide was more effective than liraglutide for weight control. With the treatment prolongation, the efficacy of GLP-1RAs on glucose control decreased, but weight control was more effective. Moreover, Weghuber et al. (17) demonstrated that in obese adolescents, semaglutide plus lifestyle intervention treatment resulted in a more significant reduction in BMI than lifestyle intervention alone. Tamborlane et al. (19) showed that liraglutide effectively improved blood sugar in T2DM adolescents.

The GLP-1RAs mainly reduce glucose through the following mechanisms. GLP-1RAs can stimulate insulin secretion to lower blood sugar (30). GLP-1RAs also increase intracellular Ca^{2+} concentration through ligand-gated calcium channels or voltage-dependent Ca^{2+} channels on the endoplasmic reticulum, enhancing insulin secretion (31,32). Notably, GLP-1RAs only increase insulin release in cases of hyperglycemia and so are not associated with hypoglycemia (33), which is confirmed again in the present study. In obesity (non-T2DM), GLP-1RAs did not significantly decrease blood glucose. Studies have suggested that GLP-1RAs induce an increase in β-cell mass through enhanced cellular regeneration and apoptosis inhibition (34,35).

GLP-1RAs can inhibit glucagon secretion in a glucose concentration-dependent manner, lowering blood sugar. Some studies have reported the possibility that GLP-1R directly mediates α-cell inhibition to suppress glucagon secretion (36). The GLP-1R can also indirectly inhibit glucagon by directly stimulating increased somatostatin secretion (37,38).

GLP-1RAs promote glycogen synthesis in liver cells, lowering blood glucose concentrations (39). GLP-1RAs balance food intake by activating multiple nuclei of the hindbrain and hypothalamus (periventricular nuclei, posterior brain area, and nucleus tractus solitarius). Moreover, GLP-1RAs activated brain regions of the mid-limbic system to inhibit reward behavior and palatability. The combined effect of GLP-1RAs on homeostasis and hedonic eating may contribute to their appetite suppression (40). Finally, GLP-1RAs could also delay gastric emptying and peristalsis of the gastrointestinal tract and reduce gastric acid secretion stimulated by pentapeptide gastrin (41).

Our study indicated that liraglutide was more effective in adolescents than exenatide regarding blood sugar control. In the LEAD-6 study, liraglutide lowered HbA1c more than exenatide (42). The probable cause was that exenatide has a short half-life and a higher plasma concentration within 4-8 hours after a single subcutaneous injection (43). However, approximately 99% of the liraglutide molecules are typically bound to plasma albumin, and the bound molecule has a half-life of 11-13 hours (41). Therefore, liraglutide concentration in plasma is more persistently high, and the hypoglycemic effect is better. Our research demonstrated that the degree of glucose reduction declined with prolonged treatment duration. The probable cause was that blood sugar does not drop continuously. Only in cases of hyperglycemia do GLP-1RAs raise insulin release to reduce blood sugar. When blood sugar drops to the normal range, the ability of GLP-1Ra to lower blood sugar only plays a role in maintaining blood sugar concentration (44).

Our research suggested that GLP-1RAs can lower weight in adolescents compared to a placebo. The weight loss mechanism is probably as follows. 1) As mentioned earlier, GLP-1RAs promote weight loss by reducing food intake and prolonging gastric emptying (40,41). 2) GLP-1RAs activate brown fat and increase rodent energy expenditure independently of locomotor activity through sympathetic nervous system (SNS) pathways. 3) GLP-1RAs also reduce peripheral lipid storage in white adipocytes in mice by a mechanism that relies on SNS activation (45). 4) In mice and monkeys, GLP-1RAs target pathways that reduce body weight and improve many metabolic parameters by producing GLP-1 bispecific molecules (46). 5) Studies have demonstrated that obese teenagers can lose weight through these mechanisms, as well as increased fat and reduced carbohydrate oxidation (47). Our study indicated that exenatide was more effective than liraglutide for weight loss. One reason may be that exenatide and lowering glucose have been shown to improve lipid homeostasis, reduce body weight, improve insulin resistance, and reduce hepatic steatosis (48,49). Another factor may be that exenatide treats obesity by regulating *CTRP3* and *PPAR*-γ gene expression, which are related to lipogenesis (50). Nevertheless, the meta-analysis conducted by Ryan et al. (12) has indicated that no significant difference existed between the effectiveness of liraglutide or exenatide for adolescent weight loss. This may be due to their inclusion of a limited number of RCTs. Our research revealed that GLP-1RAs were more effective in reducing body weight with prolonged treatment. This may be because GLP-1 produces anorexic effects on the mediation of the brainstem and hypothalamic nucleus (43). The severity of anorexia increases with therapy duration, resulting in greater weight loss.

This meta-analysis is an updated study of published RCTs on the effectiveness of GLP-1RAs in treating overweight/obesity and/or T2DM in adolescents. Our study once again confirms the effectiveness of GLP agonists in lowering glucose and weight in adolescents. In addition, we explored the different effects of exenatide and liraglutide on hypoglycemic and weight reduction in adolescents. Additionally, we found that prolonged treatment may affect the efficacy for controlling glucose and weight.

Study Limitations

Our study also has some limitations. First, our study included multiple GLP-1RAs, but subgroup analyses of all drugs were impossible because of limited data. Second, a few subgroup analyses of the included studies affected credibility to some extent. Third, because there were some differences in the included studies, the heterogeneity of the final analysis was higher, which reduced credibility. Therefore, a random-effects model was used for analysis. Fourth, the included studies were all multicenter studies in Western countries; consequently, the results could not be directly generalized to other countries.

Conclusion

This study confirmed that GLP-1RAs reduced HbA1c, FPG, and weight loss in adolescents with overweight/obesity and/or T2DM. However, GLP-1RAs had no significant effect on blood glucose reduction in obese adolescents. For adolescents with T2DM, liraglutide was superior to exenatide in lowering glucose. However, when it comes to weight control, exenatide was more effective than liraglutide. When the duration of treatment is prolonged, the magnitude of the drop in blood glucose tends to stabilize while weight loss continues.

Ethics

Ethics Committee Approval and Informed Consent: Not applicable (this paper was provided based on research in global databases).

Authorship Contributions

Concept: Cong Zhou, Design: Min Dai, Zhiyi Xiang, Siyu Lu, Cong Zhou, Data Collection or Processing: Senjie Dai, Lihu Gu, Zhiyi Xiang, Anyi Xu, Analysis or Interpretation: Min Dai, Lihu Gu, Siyu Lu, Yang Yang, Cong Zhou, Literature Search: Min Dai, Senjie Dai, Lihu Gu, Zhiyi Xiang, Anyi Xu, Siyu Lu, Yang Yang, Writing: Min Dai, Senjie Dai, Cong Zhou.

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Click the link to access Supplementary Table 1 and Supplementary Figures 1, 2:

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BRIEF REPORT

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Vitamin D Status in an Italian Pediatric Cohort: Is There a Role for Tobacco Smoking Exposure?

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What is already known on this topic?

Inadequate vitamin D status has been previously reported in children from Italy's northern and southern regions, suggesting that it is relatively independent of the latitude.

What this study adds?

Smoke exposure was found to be a significant risk factor for hypovitaminosis D. This finding highlights the importance of ensuring healthy and smoke-free environments for children.

Abstract

Objective: Vitamin D deficiency is a common public health issue worldwide. The purpose of this study was to investigate the vitamin D status and its potential determinants in children residing in Sardinia (40°N), Italy.

Methods: Children were enrolled over a 12-month period. Serum 25(OH)D was measured by an immunochemiluminescence assay. A questionnaire was used to gather information on other variables, including passive smoke exposure.

Results: A total of 182 children (males: 51.7%; median age: 9 years) were included. Mean ± standard deviation serum 25(OH)D was 25.2 ± 8.3 ng/mL for the whole group. The majority (n = 123, 67.6%) had vitamin D sufficient values > 20 ng/mL, while 32.4% (n = 59) had vitamin D insufficient/deficient values (≤20 ng/mL). Among the variables investigated, passive smoke exposure was significantly associated with insufficient 25(OH)D levels ($p < 0.0001$).

Conclusion: Our results confirm that hypovitaminosis D is common in Italian children. Furthermore, passive smoke exposure was identified as a significant risk factor for hypovitaminosis D.

Keywords: Vitamin D deficiency, hypovitaminosis D, passive smoke exposure, lifestyle habits

Introduction

Vitamin D deficiency is a common public health issue worldwide, affecting people of any age. Its etiology results from the variable and complex interactions between environmental, genetic, and epigenetic factors (1). Although the exact cut-off level for defining childhood hypovitaminosis D is still debated, vitamin D insufficiency is defined as serum 25(OH)D levels between 12-20 ng/mL and deficiency as < 12 ng/mL; both are associated with increased risk for rickets (1,2). Hypovitaminosis D has been reported to affect a majority of children in both northern and southern regions of Italy (44°- 40°N), suggesting that vitamin D status is relatively independent of region latitude (3,4).

Recent studies have shown a significant association between tobacco smoke exposure and vitamin D levels in children

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(5,6,7,8). The purpose of this study was to investigate the vitamin D status and its potential determinants in children residing in Sardinia, Italy.

Methods

Experimental Subjects

A retrospective observational study was conducted among children aged 1-16 years who lived in Northern Sardinia (40°N), Italy, enrolled from June 2018 to May 2019. This data was obtained during clinic visits to the University Hospital of Sassari. An intake of vitamin D supplements after the first year of life was the exclusion criterion. Our study was conducted in accordance with the ethical standards of the regional committee on human experimentation. Informed consent was obtained for each participant.

Collected data included blood levels of vitamin D, demographic data, body height and weight, and body mass index (BMI) Z-score. Each participant's parents filled out a specifically designed questionnaire that investigated family-related factors, residence (rural/urban), sunlight exposure, regular use of total protection sunscreens, Fitzpatrick skin types, fortified milk intake, and passive smoke exposure. The questionnaire also inquired about the presence of chronic diseases and prolonged pharmacological treatments.

Vitamin D Assay

The serum 25(OH)D levels were measured using the immunochemiluminescence Liaison® 25 OH vitamin D Total Assay (CLIA, DiaSorin Spa, Saluggia, Vatican city, Italy) following the manufacturer's instructions. Vitamin D status was classified as sufficiency [serum 25(OH)D > 20 ng/mL], insufficiency [serum 25(OH)D 12-20 ng/mL], and deficiency [serum 25(OH)D < 12 ng/mL], according to the "Global Consensus Recommendations on Prevention and Management of Nutritional Rickets" (2).

Statistical Analysis

Qualitative data were summarized as absolute and relative (percentage) frequencies. Means and standard deviation (SD) or medians and interquartile ranges (IQR) were used for quantitative variables. Comparison of quantitative variables among different levels of serum vitamin D (three groups) were performed using one-way ANOVA or its non-parametric equivalent, the Kruskal-Wallis test. Post-hoc analysis was performed using Dunn's test and Bonferroni correction. Differences in qualitative variables were assessed using Fisher's exact test. Spearman's correlation coefficients were calculated to explore the relationship between serum vitamin D levels and siblings. Moreover, univariate and multivariate logistic regression analyses were performed to assess the relationship between serum vitamin D levels (cut-off < 0.20) and sample characteristics. Stata 15 statistical software (StataCorp LLC, Texas, USA) was used for every statistical computation. P values of less than 0.05 were considered statistically significant.

Results

A total of 182 children were enrolled during the study period, median (IQR) age was 9 (6-12; range 1-16) years and 51.7% were male. Sixty-nine of the participants were siblings. Demographic and clinical characteristics of the study population, stratified by vitamin D status, are shown in Table 1.

Table 1. Demographic and clinical characteristics of study population (n = 182) stratified by vitamin D status [sufficiency, serum 25(OH)D > 20 ng/mL; insufficiency, serum 25(OH)D of 12-20 ng/mL; deficiency, serum 25(OH)D < 12 ng/mL]

Variables		< 12 ng/mL (n = 4)	12-20 ng/mL (n = 55)	> 20 ng/mL (n = 123)	p
Males, n (%)		3 (75.0)	27 (50.0)	64 (51.6)	0.68
Median (IQR) age, years		10.0 (4.5-13.5)	10 (7-12)	8 (5-11)	0.07
Median (IQR) weight, kg		29 (16.3-42.0)	36 (18.8-42.5)	24 (17.2-36.0)	**0.02**[1]
Median (IQR) height, m		1.37 (1.02-1.47)	1.40 (1.16-1.52)	1.25 (1.10-1.41)	**0.03**[2]
Median (IQR) BMI, kg/m^2		17.0 (15.2-19.9)	16.4 (15.0-19.3)	15.8 (14.6-18.1)	0.25
Median (IQR) BMI Z-score		-0.47 (-1.67; 0.60)	-0.52 (-1.09; 0.25)	-0.38 (-1.37; 0.47)	**0.03**
Residence, n (%)	Rural	0 (0.0)	9 (16.4)	9 (7.6)	0.17
	Urban	4 (100.0)	46 (83.6)	110 (92.4)	
Sun exposure, n (%)	< 15 days	1 (25.0)	2 (3.7)	7 (5.7)	0.36
	15-30 days	1 (25.0)	9 (16.7)	23 (18.9)	
	> 30 days	2 (50.0)	43 (79.6)	92 (75.4)	
Use of sunscreens, n (%)	Not exposed	1 (25.0)	6 (11.1)	11 (8.9)	0.73
	Non-regular	1 (25.0)	19 (35.2)	40 (32.5)	
	Regular	2 (50.0)	28 (53.7)	72 (58.5)	

Table 1. Continued

Variables		< 12 ng/mL (n = 4)	12-20 ng/mL (n = 55)	> 20 ng/mL (n = 123)	p
Formula milk (between 1-3 years), n (%)	No	3 (75.0)	36 (66.7)	66 (54.1)	0.22
	Yes	0 (0.0)	16 (29.6)	54 (44.3)	0.06
	Maternal	1 (25.0)	2 (3.7)	2 (1.6)	**0.02**[3]
Fitzpatrick class, n (%)	2	0 (0.0)	6 (11.1)	3 (2.4)	0.43
	3	0 (0.0)	10 (18.5)	26 (21.1)	
	4	2 (50.0)	23 (44.6)	56 (45.5)	
	5	2 (50.0)	14 (25.9)	35 (28.5)	
	6	0 (0.0)	1 (1.9)	3 (2.4)	
Nephrotic syndrome, n (%)		0 (0.0)	0 (0.0)	0 (0.0)	-
Kidney failure, n (%)		0 (0.0)	0 (0.0)	0 (0.0)	-
Liver failure, n (%)		0 (0.0)	0 (0.0)	0 (0.0)	-
Liver disease, n (%)		0 (0.0)	0 (0.0)	1 (0.8)	1.00
Antiepileptic drugs, n (%)		0 (0.0)	3 (5.6)	0 (0.0)	**0.03**[4]
Systemic corticosteroids, n (%)		0 (0.0)	0 (0.0)	0 (0.0)	-
Rifampicin, n (%)		0 (0.0)	0 (0.0)	0 (0.0)	-
Highly active antiretroviral therapy, n (%)		0 (0.0)	0 (0.0)	0 (0.0)	-
Celiac disease, n (%)		0 (0.0)	2 (3.6)	1 (0.8)	0.28
Inflammatory bowel disease, n (%)		0 (0.0)	0 (0.0)	0 (0.0)	-
Asthma, n (%)		0 (0.0)	3 (5.5)	7 (5.7)	1.00
Diabetes mellitus type 1, n (%)		0 (0.0)	2 (3.6)	1 (0.8)	0.28
Passive smoke exposure, n (%)		1 (50.0)	15 (53.6)	11 (17.7)	**0.001**[5]
Mean (SD) serum 25(OH)D level, ng/mL		8.8 (0.5)	16.7 (2.6)	29.5 (6.3)	**< 0.0001**

[1] < 10 ng/mL versus > 20 ng/mL, p value = 0.02.
[2] < 10 ng/mL versus > 20 ng/mL, p value = 0.01.
[3] < 10 ng/mL versus > 20 ng/mL, p value = 0.002.
[4] < 10 ng/mL versus > 20 ng/mL, p value = 0.008.
[5] < 10 ng/mL versus > 20 ng/mL, p value = 0.002.
SD: standard deviation, BMI: body mass index, IQR: interquartile ranges

Mean ± SD serum 25(OH)D value was 25.2 ± 8.3 ng/mL for the whole group. The majority (n = 123, 67.6%) of children had vitamin D sufficient values > 20 ng/mL. Of these 56 (30.8%) had values ≥30 ng/mL, and 67 (36.8%) had values in the 21 to 29 ng/mL range.

Among the children with serum vitamin D values ≤20 ng/mL (n = 59, 32.4%), 55 had insufficiency [25(OH)D, 12-20 ng/mL], and only 4 (2.2%) had deficiency (< 12 ng/mL). The latter underwent further laboratory investigations to rule out active rickets.

A history of daily tobacco smoke exposure was found in 27 (14.8%) children, of whom 16 (59.2%) had vitamin D ≤20 ng/mL. Only 43 (27.7%) of the 155 children not exposed to tobacco smoke had vitamin D ≤20 ng/mL (p = 0.001). Among the lifestyle factors investigated through the questionnaire, only smoke exposure showed a significant association with vitamin D status. Multivariate logistic regression analysis confirmed a significantly increased risk [odds ratio (OR): 6.0; 95% confidence interval (CI): 2.1-17.6; p = 0.001] of hypovitaminosis D [serum 25(OH)D levels ≤20 ng/mL] in children exposed to passive smoke (Figure 1, Table 2).

Discussion

Consistent with the results of our preliminary report of a smaller cohort of children (3), we found about one third of the healthy children living in Northern Sardinia had hypovitaminosis D. In our study population, the median global serum 25(OH)D value was 25.2 ng/mL, substantially similar to the value of 28.2 ng/mL reported in a recent Italian cross-sectional study by Galeazzi et al. (4). Consistent with Galeazzi et al. (4), we observed 25(OH)D levels were highest in summer and lowest during winter and spring, reflecting seasonal variations in sun exposure. However, unlike other studies, we documented mean values above the threshold of 20 ng/mL in winter.

The results of this questionnaire-based study showed that tobacco smoke exposure was a significant risk factor for hypovitaminosis D. To the best of our knowledge, this is

the first time such an association has been demonstrated in Italian children.

Previous studies have identified active smoking as a risk factor for vitamin D insufficiency in adolescents (9) but only a few studies have reported the effects of passive tobacco smoke exposure on vitamin D status in otherwise healthy children (6,7,8). In a US study of 2,263 subjects aged 3-17 years, vitamin D deficiency was observed in 15.1% of children not exposed to tobacco smoke, 20.9% of children exposed to secondhand smoke, and 18.0% of adolescent smokers (7). A Danish study investigated environmental, dietary, and genetic determinants of serum 25(OH)D levels during pregnancy and early childhood. In 298 children aged 4 years, the following determinants were identified: lower maternal age at birth, higher pre-pregnancy BMI, lower genetic vitamin D score, older siblings, tobacco smoke exposure, and female sex (5). More recently, a Japanese questionnaire-based study evaluated the association between smoke exposure and vitamin D deficiency in a large cohort of young children, showing that the two factors were significantly associated with each other (OR: 1.35; 95% CI: 1.14-1.59) (6).

The mechanisms by which tobacco smoke exposure might affect vitamin D status are likely complex and not fully understood. It has been hypothesized that tobacco smoke might interfere with vitamin D metabolism in multiple ways, including skin and renal activation of vitamin D and dysfunction in the parathyroid hormone (PTH)-vitamin D axis (10). Smoke exposure has been reported to be associated with altered dietary intake of vitamin D and calcium, through malabsorption but also by modifying taste (10). Moreover, through a yet unknown mechanism, smoke exposure alters normal PTH response to low vitamin D levels, resulting in simultaneous decreases in vitamin D, calcium and PTH (10). Whether this is secondary to PTH impaired secretion or to faster degradation, or both, is not known, but the consequence is certainly hypocalcemia and low bone mineral density. In this regard, a study cohort of 1,422 individuals (age 3 to 18 years) followed for 28 years observed that exposure to passive smoking in childhood, determined by parental smoking and serum cotinine (metabolite of nicotine) concentrations, was an important determinant

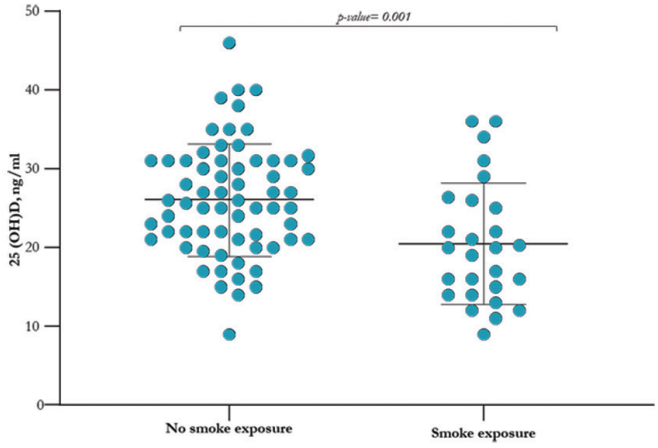

Figure 1. Serum 25(OH)D values in children with and without tobacco smoke exposure

Table 2. Relationship between hypovitaminosis D [serum 25(OH)D levels ≤20 ng/mL] and variables analyzed

Variables		Univariate analysis		Multivariate analysis	
		OR (95% CI)	p	OR (95% CI)	p
Male		1.0 (0.5-1.8)	0.88	0.9 (0.3-2.3)	0.83
Age, years		1.1 (1.0-1.2)	**0.02**	1.04 (0.89-1.21)	0.64
Weight, kg		1.0 (1.0-1.0)	**0.05**	-	
Height, m		6.7 (1.5-29.6)	**0.01**	-	
BMI, kg/m^2		1.1 (1.0-1.1)	0.30	-	
BMI Z-score		1.06 (0.87-1.30)	0.56	-	
Urban residence		0.5 (0.2-1.2)	0.12	-	
Sun exposure		1.1 (0.6-1.9)	0.76	-	
Regular use of sunscreens		0.8 (0.4-1.5)	0.52	-	
Formula milk		0.5 (0.2-0.9)	**0.03**	1.0 (0.4-2.9)	0.96
Fitzpatrick class	2	4.6 (1.1-19.1)	**0.04**	9.6 (0.9-105.3)	0.06
	3	0.9 (0.7-1.2)	0.54	-	
	4	1.0 (0.5-1.8)	0.87	-	
	5	0.9 (0.5-1.7)	0.76	-	
	6	0.7 (0.1-6.9)	0.76	-	
Passive smoke exposure		5.3 (2.0-14.0)	**0.001**	6.0 (2.1-17.6)	**0.001**

BMI: body mass index, CI: confidence interval

of impaired bone health with reduced bone mass, density, and strength indices measured later in adulthood (11). In addition, the toxicity of high cadmium and lead contents in cigarette smoke was associated with low levels of vitamin D by impairing both its intake and its activation, as this toxicity causes renal glomerular and tubular dysfunction. Vitamin D activation also decreases through skin aging and by dysregulation of the cytochrome P450 genes related to its metabolism, due to smoke exposure (10).

Some *in vitro* studies have provided further insight into the mechanism by which tobacco smoke may affect vitamin D status. A Korean study demonstrated that cigarette smoke extracts can inhibit the vitamin D-induced translocation of vitamin D receptor (VDR) in human alveolar basal epithelial cells. The subsequent treatment of 1,25-(OH)2-D3 induced translocation of VDR from nucleus to microsomes in a dose-dependent manner (12). More recently, Mathyssen et al. (13) found that cigarette smoking reduced the production of the active form of vitamin D in lung epithelial cells and also altered the normal expression of the VDR.

Study Limitations

The findings of this study should be interpreted in light of some limitations and biases mainly due to its nature, stemming from the retrospective observational design, where information collected through questionnaire was self-reported. Another limitation was the lack of a follow-up evaluation, which would be necessary to describe and investigate the medium-term implications for vitamin D deficiency. Other limitations are related to the small sample size, non-representative of the overall Italian pediatric population.

Conclusion

Our results provide further evidence that hypovitaminosis D is common in the Italian pediatric population, and suggest that smoke exposure is a significant risk factor for hypovitaminosis D. Given that vitamin D plays a crucial role in various physiological processes, including the development and maintenance of a healthy skeleton, mineral homeostasis, and immune system regulation, our findings are relevant to both clinical practice and public health, even more so considering that smoke exposure and other unhealthy lifestyle habits are preventable environmental factors.

Taken together, the available data suggest the complexity of the factors influencing serum vitamin D, the levels of which in children result from a variable combination of environmental factors, family lifestyle habits, epigenetic and genetic determinants. Experimental and observational prospective studies are needed to further evaluate causal relationship between smoke exposure and vitamin D status in children.

Ethics

Ethics Committee Approval: This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the regional committee on human experimentation (Comitato Etico ATS Sardegna, 29 May 2018, Protocol number: PG/2018/68).

Informed Consent: Parents gave informed consent for each child participating in the study.

Authorship Contributions

Concept: Maria Grazia Clemente, Dario Argiolas, Lino Argiolas, Roberto Antonucci, Design: Maria Grazia Clemente, Dario Argiolas, Lino Argiolas, Mary E. Blue, Roberto Antonucci, Data Collection or Processing: Dario Argiolas, Stefania Bassu, Angela Bitti, Mauro Argiolas, Lino Argiolas, Laura Saderi, Mariangela V. Puci, Giovanni Sotgiu, Analysis or Interpretation: Dario Argiolas, Stefania Bassu, Angela Bitti, Mauro Argiolas, Laura Saderi, Mariangela V. Puci, Giovanni Sotgiu, Cristian Locci, Literature Search: Maria Grazia Clemente, Stefania Bassu, Mauro Argiolas, Laura Saderi, Mariangela V. Puci, Giovanni Sotgiu, Cristian Locci, Writing: Maria Grazia Clemente, Angela Bitti, Giovanni Sotgiu, Mary E. Blue, Roberto Antonucci, Cristian Locci.

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CASE REPORT

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Mitotically Active Follicular Nodule in Early Childhood: A Case Report with a Novel Mutation in the Thyroglobulin Gene

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What is already known on this topic?

Loss-of-function mutations in the thyroglobulin (*TG*) gene are a rare cause of dyshormonogenesis. Cancer development due to TG mutations is rare and mostly occurs in adulthood. Long-term elevated thyroid stimulating hormone causes the growth of thyroid follicular cells. It may play a role in the development of malignant tumors, especially in adulthood.

What this study adds?

A novel compound heterogeneous mutation [c.2149C>T (p.R717*) (P.Arg717Ter)/c.5361_5362delCCinsG(p.H1787Qfs*3) (p.His1787GlnfsTer3)] in the *TG* gene was identified. This patient had a premalignant thyroid lesion in early childhood. A mitotically active follicular nodule, of which pathological features were not previously defined in the literature, is reported.

Abstract

Dyshormonogenesis (DG) is the failure of thyroid hormone production due to a defect in thyroid hormonogenesis. Loss-of-function mutations in the thyroglobulin (*TG*) gene are a cause of DG, leading to gland stimulation by thyroid-stimulating hormone (TSH), resulting in goiter. We report a mitotically active follicular nodule in an 11-year-old female with a novel mutation in the *TG* gene. The patient had been under follow-up for congenital hypothyroidism (CH) since the neonatal period, and she had normal TSH levels on replacement therapy. Genetic test revealed a novel compound heterogeneous mutation [c.2149C>T (p.R717*) (P.Arg717Ter) / c.5361_5362delCCinsG (p.H1787Qfs*3) (p.His1787GlnfsTer3)] in the *TG* gene. She underwent total thyroidectomy for a thyroid nodule that was reported as Bethesda IV on fine needle aspiration biopsy (FNAB) and noted as suspicious for noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). Pathological examination revealed a 16 mm, well-demarcated follicular nodule with a solid/insular pattern. Mitotic activity and Ki67 proliferation index were unusually high (10 mitoses/mm^2 and 10%, respectively). Marked cellular pleomorphism and nuclear atypia are well-known diagnostic pitfalls in patients with dyshormonogenetic goiter. However, high mitotic activity is a feature that is less commonly reported in dyshormonogenetic goiter and may raise suspicion of poorly differentiated carcinoma when observed together with a solid pattern. The absence of signs of invasion, history of CH, and awareness of the presence of mutations compatible with dyshormonogenetic goiter can prevent the overinterpretation of such lesions. The risk of cancer development in the dyshormonogenetic thyroid gland is possible in childhood. The close follow-up is life-saving and prevents morbidities and possible mortality.

Keywords: Congenital hypothyroidism, thyroglobulin synthesis defect, thyroglobulin (*TG*)

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Introduction

Dyshormonogenesis (DG) is the failure of thyroid hormone production in a structurally intact gland due to a defect in thyroid hormonogenesis, leading to congenital hypothyroidism (CH). DG is a rare but significant risk factor associated with developing thyroid cancer (1,2). The thyroglobulin (*TG)* gene is on chromosome 8q24.2-8q24.3. TG is essential for promoting thyroid hormone synthesis, and storage of iodine and inactive thyroid hormones. The incidence of thyroid DG due to *TG* mutations is approximately 1 in 100,000 newborns (3). A TG synthesis defect causes chronic stimulation of the gland by thyroid-stimulating hormone (TSH) (2). Long-standing TSH stimulation often leads to goiter. However, thyroid cancer development is mostly in adulthood (4).

The first mutation identified in *TG* was g.IVS3-3C>G in a family with congenital goiter in 1991 (5). Since then, 52 more mutations (11 splice site mutations, 11 nonsense mutations, 23 missense mutations, six deletions, and one single nucleotide insertion) have been identified (4). All patients with mutations in *TG* had a similar phenotype, such as low/absent serum TG, high levels of serum TSH, low levels of thyroid hormones, and enlarged thyroid gland (4).

This paper presents clinical, biochemical, and pathological characteristics and an eleven-year follow-up of a case of primary CH with TG synthesis deficiency. Additionally, the patient had a novel mutation in the *TG* gene, which caused the early development of a mitotically active follicular nodule.

Case Report

An 11-day-old female patient was admitted to our outpatient clinic due to elevated TSH (75.5 µIU/mL), which was detected by a neonatal screening test on the seventh day of life. Her medical history showed that she was born to non-consanguineous parents at 38 weeks of gestation, with a birth weight of 3820 g. A physical examination revealed a weight of 3820 g (68%), a length of 53 cm (85%), and a head circumference of 35.5 cm (51%). The anterior fontanelle was 3x3 cm, and the posterior fontanelle was 1x1 cm. Laboratory tests showed normal hemogram and liver and kidney function and blood glucose. Thyroid function test (TFT) confirmed primary hypothyroidism with a free (f)T4 of 5.58 pmol/L (7-16 pmol/L) and TSH >100 mIU/mL (0.34-5.36 mIU/mL). The urinary iodine level was 159 µg/L (normal value 100-200 µg/L). TG level was <0.1 ng/mL (1.15-50.03 ng/mL). Her thyroid volume was 2.07 mL [4.45 standard deviation (SD) score (SDS)], which excluded thyroid agenesia. Treatment with L-thyroxine (L-T4), 10 µg/kg per day, was initiated on the tenth day of life. One month later, the TSH, fT4, and fT3 levels were normal.

Molecular analysis revealed a novel compound heterogeneous *TG* mutation [c.2149C>T(p.R717*) (P.Arg717Ter) / c.5361_5362delCCinsG (p.H1787Qfs*3) (p.His1787GlnfsTer3)]. The mutation was assessed by Franklin by Genoox. *TG*: c.2149C>T(p.R717*) was pathogenic and *TG*:c.5361_5362delCCinsG likely pathogenic (6).

She was followed up every three months. TSH was carefully managed to remain in the lower part of the normal range (Table 1). She had normal growth and puberty. Neurological evaluation revealed normal language, cognitive, social, and fine motor development.

She underwent periodical ultrasound (US) investigation once a year. At age 10 years, thyroid US revealed a hypoechoic, well-defined nodule of approximately 7x6 mm in size, with high internal hypervascularity in the homogenous parenchyma of the left inferior thyroid lobe, without any sign of calcification. Lymphadenopathy was not observed. She was on L-T4, 2 µg/kg per day. Her TFT at the time was: serum TSH 6 µIU/mL (0.6-4.64 µIU/mL) and fT4 21.8 pmol/L (11-22 pmol/L). Fine-needle aspiration biopsy was performed and interpreted as "suspicious for follicular neoplasm". Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) was suggested as a possible diagnosis. The patient underwent a total thyroidectomy six months later when the family agreed to the operation. Histopathological examination revealed a 16 mm, well-demarcated follicular nodule with an insular and solid pattern in the left lobe. Mitotic activity and Ki67 proliferation index were unusually high (10 mitoses/mm^2 and 10%, respectively). No vascular nor capsular invasion was observed in the comprehensive examination of the nodule, and nuclear features were not adequate for a diagnosis of papillary carcinoma. The case was reported with a descriptive diagnosis as a "mitotically active follicular tumor".

The patient is now 11 years of age and on 100 µg of L-T4 daily (2.3 µg/kg/day). She weighs 42.7 kg (0.55 SD) and her height is 154.3 cm (1.24 SDS). BMI is 17.9 kg/m^2 (-0.05 SDS). Her growth, pubertal and mental development are normal. Her TFT is now: serum TSH 2.64 µIU/mL (0.6-4.64 µIU/mL) and fT4 22 pmol/L (11-22 pmol/L), anti-TPO antibody (Ab): 2.3 IU/mL (0-34 IU/mL), anti-TG Abs 10 IU/mL (0-115 IU/mL). She is under close follow-up by physical examination every three monthly, with periodic neck US. No lymphadenopathy or metastases were observed.

Table 1. Eleven year follow-up of the patient

Age at screening	TSH (0.6-4.64 µIU/mL)	free T4 (11-22 pmol/L)	free T3 (3.8-6 pmol/L)	LT4 dose (µg/kg)	Thyroid volume (SDS) on US*
11 days	74.3	5.58	6.27	10	2.07 mL (4.45 SD)
1 month	5.28	14.9	5.3	10	-
1 year	1.3	18.35	6.2	3.5	2 mL (2.14 SD)
2 years	2.9	18.5	6.8	3.2	-
3 years	2.3	18	7	3	5.53 mL (2.9 SD)
4 years	5.4	17.04	-	2.8	-
5 years	2.8	19	-	2.5	6.1 mL (3.5 SD)
6 years	1.3	21.8	-	2.4	-
7 years	5.3	18	-	2.3	7.5 mL (2.6 SD)
8 years	5.2	16.6	6.8	2.4	7.1 mL (2.4 SD)
9 years	6.8	18	7.3	2.2	9.7 mL (4.1 SD)
10 years	6	21.8	7.2	2.2	10.2 mL (4.4 SD)**
11 years	2.64	22	6.3	2.3	Total thyroidectomy

*Thyroid SD measurements were calculated using age and gender for the Turkish population and an online calculator then available online at Turkish Society for Pediatric Endocrinology and Diabetes online (10,11).
**A nodule of 7x6 mm in size, hypoechoic, well-defined with high internal hypervascularity on the homogenous parenchyma of the left inferior thyroid lobe.
SDS: standard deviation (SD) score US: ultrasound, TSH: thyroid-stimulating hormone

Discussion

We describe a case with a novel, compound heterogeneous mutation in the *TG* gene. Her histopathological findings were unusual. We observed a mitotically active follicular nodule in the context of a dyshormonogenetic goiter. To the best of our knowledge, these pathological features were not previously reported in the literature. Mitotic activity and Ki67 proliferation index were unusually high in the present case. High mitotic activity is a feature that is less well-known in DG and may be more alarming for the pathologist, raising suspicion of poorly differentiated carcinoma, especially when observed together with a solid insular trabecular (STI) pattern. In the recent World Health Organization classification of thyroid tumors, two types of high-grade follicular-derived carcinomas are described. Differentiated high-grade thyroid carcinoma is a papillary or follicular carcinoma with increased mitotic counts (≥5 mitosis/mm^2) or tumor necrosis. The second category is poorly differentiated thyroid carcinoma (PDTC), and it is defined as a malignant tumor of follicular cells with a STI pattern, without typical papillary carcinoma nuclei, and with the presence of convoluted nuclei or necrosis or high levels of mitosis (≥3 mitosis/mm^2) (7). Although the present nodule shows a much higher mitotic activity and a STI pattern, it did not exhibit any clear-cut histopathological features of malignancy. Although high mitotic activity is a worrisome histopathologic feature in a STI patterned follicular nodule, according to our clinical experience the diagnosis of PDTC should not be made, especially in a pediatric patient, based solely on the presence of a STI pattern and high mitotic activity in a completely well-circumscribed nodule without capsular or vascular invasion. The absence of signs of invasion, history of CH, and awareness of the presence of mutations compatible with DG may prevent the overinterpretation of such lesions. Although it is not possible to diagnose such a nodule as a malignant tumor in the presented case, the definitive nature of the present tumor remains to be characterized by follow-up of similar cases. Since thyroidectomy was performed in this case, it is not possible to comment on whether this particular nodule would have developed an invasive character. The high mitotic activity and high Ki67 index observed in the present case may be related to the novel TG mutation. We believe that radio-iodine ablation or lymph node dissection was unnecessary. However, since follow-up information on similar cases has not been reported to date, it will be safer to keep the patient under close follow-up, for early detection of a recurrence, albeit with a low probability.

Thyroid US and TG levels are some of the considerabletools to determine the etiology of CH, since all published patients with DG due to *TG* variants present with low/absent serum TG, high levels of serum TSH, low levels of thyroid hormones, and enlarged thyroid gland. Few patients develop a fetal goiter, diagnosed by antenatal US, and need intrauterine hormone replacement. However, others present with goiter at a later age (4). In the present case, we showed a mitotically active follicular nodule at an early age, which may have been related to the novel mutation.

Dyshormonogenetic goiter is a rare risk factor for developing thyroid cancer. In a study, 56 cases of dyshormonogenetic goiters with ages ranging from newborn to 52 yr were evaluated (8). Ten cases (18%) were diagnosed with thyroid cancer. Follicular type thyroid cancer was mainly seen, and almost all ten patients were diagnosed with thyroid cancer in adulthood. None of them had TG synthesis defects. Long-term elevated TSH causes the growth of thyroid follicular cells, and it might have a role in developing malignant tumors. However, cancer occurs after a long time under TSH stimulation, especially in adulthood (9). The most exciting aspect of the present case, from our point of view, was that her TSH level was normal from the beginning of her life with the exception of the last three years before presentation with the nodule. However, it was never markedly elevated under treatment (Table 1). This observation is significant in considering the genotype effect on tumorigenesis. Defining underlying genetic mechanisms will be more helpful in understanding the progression of the disease.

Conclusion

It can be speculated that we may have detected the nodule before the development of an aggressive tumor in adulthood. We want to emphasize two points about the present case. Firstly, an annual thyroid US examination was significant and potentially lifesaving. We believe that serial thyroid US examination was to our advantage for the early diagnosis of this nodule. Secondly, in the last three years of the follow-up, we realized that this patient had elevated TSH levels, although fT4 was in the normal range (Table 1). TSH stimulation might have initiated tumor development.

Ethics

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

Authorship Contributions

Surgical and Medical Practices: Sirmen Kızılcan Çetin, Zehra Aycan, Zeynep Şıklar, Serpil Dizbay Sak, Serdar Ceylaner, Elif Özsu, Merih Berberoğlu, Concept: Sirmen Kızılcan Çetin, Zehra Aycan, Merih Berberoğlu, Design: Sirmen Kızılcan Çetin, Zeynep Şıklar, Serdar Ceylaner, Merih Berberoğlu, Data Collection or Processing: Sirmen Kızılcan Çetin, Serpil Dizbay Sak, Serdar Ceylaner, Elif Özsu, Merih Berberoğlu, Analysis or Interpretation: Sirmen Kızılcan Çetin, Serpil Dizbay Sak, Serdar Ceylaner, Literature Search: Sirmen Kızılcan Çetin, Zehra Aycan, Serpil Dizbay Sak, Serdar Ceylaner, Elif Özsu, Writing: Sirmen Kızılcan Çetin, Zehra Aycan, Zeynep Şıklar, Merih Berberoğlu.

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CASE REPORT

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A New Variant of the *IER3IP1* Gene: The First Case of Microcephaly, Epilepsy, and Diabetes Syndrome 1 from Turkey

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What is already known on this topic?

MEDS1 manifests as microcephaly with simplified gyral pattern in combination with severe infantile epileptic encephalopathy and early-onset permanent diabetes.

What this study adds?

This is the first case reported from Turkey. It differs from previously reported cases due to the absence of a typical simplified gyral pattern on early brain magnetic resonance imaging, the late onset of diabetes, and the presence of a new genetic variant.

Abstract

Microcephaly, epilepsy and diabetes syndrome 1 (MEDS1) is a rare autosomal recessive disorder caused by defects in the immediate early response 3 interacting protein 1 *(IER3IP1)* gene. Only nine cases have been described in the literature. MEDS1 manifests as microcephaly with simplified gyral pattern in combination with severe infantile epileptic encephalopathy and early-onset permanent diabetes. A simplified gyral pattern has been described in all cases reported to date. Diagnosis is made by demonstration of specific mutations in the *IER3IP1* gene. In this study, we present an additional case of a patient with MEDS1 who was homozygous for the c.53C > T p.(Ala18Val) variant. This case, the first to be reported from Turkey, differs from other cases due to the absence of a typical simplified gyral pattern on early brain magnetic resonance imaging, the late onset of diabetes, and the presence of a new genetic variant. The triad of microcephaly, generalized seizures and permanent neonatal diabetes should prompt screening for mutations in *IER3IP1*.
Keywords: Developmental delay, diabetes mellitus, epilepsy, *IER3IP1*, MEDS1

Introduction

The term "monogenic diabetes (infantile-onset diabetes)" refers to diabetes associated with a monogenic defect and is diagnosed in the first six months of life (1). Recent developments in the field of molecular genetics indicate that diabetes occurring very early in life is mostly caused by underlying monogenic defects (2,3). Current studies suggest that monogenic diabetes should be considered in cases of diabetes diagnosed in the first two years of life, and these studies report that approximately 1-6 % of pediatric diabetes cases are actually neonatal diabetes (2,3,4). Microcephaly, epilepsy, and diabetes syndrome 1 (MEDS1) is an autosomal recessive neurodevelopmental disorder that was first described by Poulton et al. (5) in 2011 and is characterized by microcephaly, simplified gyral pattern, severe epilepsy, and infantile diabetes. The disease is known to result from

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homozygous and compound heterozygous mutations in the immediate early response 3 interacting protein 1 (*IER3IP1)* gene, and it has so far been reported in a total of nine cases (5,6,7,8,9).

In this study, we present an additional case of a patient with MEDS1 who was homozygous for the c.53C > T p.(Ala18Val) variant. The case, the first to be reported from Turkey, differs from the other previously reported cases due to the absence of a typical simplified gyral pattern on early brain magnetic resonance imaging (MRI), the late onset of diabetes, and the presence of a new genetic variant. The triad of microcephaly, generalized seizures and permanent neonatal diabetes should prompt screening for mutations in *IER3IP1*.

Case Report

The patient was the first live singleton birth from the first pregnancy of a healthy father and mother, who were first cousins. He was born at term, and had no complications during pregnancy or the perinatal period. He had a birth weight of 3.400 kg [-0.24 standard deviation (SD)] with a length of 49 cm (-0.37 SD) and a head circumference of 34 cm (-0.42 SD). At the age of 11 weeks, he presented to the pediatric neurology outpatient clinic with complaints of spasms, crying, and restlessness. Neurological examination performed at presentation revealed that he had no eye contact, object tracking, and head control and that he had central hypotonia and flexor spasms. On physical examination he weighed 4,700 kg (-0.83 SD), measured 55 cm (-1.31 SD), and had a head circumference of 35.5 cm (-2.46 SD) with a conspicuous microcephaly. He had hypertelorism, depressed nasal bridge and micrognathia (Figure 1). Laboratory tests revealed blood amino acid, cerebrospinal fluid (CSF) amino acid, acyl-carnitine, and urine organic acid levels within normal limits. Electroencephalogram showed a burst suppression pattern. Brain MRI performed at the age of two months revealed normal cortical sulci and gyri with normal widths for age, as well as normal ventricular system with normal width for age (Figure 2). Diagnosed with epilepsy, he was started on vigabatrin and pyridoxal 5 phosphate therapy. Owing to first-degree consanguineous marriage in the family and the co-occurrence of epilepsy and dysmorphic features, whole-exome sequencing (WES) was performed on DNA obtained from the proband and parents. He was found to have a homozygous missense variation (c.53C > T/p.Ala18Val) in *IER3IP1*. We could not find any phenotype-genotype study about this mutation in the literature. Homozygous pathogenic variants of this gene have been associated with autosomal recessive microcephaly, epilepsy, and diabetes syndrome type 1 (OMIM: 614231), and *IER3IP1* is a highly conserved protein with marked expression in the cerebral cortex and in beta cells. As the patient's seizures did not respond to the initial treatment, other drugs were introduced in the following order: levatiracetam, topiramate, clonazepam, and clobazam. Brain computed tomography (CT) performed at the age of nine months revealed increased distance in the CSF space, particularly remarkable in the frontal lobe (Figure 3).

While there were no clinical signs of hypogonadism that might occur with MEDS1, thyroid function tests revealed a thyroid stimulating hormone level of 0.89 IU/mL (normal range: 0.27-4.2) and free T4 level of 0.96 ng/mL (normal range: 0.87-1.76). At the age of 18 months, he presented with

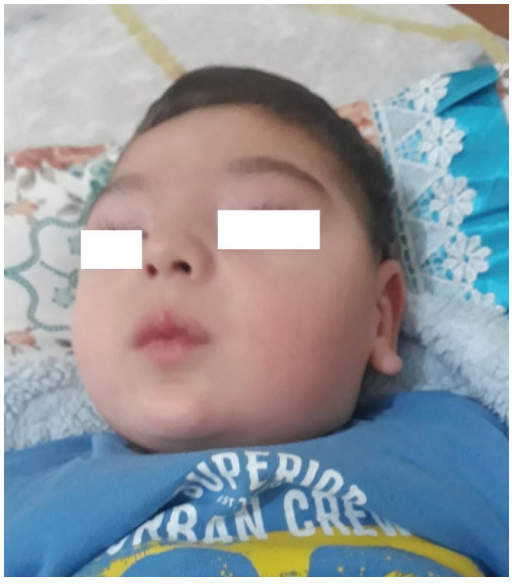

Figure 1. Patient's facial appearance

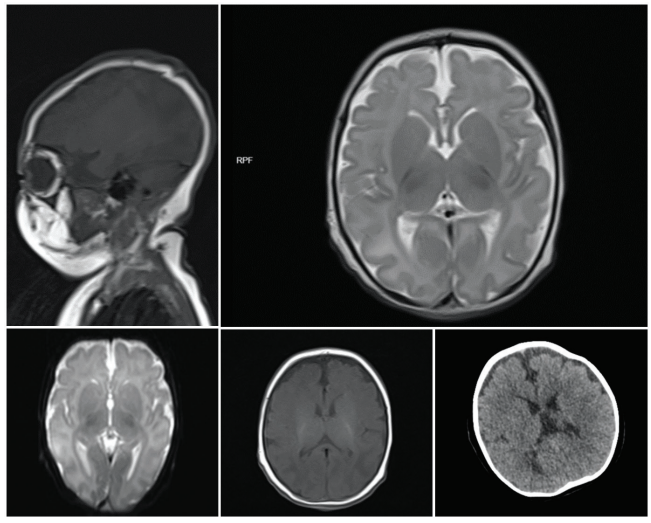

Figure 2. Brain magnetic resonance imaging of the patient at the age of three months old

rapid breathing, and further tests revealed a blood glucose level of 311 mg/dL, insulin level of 4.9 mIU/L, C-peptide level of 0.43 µg/L, and HbA1c level of 7.09%. Upon establishing a diagnosis of diabetes, insulin therapy was started at a dose of 0.4 U/kg. Diabetes antibodies (anti-glutamic acid decaboxylase, anti-insulin, and islet antibodies) were negative. There was no acidosis or ketonuria. Medical history was negative for polydipsia, polyuria, and significant weight loss. The c.53C>T/p.Ala18Val variant in *IER3IP1* has not been previously reported. Both parents were found to be heterozygous for the mutation.

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

Preparation for Genetic Analysis

Genomic DNA extraction was performed according to manufacturer's instruction (Maxwell RSC Blood DNA Kit, Promega, USA) using Maxwell RSC Instrument (Promega, USA). 30 µL of proteinase K (PK) solution was added into a 200 µL blood sample. Then 300 µL of lysis buffer was added to the blood and PK mix and incubated at 56 °C for 20 minutes. After this step, each blood lysate sample was transferred to the cartridges. At the end of assay in the instrument, 50 µL of DNA was eluted. The concentration of DNA was determined spectrophotometrically by measurement of the absorbance at 260/280 nm using a Nanodrop 1000 apparatus (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The concentration of DNA samples for libraries were determined by using Qubit 3.0 (Thermo Fisher Scientific). The sequencing libraries for exome sequencing were prepared according to Twist Human Core Exome Kit protocol (Twist Bioscience, South San Francisco, USA) Paired-end 150 bp read sequencing was performed on a NovaSeq system (Illumina, San Diego, USA).

Results

Raw data were uploaded to the Sophia DDM (Sophia Genetics, Lausanne, Switzerland) platform for further analysis, which detected a homozygous *c.53C>T* p.(Ala18Val) (NM_016097) variation in *IER3IP1*. This detected variant could not be found in any literature report or in the healthy population database (gnomAD; https://gnomad.broadinstitute.org/). However, *in silico* prediction databases (MutationTaster, PROVEAN, SIFT) stated, in consensus, that the variation was "deleterious." The American College of Medical Genetics 2015 criteria qualified the variant as "Class 3 - variant of uncertain clinical significance" (10). The segregation analysis for the variation was performed with Sanger sequencing and Integrative Genomics Viewer using samples collected from the patient's parents, and both parents were found to be heterozygous carriers of the mutation (Figures 4, 5). As the patient's clinical findings were similar to the expected symptoms of MEDS1 (OMIM: 614231) phenotype, known to be caused by homozygous pathogenic variants in *IER3IP1*, this mutation was thought to account for the patient's phenotypic features.

Discussion

This case report presents a male patient with homozygous variation in *IER3IP1* and this is the first case reported from Turkey and the 10th case reported globally. The present case differs from other previously reported cases due to the absence of the typical simplified gyral pattern on brain MRI and the presented case had a later onset of diabetes compared with other reported cases.

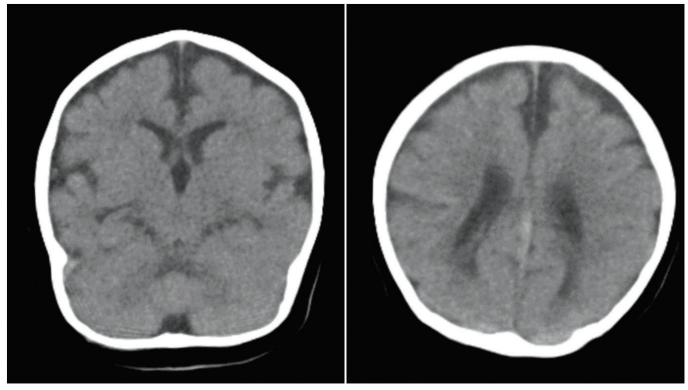

Figure 3. Brain computed tomography at the age of 9 months old

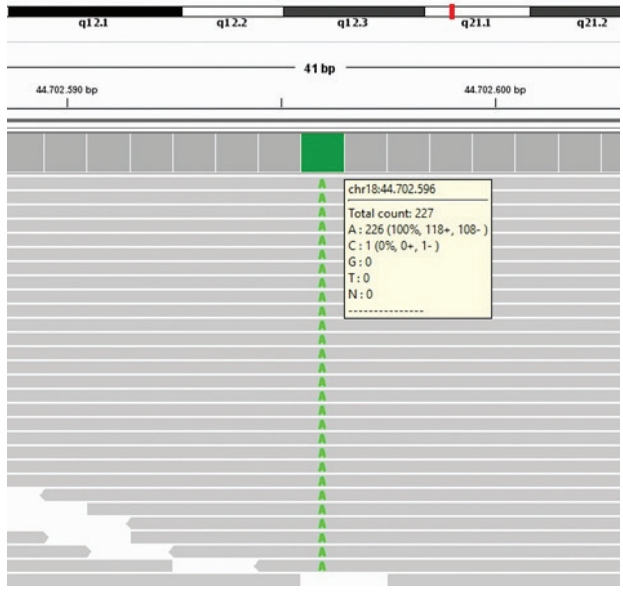

Figure 4. Results of next-generation sequencing at the mutation locus c.53C>T p.(Ala18Val)

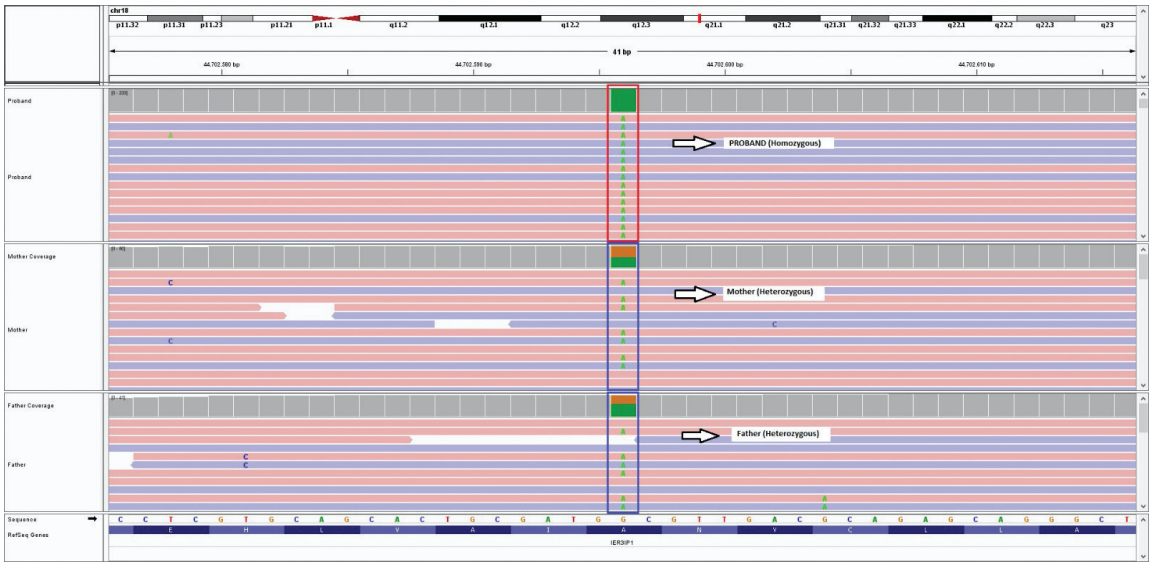

Figure 5. Integrative Genomics Viewer were used to analyse the characteristics of the mutated MEDS1 protein

MEDS1: microcephaly, epilepsy, and diabetes syndrome 1

MEDS1 syndrome was first reported by Poulton et al. (5) in 2011 in two cases from two unrelated families. Common findings in these cases were microcephaly with simplified gyral pattern in combination with severe infantile epileptic encephalopathy and early-onset permanent diabetes. An autopsy specimen from one patient showed increased apoptosis in the cerebral cortex and pancreas beta cells, implicating premature cell death as the pathogenetic mechanism (5).

MEDS1 (OMIM: 614231), which shows an autosomal recessive pattern of inheritance, results from a defect in the production of *IER3IP1* expressed in beta cells of the cerebral cortex and pancreas. *IER3IP1* is localized to the endoplasmic reticulum (ER) and is thought to play a role in the transport of proteins between the ER and Golgi apparatus and to be involved in the ER stress response (5). The association of neonatal diabetes with *IER3IP1* mutations suggests that *IER3IP1* regulates β-cell survival and/or function. Increased apoptosis in the cerebral cortex and pancreatic beta cells in autopsy samples with *IER3IP1* mutation points to early apoptosis as the pathogenic mechanism (5,7).

Neonatal diabetes refers to diabetes that is associated with a monogenic defect and is usually diagnosed in the first six months of life. The age at diagnosis of diabetes in the reported MEDS1 cases ranges from 14 days to 2 months (Table 1). In our case, however, diabetes emerged at the age of 18 months, substantially later than in other reported cases (6,7,9). Although current studies have shown that monogenic diabetes usually occurs in the first six months, recent studies have shown that it can rarely occur at the age of 12 or even 24 months (3,11,12). The important characteristics of the cases reported in the literature are summarized in Table 1.

The detection of c.62 T > G and c.233 T > C variants in all but one of the cases reported to date, and the fact that most of the cases are in Middle Eastern and North African countries or in countries receiving immigration from these regions, indicate that these variants are probably not mutational hotspots, but rather are rare ancestral variants unique to these regions. In the case reported by Shalev et al. (8), the common c.62 T > G missense variant and the novel c.79delT frameshift variant were compound heterozygous, and although this novel variant was a frameshift variant, the patient was more mildly affected than previously reported ones and survived to 8 years of age. This shows that variants other than the two most common mutations can result in different phenotypes. The c.62 T > G (p.Val21Gly) variant affects the first transmembrane hydrophobic domain of the protein, and the c.233T > C (p.Leu78Pro) variant affects the second transmembrane hydrophobic domain of the protein, impairing the protein's expression and/or function. The variant in our patient affects amino acid at position 18 in the first transmembrane hydrophobic domain, probably its mechanism of action is similar to c.62T > G, which affects amino acid at position 21. In addition, the c.62T > G variant is adjacent to the protein cleavage site. The milder phenotype of our case may be due to the fact that the variant found in the presented case was not so close to this cleavage site (5). Another piece of evidence supporting the pathogenicity of the variant in our patient is that the residues affected by both our patient's variant (18^{th} residue) and the c.62T > G (21^{st} residue) variant are located within a highly conserved 12-residue region across species, as shown below.

Table 1. The important characteristics of the cases reported in the literature

	Poulton et al. (5) (2011)		Abdel-Salam et al. (6) (2012)				Shalev et al. (8) (2015)	Valenzuela et al. (7) (2017)	Rjiba et al. (9) (2021)	Current case
			Family 1251		Family 1578					
	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
Gender	M	M	M	F	F	F	M	M	M	M
Consanguinity	+	+	+	+	+	+	-	-	+	+
MRI	Simplified gyral pattern	Simplified gyral pattern	Simplified gyration, cortical atrophy, hypoplastic corpus callosum, cerebellar vermis hypoplasia	Simplified gyral pattern and agenesis of corpus callosum with mild cerebellar vermis hypoplasia without atrophy	Cerebral atrophywith simplified gyral pattern, and agenesis of the corpus callosum	Simplified gyral pattern and agenesis of corpus callosum with normal cerebellum	Simplified gyral pattern	Simplified gyral pattern, ventriculomegaly and hypoplastic corpus callosum	Atrophy of the supra-tentorial level without pituitary anomaly	Normal
EEG	High voltage asymmetric multifocal activity with abnormal background	Hypsarrythmia	Polyspikes and slow waves with burst suppression	Generalized epileptic abnormalities with sharp and slow-waves	Burst suppression pattern	Burst suppression pattern	Hypsarrythmia	Low-amplitude background in the theta-delta range, suggesting diffuse neuronal dysfunction, with no epileptiform discharges	NA	Burst suppression pattern
Genetic analysis	c.62 T > G p.Val21Gly Homozygous	c.233 T > C p.Leu78Pro Homozygous	c.233 T > C p.Leu78Pro Homozygous	c.233 T > C p.Leu78Pro Homozygous	c.233 T > C p.Leu78Pro Homozygous	c.233 T > C p.Leu78Pro Homozygous	c.62 T > G/ p.Val21Gly and c.79delT/p. Phe27fsSer*25 Compound heterozygous	c.233 T > C p.Leu78Pro Homozygous	c.62 T > G p.Val21Gly Homozygous	c.53C > T p.(Ala18Val) Homozygous
Diabetes	+	+	+	+	+	+	+	+	+	+
Age at diabetes onset	NA	NA	NA	NA	40 days	14 days	NA	5 weeks	2 months	18 months
Hypogonadism	+	NA	Bilateral undescended testes	NA	NA	NA	Retractile testes	NA	Unilateral cryptorchidism and small genitalia	-
Skeletal findings	NA	NA	Osteoporosis, metaphyseal changes	Pathological fracture	Poor modeling of long bones and osteopenia	NA	NA	-	NA	-
Death	18 months	27 months	5 1/2 years	26 months	3 1/2 years	-	8 years	7 weeks	1 year	-

MRI: magnetic resonance imaging, EEG: electroencephalogram, NA: not available, M: male, F: female

11	18	21	32
AALLCVN	A	IA	V	LHEERFLKNIG	human
AALLCVN	A	IA	V	LHEERFLKNIG	mouse
TAILFTN	A	IA	V	LHEERFLSKIG	zebrafish
AALLCVN	A	IA	V	LHEERFLKNIG	cow
AALLFVN	A	IA	V	LHEERFLRR	blue-ringed sea krait

In differential diagnosis, Wolcott-Rallison syndrome has been reported to be the most common cause of neonatal diabetes in families with consanguineous marriages (13). This syndrome, which results from a homozygous mutation in *EIF2AK3*, is characterized by insulin-dependent diabetes mellitus before six months of age, skeletal dysplasia after six months of age, and liver failure. This syndrome manifests as renal failure, microcephaly, epilepsy, and central hypothyroidism and it must be ruled out in the differential diagnosis of MEDS1 (14). Increased ER stress, and thus beta-cell death, constitutes the pathogenesis of the disease, and management requires insulin replacement (15). This syndrome may also include episodes of liver failure and skeletal anomaly in later ages, indicating the importance of early genetic diagnosis. A study that presented four cases of MEDS1 reported that three of the patients had skeletal findings, including osteoporosis, metaphyseal changes, osteopenia, pathological fractures, and poor modeling of long bones (6). Our patient had no skeletal anomaly.

IER3IP1 has an unclear role in the development of the cortex and in the pathogenesis of epilepsy and diabetes, but it is thought to be required during early stages of neural development, for instance, during neural progenitor proliferation. Presence of microcephaly with simplified gyration has a distinctive role in differential diagnosis and already exists during gestation. Severe infantile epileptic encephalopathy is highly unusual in primary microcephaly and has been reported only in patients with *WDR62* mutations (16). In addition, a rare combination of primary microcephaly and severe infantile epilepsy in patients with *PNKP* mutations has been reported (17).

As our patient had refractory epilepsy, microcephaly, and axial hypotonia at the time of presentation, no specific complication at birth, and was born of parents who were first cousins, he underwent early WES and was subsequently diagnosed with MEDS1. The patient's family was asked to be vigilant about symptoms of potential diabetes with blood glucose being monitored. Diabetes emerged later than reported in other cases in the literature, but early genetic analysis allowed for diagnosing diabetes before acidosis developed. The patient did not present with microcephaly at birth, but with increasing age, it became evident. It was remarkable that the simplified gyral pattern, which was detected in all other cases, was absent on early MRI. Continued apoptosis in the postnatal period was thought to be the cause of MRI findings and microcephaly. This hypothesis was supported by increased distance in the CSF space detected on brain CT performed at the age of nine months.

Conclusion

In conclusion, this is the first case of MEDS1 from Turkey and is the first report of a variant that has not been previously described. Although the simplified gyral pattern, which co-occurs with the triad of microcephaly, epilepsy, and diabetes, may guide the diagnosis of MEDS1, manifestation of the symptoms may sometimes take time. Early genetic counseling should be considered in families where consanguineous marriage is accompanied by epilepsy and microcephaly.

Ethics

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

Authorship Contributions

Surgical and Medical Practices: Elif Söbü, Gül Demet Kaya Özçora, Bahtiyar Şahinoğlu, Feride Tahmiscioğlu Bucak, Concept: Gül Demet Kaya Özçora, Feride Tahmiscioğlu Bucak, Design: Elif Söbü, Data Collection or Processing: Elif Söbü, Gül Demet Kaya Özçora, Bahtiyar Şahinoğlu, Analysis or Interpretation: Elif Söbü, Gül Demet Kaya Özçora, Bahtiyar Şahinoğlu, Feride Tahmiscioğlu Bucak, Elif Yılmaz Güleç, Literature Search: Elif Söbü, Gül Demet Kaya Özçora, Writing: Elif Söbü, Feride Tahmiscioğlu Bucak, Elif Yılmaz Güleç.

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CASE REPORT

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Painless Footdrop in a Child with Newly Diagnosed Type 1 Diabetes Mellitus: Case Report

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What is already known on this topic?

Diabetic neuropathy is a major cause of morbidity amongst diabetics. Its presentation is usually regarded as a late-stage complication of diabetes, mostly affecting patients with advancing age

What this study adds?

This report describes a rare case of a pediatric patient with newly diagnosed type 1 diabetes mellitus (T1DM) who presented with signs of mononeuropathy. It highlights that T1DM may present atypically as acute onset neuropathy in pediatric patients, making it an important differential diagnosis.

Abstract

Diabetic neuropathy is a major cause of morbidity among diabetics, usually affecting patients with long-standing diabetes and advancing age. We present a case of atypical first clinical presentation of type 1 diabetes mellitus (T1DM) in a pediatric patient. A 15-year-old male patient presented to the emergency department with complaints of right foot weakness associated with mild paresthesia of 1-week duration. There were complaints of polyuria, polydipsia and weight loss in the same timeframe. On subsequent examination, the patient exhibited signs of right-sided foot drop with weak ankle dorsiflexion and eversion, accompanied by impaired sensation over the dorsum of the right foot. Lab results confirmed a diagnosis of T1DM and the patient was started on subcutaneous insulin injections. The patient's foot drop recovered within one month of insulin initiation. This case highlights that T1DM may present atypically as acute onset neuropathy in pediatric patients, making it an important differential diagnosis.

Keywords: Case report, diabetes mellitus, footdrop, mononeuropathy

Introduction

Diabetic neuropathy (DN) is a major cause of morbidity amongst diabetics (1). It is a well-known process that over half the individuals with diabetes develop over time (1). Its presentation is usually regarded as a late-stage complication of diabetes, mostly affecting patients with advancing age (1). In pediatric patients, DN is rarely seen, especially as an initial presentation of type 1 diabetes mellitus (T1DM). This paper describes a rare case of a pediatric patient with newly diagnosed T1DM who presented with Foot drop.

Case Report

A previously healthy 15-year-old Arab male presented to our hospital with the chief complaint of right foot weakness of 1-week duration. The weakness was associated with a mild tingling sensation in his right foot but no numbness.

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The patient also reported feeling unbalanced, often tripping over, as well as dragging his right foot for the past week. During this period, symptoms of polydipsia, polyuria, and polyphagia were also present.

Upon detailed history questioning, the patient reported right knee trauma two months prior to this presentation. He described two days of pain and clicking sounds in the affected knee, which resolved spontaneously and has been asymptomatic since. No cast was worn. No recent infections were reported. Past medical history and family history were unremarkable.The patient did not take any regular medications.

The patient was seen by the neurology team in view of his presenting symptoms. The neurological examination revealed a high steppage gait with right-sided foot drop and absent heel strike. Mild wasting of calf muscles was noted in the right leg. Examination of the right foot showed a grade 0/5 ankle dorsiflexion and 0/5 ankle eversion with normal ankle plantar flexion and normal ankle inversion. Ankle reflex was absent on the right and superficial sensation was also impaired over the dorsum of the right foot. The remainder of the general physical and neurological exam findings were normal, including left foot, upper limbs, cerebellar and cranial nerve functions.

Nerve conduction studies (NCS) were performed twice on bilateral peroneal, tibial, sural and superficial peroneal nerves (Tables 1, 2, 3). The tests showed normal distal latencies, conduction velocities and amplitudes from all of the nerves tested.

The diagnosis of T1DM was made after the laboratory reported a random serum glucose level of 247 mg/dL (normal 73-112 mg/dL) with hemoglobin A1c (HbA1c) of 11.7% (normal 4.3-5.7%) and anti-glutamic acid decarboxylase antibody titers of 51.9 units/mL (normal <5 units/mL).

Table 1. Motor nerve conduction study findings. Peroneal nerve responses to the extensor digitorum brevis muscle and tibial nerve responses to the abductor hallucis muscle were symmetrical with normal responses and amplitude, bilaterally

Site	Lat. (ms)	Dur. (ms)	Amp. (mV)	Area (mVms)	Stim. (mA)	Dist. (mm)	Intvl. (ms)	NCV (m/s)
Left peroneal								
Ankle	4.3	12.5	8.5	26.5	25		4.3	
Head of fibula	11.6	16.7	7.5	26	37	320	7.4	43.5
Right peroneal								
Ankle	4.1	12.3	8.9	23.9	21		4.1	
Head of fibula	11.5	12.2	7.6	20.9	32	340	7.4	45.9
Left tibial								
Ankle	3.7	14.2	21.4	81.9	21		3.7	
Popliteal	13.3	16	14.5	71.8	50	400	9.6	41.7
Right tibial								
Ankle	4.3	12.1	22.3	77.8	20		4.3	
Popliteal	13.5	13	15.5	64.8	41	380	9.2	41.3

Table 2. Sensory nerve conduction study results. Sensory responses from the superficial peroneal and sural nerves bilaterally showed normal amplitudes

Nerve	Lat. 1 (ms)	Lat. 2 (ms)	Amp. (uV)	Area (mVms)	Stim. (mA)	Dist. (mm)	Intvl. (ms)	NCV (m/s)
Left sural	2.4	3.1	33.8	2.1	23	110	2.4	45.8
Right sural	2.9	3.5	40.2	2	18	120	2.9	42.1
Left superficial peroneal	2.2	2.7	23.6	0.8	22	110		43.5
Right superficial peroneal	3.1	2.2	14.3	1.5	24	120		45

Table 3. F-wave latency results. Bilateral peroneal and tibial F-wave latencies were within normal limits

Nerve	Side	Stim. Site	F-Lat.	F-M Lat.
Peroneal	Left	Ankle	46.4 ms	42.2 ms
Peroneal	Right	Ankle	46.9 ms	46.9 ms
Tibial	Ankle	Ankle	49.2 ms	45.9 ms
Tibial	Ankle	Ankle	46.9 ms	43.5 ms

Venous blood gas showed normal pH and bicarbonate values. Moderate glycosuria and ketonuria were noted on urinalysis. Other laboratory findings, including thyroid functions, infectious workup, electrolytes, celiac screen and full blood counts were within normal ranges. Trauma and masses as a cause of peroneal neuropathy were ruled out by normal findings on magnetic resonance imaging (MRI) of the right knee. Central causes, including space-occupying lesions, infarction, bleeding, and inflammation processes were all ruled out by normal MRI brain and spine findings.

The patient was admitted and started on long acting insulin Degludec once daily and short acting insulin Aspart with meals at a total daily dose of 1.2 units/kg/day. His insulin doses were adjusted based on his blood sugar readings during hospital admission. He also underwent a few sessions of physiotherapy during his admission.

Within three days his glucose levels had normalized and he had mild improvement of his right foot weakness. The patient was discharged four days after his admission with a plan to follow up in physiotherapy, endocrinology and neurology outpatient clinics. Both the patient and his parents were given comprehensive education about diabetes and plans for follow up.

Two months later, in the follow up endocrinology clinic visits, the patient's glycemic control had significantly improved (HbA1c 6.4%) and he was asymptomatic.

Discussion

DM is one of the most commonly diagnosed endocrine disorders among children (2). T1DM is characterized by elevated levels of blood glucose as a result of autoimmune destruction of pancreatic beta cells, which cause insufficient insulin production (3). There has been a steady increase in the incidence of DM worldwide, with T1DM rising 3% annually in the last few decades (4). The United Arab Emirates and the Middle East in particular are facing an epidemic, with the Middle East and North Africa region currently exhibiting the highest prevalence of diabetes in the world after age-standardization (5).

The first presentation of DM usually involves osmotic symptoms, such as polyuria, polydipsia and weight loss (6). If left undiagnosed for long, patients may also present with diabetic ketoacidosis (DKA), which poses significant risks to the patient's morbidity and may even be fatal. This makes the early diagnosis of diabetes and recognition of atypical symptoms very important (7).

DN is a widely known complication of long-standing diabetes (1). Multiple mechanisms have been suggested to play a role in the pathogenesis of DN. These include hyperglycemia-induced oxidative damage, nerve ischemia due to endothelial dysfunction and the loss of insulin and its role as a neurotropic peptide (8,9,10).

DN can be divided into two broad categories. The first is generalized neuropathy, which encompasses diabetic sensorimotor polyneuropathy. This group has a chronic presentation that typically correlates with longstanding diabetes and advancing age (11). The second group encompasses a more acute presentation, has a self-limiting course, and is not associated with duration, intensity of diabetes or hyperglycemia. Entities within the second group include painful sensory neuropathy with weight loss (or diabetic cachexia), treatment related (insulin neuritis), polyneuropathy after ketoacidosis and hyperglycemia neuropathy (12).

The present case report appears appropriate for the second group, as it was acute in nature and had a rapid resolution following treatment. The presented patient had a predominant motor involvement, and no reports of weight loss, which makes diabetic cachexia less likely as it is mainly a sensory neuropathy (12).

Baszyńska-Wilk et al. (13) described a 9-year-old patient who developed symmetric lower limbs paresis with new onset T1DM after being admitted with severe DKA. During the course of her stay, the authors reported findings of brain edema and multifocal vasogenic brain lesions on further imaging. Their case exhibited a clinical feature of peripheral neuropathy after DKA, a complication that can be a consequence of peripheral nerve ischemia or other hemodynamic and metabolic changes that are linked to DKA (12). In our case, this diagnosis was taken into consideration, however our patient had no laboratory results suggestive of DKA and also showed no signs of central nervous system abnormalities, which are expected in DKA.

Multiple non-diabetic etiologies were also considered. Peroneal nerve injury as a result of trauma was investigated. However, the time frame of the patients' knee injury in relation to the onset of the symptoms made this very unlikely. Furthermore, our patients' symptoms coincided with the classic presenting symptoms of T1DM and rapidly improved with insulin treatment, all pointing to DN as the cause of his symptoms.

Few cases of pediatric neuropathy in the setting of undiagnosed diabetes have been observed and are accessible in the literature (14,15,16). Our case closely resembles the aforementioned cases in the signs and symptoms of our patients, aside from a normal NCS result. Although abnormal NCS results may be frequently found in

neuropathies, it has been reported to require a longer course of diabetes and a higher severity of hyperglycemia (17). In addition, the presented patient's neuropathic symptoms also completely recovered following glycemic control. This reinforces our diagnosis of DN as an atypical presentation of diabetes.

Conclusion

This case highlights that T1DM may present atypically as acute onset neuropathy in pediatric patients. It is important to recognize these clinical features as early recognition can reduce the risk of further complications and allows patients to receive appropriate treatment.

Ethics

Informed Consent: Written informed consent was obtained from the patient's guardian.

Authorship Contributions

Surgical and Medical Practices: Manal Mustafa, Samar Almuntaser, Concept: Maryam Jafari, Samar Almuntaser, Design: Maryam Jafari, Ahmedyar Hasan, Jessie Joseph, Data Collection or Processing: Maryam Jafari, Manal Mustafa, Samar Almuntaser, Analysis or Interpretation: Maryam Jafari, Samar Almuntaser, Literature Search: Maryam Jafari, Ahmedyar Hasan, Jessie Joseph, Samar Almuntaser, Writing: Maryam Jafari, Ahmedyar Hasan, Jessie Joseph, Manal Mustafa, Samar Almuntaser.

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CASE REPORT

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Elemental Milk Formula as a Possible Cause of Hypophosphatemic Rickets in Wiedemann-Steiner Syndrome

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What is already known on this topic?

Nutritional phosphate deficiency is not a common cause of hypophosphatemic rickets; rather, excessive phosphate wasting, which can be caused by an excess of fibroblast growth factor 23, as in X-linked hypophosphatemic rickets, is the most common cause. Nutritional hypophosphatemia can occur in certain conditions, such as premature babies, malabsorption disorders, or if a child is taking medication that interferes with phosphate intestinal absorption.

What this study adds?

We describe a patient with multiple co-morbidities and Wiedemann-Steiner syndrome who developed hypophosphatemic rickets after being exclusively fed elemental milk formula, which was resolved by switching formulas. In the literature, this formula-associated effect was only described in a limited number of patients. Further research is needed to determine whether some patient-related factors, such as the very rare syndrome described in our patient, could influence this effect.

Abstract

Phosphate has a fundamental role in bone mineralization, and its chronic deficiency has multiple negative consequences in the body, including defects in bone mineralization that will manifest in children as rickets and osteomalacia. Here we present a young boy known to have Wiedemann-Steiner syndrome with multiple co-morbidities that necessitated gastric tube feeding. The child at 22 months was found to have hypophosphatemia and a high alkaline phosphatase level associated with rachitic skeletal manifestations that were attributed to low phosphate intake and/or gastrointestinal absorption, as there was no evidence of excessive phosphate wasting based on appropriate tubular renal re-absorption of phosphate. The primary nutritional source was an elemental amino acid-based milk formula (Neocate®) from 12 months of age. After switching from Neocate® to another elemental amino-acid based milk formula, all biochemical and radiological abnormalities returned to normal, indicating that the Neocate® formula was the possible cause of the patient's low phosphate intake. However, in the literature, this formula-associated effect was only described in a limited number of patients. Whether or not some patient-related factors, such as the very rare syndrome described in our patient, could influence this effect warrants further exploration.

Keywords: Phosphopenic rickets, osteomalacia, Neocate®

Introduction

Phosphate is mainly an intracellular anion involved in various metabolic processes that occur during normal physiologic activity (1). Serum phosphate levels in healthy individuals are kept within a narrow range, primarily regulated by fibroblast growth factor 23 (FGF23), parathyroid hormone (PTH), and 1,25 dihydroxyvitamin D [1,25$(OH)_2$D] (2).

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Acute hypophosphatemia is relatively common, particularly among pediatric patients admitted to the intensive care unit (3). Acute illnesses can result in transient hypophosphatemia, which occurs due to a number of mechanisms but does not deplete the body's total phosphate store (4). Prolonged hypophosphatemia negatively impacts multiple body systems, with bone and the musculoskeletal system bearing the brunt of the damage (5). Phosphate is required for the maturation of the growth plate and bone mineralization. Phosphate is the leading factor of apoptosis in terminally differentiated hypertrophic chondrocytes in the growth plate. It also forms hydroxyapatite crystals with calcium, the major mineral component of bone. As a result, chronic hypophosphatemia can cause hypertrophic chondrocyte accumulation at the growth plate, resulting in classic rickets signs, as well as affect bone mineralization, resulting in osteomalacia, which can lead to bone deformities and recurrent fractures (6).

Chronic hypophosphatemia commonly develops as a result of increased urinary phosphate loss caused by hyperparathyroidism secondary to vitamin D deficiency, excess FGF23, renal tubulopathy, or as a side effect of certain medications. Hypophosphatemia, due to decreased phosphate intake, is rare because most foods are high in phosphate, but it can occur in certain conditions such as premature babies, malabsorption disorders, or if a child is taking medication that interferes with phosphate intestinal absorption (2).

There is emerging evidence that Neocate® formula contributes to the development of hypophosphatemic rickets due to reduced phosphorus bioavailability (7,8,9,10). Neocate® is an elemental amino-acid-based milk formula that is used to treat gastrointestinal disorders that interfere with optimal nutritional requirements. Since 1995, the U.S. Food and Drug Administration has approved Neocate® for use, and it contains a comparable amount of phosphate to other formulas (11). We described a patient who developed hypophosphatemic rickets after being exclusively fed with Neocate® formula, which was resolved by switching formulas.

Case Report

A four-year old boy who had been diagnosed with Wiedemann-Steiner syndrome, had multiple co-morbidities including global developmental delay, hypotonia, bilateral sensorineural hearing loss, large patent ductus arteriosus status post ligation, right multicystic dysplastic kidney, chronic lung disease due to chronic micro aspiration syndrome on home oxygen, severe gastroesophageal reflux disease with severe oral dysphagia, and excessive oral secretion on esomeprazole. The patient had undergone Nissen fundoplication and gastric tube (GT) insertion at the age of 14 months.

He was born via cesarean section at 34 weeks of gestation and remained in the hospital for one month after birth due to respiratory distress syndrome. Since birth, he had difficulty feeding, was not growing well, with length and weight of 66 cm and 5.3 kg respectively at the age of one year, and was frequently admitted to the hospital due to recurrent aspiration pneumonia. In terms of nutritional management, he was fed orally and occasionally required nasogastric tube feeding. At the age of 2 months, he was started on high calorie milk formula (Infantrini®), which was changed to elemental formula (Neocate®) at the age of 12 months, and at the age of 14 months, he underwent Nissen fundoplication and GT insertion due to recurrent episodes of aspiration pneumonia.

At the age of 22 months, he was discovered to have low serum phosphate following admission to the hospital. Table 1 shows the initial laboratory findings. Based on radiological changes (Figure 1), low serum phosphate, normal PTH,

Table 1. Laboratory results at baseline and in response to changing the feeding milk formula

Variable	Reference range	Baseline	1 week follow-up	2 weeks follow-up	2 months follow-up	6 months follow-up	2 years follow-up
Calcium (mmol/L)	2.2-2.7	2.42	2.35	2.40	2.51	2.43	2.28
Phosphate (mmol/L)	1.39-1.74	0.85	2.1	1.66	1.61	1.73	1.57
Magnesium (mmol/L)	0.7-0.95	0.83	1.11	0.96	0.86	0.84	0.87
Creatinine (umol/L)	27-62	36	34	36	35	37	39
PTH (pmol/L)	1.59-7.24	2	-	-	3.17	2.86	3.63
ALP (IU/L)	156-369	1183	-	849	181	144	91
25(OH)D (nmol/L)		127.6	-	-	91.6	-	107.9
$1,25(OH)_2D$	62.6-228	552.4	-	-	-	-	-
Urine phosphate (mmol/L)		< 1.62	-	-	-	-	-
Urine creatinine (mmol/L)		7.9	-	-	-	-	-

PTH: parathyroid hormone, ALP: alkaline phosphatase, 25(OH)D: 25-OH vitamin D, $1,25(OH)_2D$: 1,25 dihydroxyvitamin D

normal calcium, normal 25-hydroxyvitamin D levels, and tubular renal re-absorption of phosphate of 99%, he was diagnosed with hypophosphatemic rickets due to low phosphate intake or reduced phosphate bioavailability.

The treatment for low phosphate was initially an oral phosphate supplement in the form of sodium glycerophosphate, which provided 55 mg/kg/day of elemental phosphate, and after the first dose of sodium glycerophosphate, serum phosphate increased to 2.33 mmol/L, which was associated with secondary hypocalcemia (serum calcium 2.17 mmol/L) and secondary hyperparathyroidism (PTH level 29.5 pmol/L). Following that, sodium glycerophosphate was reduced to provide 25 mg/kg/day of elemental phosphate, which kept calcium and phosphate within normal limits (Table 1). Neocate® was administered via GT and provided him with nearly 100 Kcal/kg/day. He had no diarrhea or other gastroenterology symptoms. Due to the suspicion that Neocate® had low phosphate bioavailability, it was replaced with another elemental amino-acid based milk formula, which resulted in a significant increase in serum phosphate levels and a decrease in alkaline phosphatase levels that persisted even after the oral phosphate supplement was discontinued. A repeat radiograph one year later revealed improved bone density and rickets signs that had healed. Currently, the phosphate level and the other biochemical profiles are normal for the patient age (Table 1).

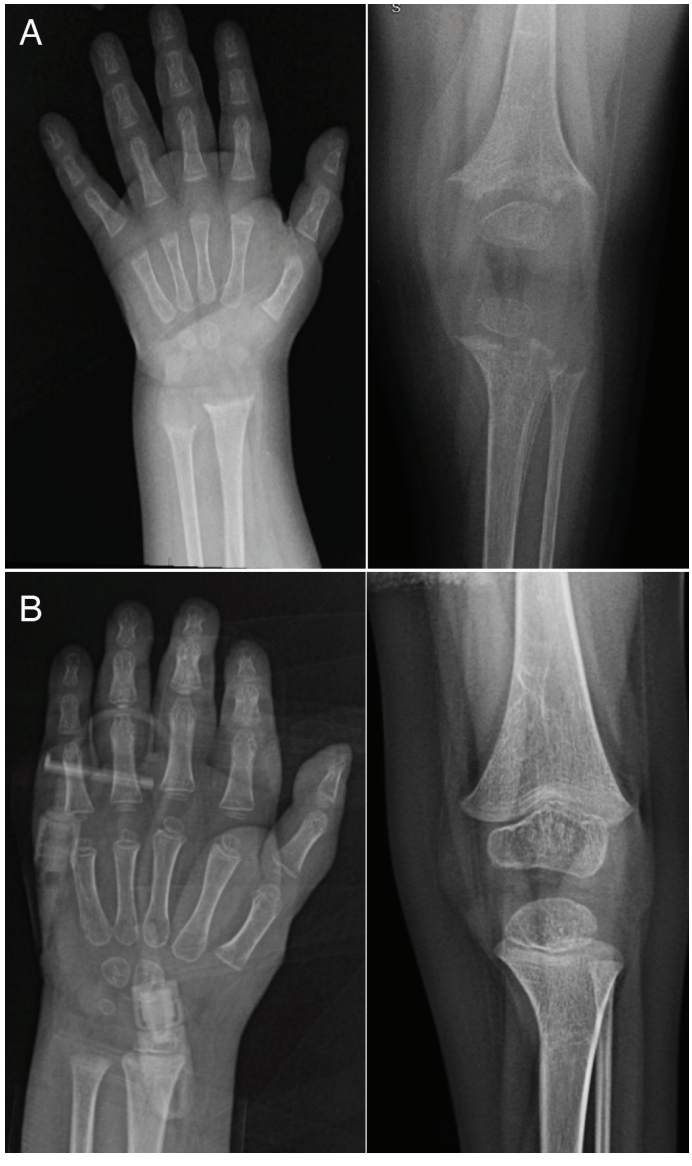

Figure 1. A and B show the baseline and follow-up radiographs of the left lower extremity and hand. A) The baseline image shows metaphyseal lucencies, cupping, and fraying of the distal femur, radius and ulna and the proximal tibia, as well as reduced osseous mineralization. B) One year follow-up shows improved mineralization and healing of rickets

Discussion

The presented patient's clinical and biochemical abnormalities are consistent with hypophosphatemic rickets, which are caused by nutritional phosphate deficiency, as evidenced by low phosphate in the urine. The fact that all biochemical and radiological abnormalities returned to normal after switching from the Neocate® formula to another formula suggests that our patient's low phosphate was possibly caused by the Neocate® formula.

Neocate® and other elemental formulas are commonly used in pediatrics to treat a variety of gastrointestinal disorders. It is an elemental amino-acid-based milk formula that is allergen-free (12). Neocate® has been used in children with milk protein allergy who are otherwise healthy, and it has not been found to cause mineral deficiencies (13). Almost all cases of hypophosphatemic rickets linked to Neocate® were in patients with multiple medical illnesses (7-10), indicating that a subset of patients may be vulnerable to impaired phosphate absorption from the Neocate® formula for reasons that are still unknown. In a recent randomized crossover trial, Neocate® was found to have comparable bioavailability of calcium and phosphorus to other elemental milk formulas in a healthy adult (14).

Our patient has multiple co-morbidities and was diagnosed with Wiedemann-Steiner syndrome, an autosomal dominant disorder caused by a mutation in the *MML* gene that results in a variety of medical problems, such as developmental delay, hypotonia, short stature, distinctive facial features, hypertrichosis cubiti and feeding difficulties that necessitate feeding support (15). In reports of a large French and Chinese cohort of patients with Wiedemann-Steiner syndrome, the observed skeletal manifestations were

advanced skeletal maturation, rib anomalies, brachydactyly, clinodactyly, tapering fingers, sacral dimple, and vertebral blocks; rickets or hypophosptaemia were not reported (16,17). The complexity of our patient's medical condition is consistent with previously reported cases of Neocate®-induced hypophosphatemic rickets, with the majority of those cases having multiple medical problems.

Given that not all patients on Neocate® develop hypophosphatemic rickets, many hypotheses have been proposed to explain these associations in a larger cohort of patients, including formula mineral bioavailability and the effect of medication such as proton pump inhibitors on absorption (10). However, these associations cannot be fully explained since the phosphorus concentration is comparable with other elemental formulas and the condition improved after substituting the formula while the patient was on the same medication, indicating that there may be other contributing factors that have yet to be discovered. A prospective study to explain these associations is needed.

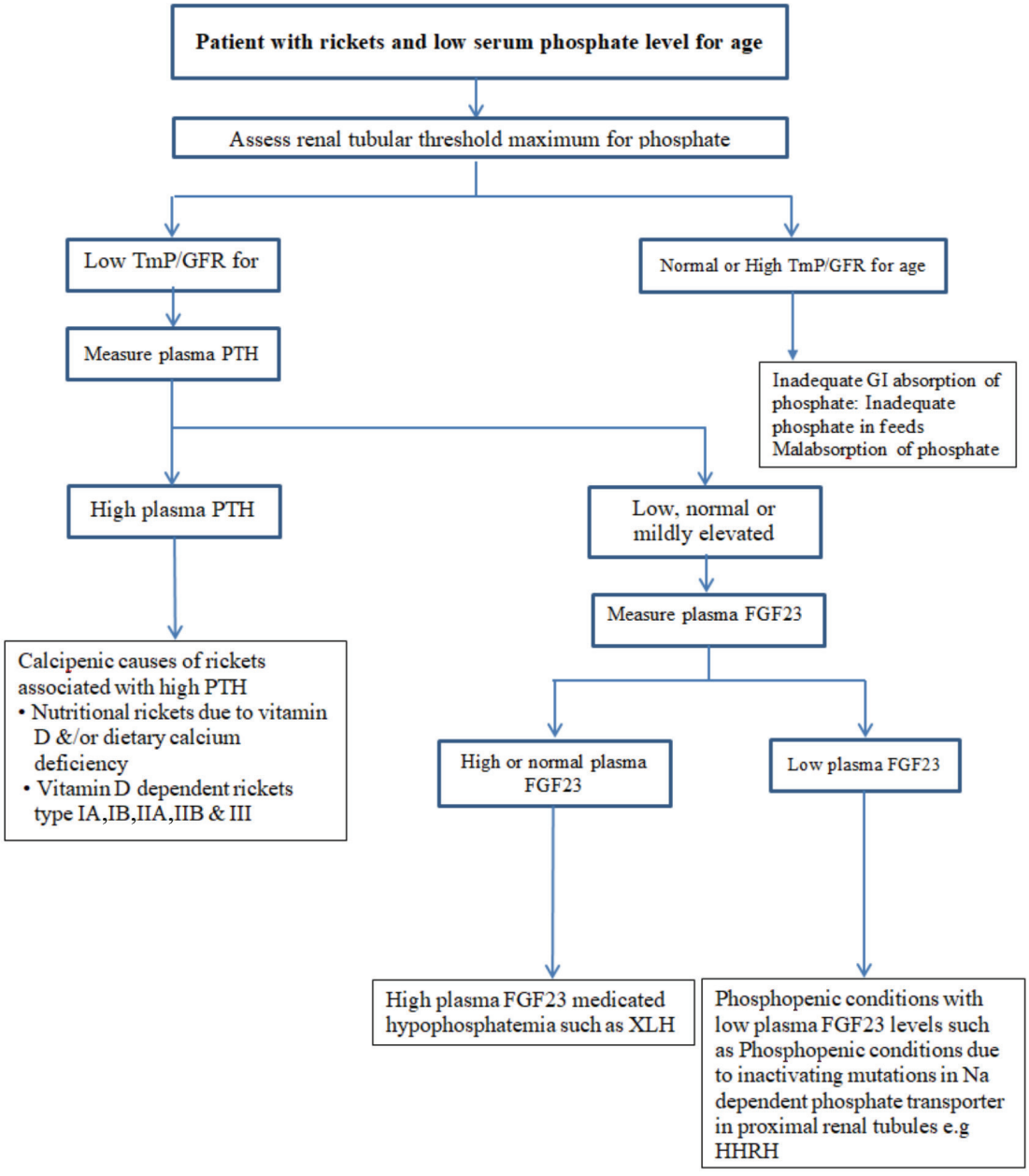

Figure 2. A biochemical algorithm for the assessment of a patient with rickets and low phosphate level for age

TmP/GFR: renal tubular threshold maximum for phosphate, PTH: parathyroid hormone, FGF23: fibroblast growth factor 23, XLH: X linked hypophosphatemic rickets, HHRH: hereditary hypophosphatemic rickets with hypercalciuria

Nutritional phosphate deficiency is not a common cause of hypophosphatemic rickets; instead, the majority of cases are caused by excessive phosphate wasting, which can be caused by an excess of FGF23, as in X-linked hypophosphatemic rickets, or by a primary defect in the Na-Po4 cotransporter, as in Dent disease (2). Given that the majority of cases reported in relation to Neocate® usually have multiple medical problems, and hypophosphatemia can be explained by a variety of factors such as prematurity and malabsorptive disorders, there is a tendency to delay in reporting these cases, which is understandable given that the majority of cases are recently reported.

Using a standard biochemical approach to hypophosphatemia treatment is one way to detect these cases early. Figure 2 depicts the stepwise biochemical approach for rickets. It is recommended that any patient with hypophosphatemia have their PTH level checked; if it is high, this means the primary defect is calcium deficiency, which could be caused by calcium or vitamin D deficiency, with nutritional vitamin D deficiency being the most common cause. If the PTH level is normal, the phosphate level in the urine should be evaluated; if it is low, it is due to nutritional deficiency or gut malabsorption; if it is high, it is due to excessive FGF23 or primary renal tubulopathy (18). The high $1,25(OH)_2D$ level observed in our patient could be confused with other vitamin D-related disorders, such as vitamin D-dependent rickets type 2, which is caused by a mutation in the vitamin D receptor. The high $1,25(OH)_2D$ level in our patient was related to a decrease in oral phosphate absorption, which leads to increased expression of one alfa hydroxylase enzyme in the kidney, which is responsible for converting 25(OH) vitamin D to its active form, $1,25(OH)_2D$.

The treatment of nutritional hypophosphatemia caused by Neocate® is not well established; there is a tendency for hyperphosphatemia after phosphate administration or formula substitution, which is explained by the expression of the Na-PO_4 cotransporter in the gut and kidney as a result of chronic hypophosphatemia and low FGF23 (19). To avoid hyperphosphatemia and secondary hypocalcemia, the phosphate dose should be gradually increased while calcium and phosphate levels are closely monitored. Rebound hypophosphatemia and hypocalcemia can also occur as a result of hungry bone syndrome caused by longstanding bone mineral depletion (20).

Conclusion

In patients with multiple co-morbidities, chronic hypophosphatemia due to the possibility of reduced phosphate bioavailability in Neocate® formula should be considered. We recommend that these patients taking Neocate® formula have their minerals and electrolytes checked on a regular basis. A prospective randomized study with a homogeneous group of patients should be performed to explore the potential patient-related factors that could increase the risk of developing hypophosphatemia, which could help in a better understanding of this association.

Ethics

Informed Consent: Informed consent was taken from the father for this material to be published.

Authorship Contributions

Concept: Fahad Al-Juraibah, Maali Melha, Azam Alromaih, Design: Fahad Al-Juraibah, Maali Melha, Azam Alromaih, Data Collection or Processing: Fahad Al-Juraibah, Maali Melha, Azam Alromaih, Analysis or Interpretation: Fahad Al-Juraibah, Fahad Al-Juraibah, Maali Melha, Azam Alromaih, Areej Al-Sunaid, Literature Search: Fahad Al-Juraibah, Fahad Al-Juraibah, Maali Melha, Azam Alromaih, Areej Al-Sunaid, Writing: Fahad Al-Juraibah, Fahad Al-Juraibah, Maali Melha, Azam Alromaih, Areej Al-Sunaid

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ORIGINAL ARTICLE

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Mild Aromatic L-Amino Acid Decarboxylase Deficiency Causing Hypoketotic Hypoglycemia in a 4-year-old Girl

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What is already known on this topic?

Aromatic L-amino acid decarboxylase (AADC) deficiency is an inherited metabolic disease that leads to a deficiency of serotonin, dopamine, epinephrine, and norepinephrine. Neurological findings are dominant due to deficiencies in neurotransmitter synthesis, but hypoglycemia can occur because of autonomic dysfunction.

What this study adds?

The first finding in this case with mild AADC deficiency was hypoglycemia. In patients presenting with hypoglycemia, AADC deficiency should be considered in the differential diagnosis, even if there are no neurological findings.

Abstract

Aromatic L-amino acid decarboxylase (AADC) deficiency is a disease in which neurological findings are dominant due to deficiencies in neurotransmitter synthesis. Hypoglycemia caused by autonomic dysfunction is one of the symptoms that may be encountered. Here we report a case of mild AADC deficiency presenting with hypoglycemia without any neurological signs. A 4-year-old girl presented with recurrent hypoglycemia. Her growth and development were normal. Plasma insulin and cortisol values were normal in the sample at the time of hypoglycemia. C8:1-Carnitine elevation was detected in the acylcarnitine profile. A clinical exome panel was performed with the suggestion of a fatty acid oxidation defect. However, a homozygous variant in the *DDC* gene was detected. Furthermore, cerebrospinal fluid neurotransmitter analysis revealed low 5-hydroxyindolacetic acid and homovanillic acid and high 3-O-methyl-dopa and methyltetrahydrofolate (5 MTHF) consistent with AADC deficiency. Plasma AADC enzyme activity was low. The episodes of hypoglycemia were treated with uncooked cornstarch. This case suggests that AADC deficiency should be considered in some patients with hypoglycemia.

Keywords: Aromatic L-amino acid decarboxylase deficiency, AADC deficiency, hypoglycemia, neurotransmitter deficiency

Introduction

Hypoglycemia is a common biochemical findings in endocrine and inherited metabolic disorders. Well-known, specific metabolic disorders causing hypoglycemia include glycogen storage diseases, gluconeogenesis disorders, fatty acid oxidation defects, and ketolysis defects (1). Identifying and treating the cause of hypoglycemia is of great importance. Therefore, the extremely rare metabolic causes of hypoglycemia, such as neurotransmitter disorders and in particular aromatic L-amino acid decarboxylase (AADC) deficiency, should be kept in mind (2). AADC is an essential

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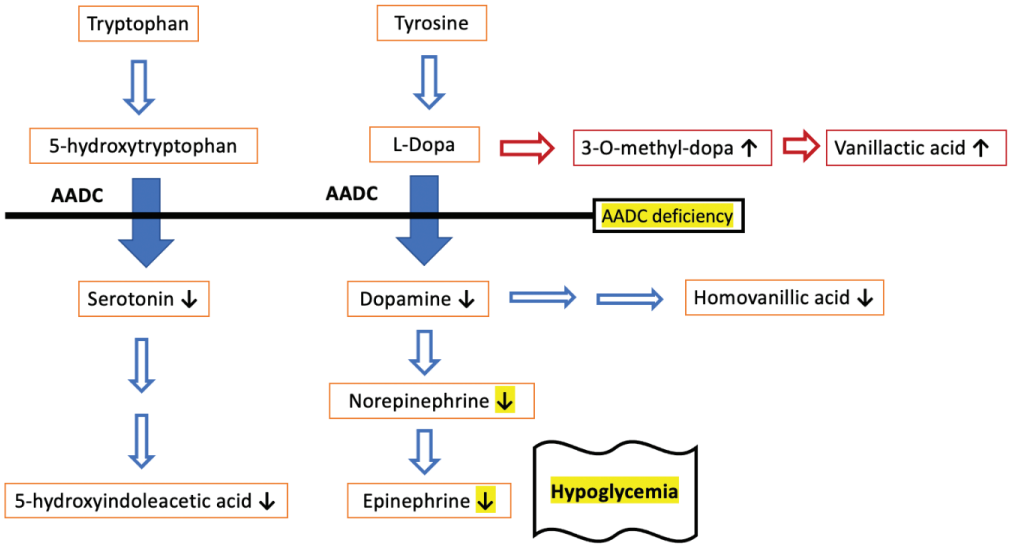

Figure 1. Norepinephrine (NE) and epinephrine (E) maintain glycemia levels by stimulating glucagon release, glycogenolysis, and food consumption and inhibiting insulin release. Studies by Ste Marie and Palmiter (14) found that the absence of catecholamines in dopamine β-hydroxylase-null mice resulted in chronically low blood glucose levels, impaired glucagon response to hypoglycemia, and elevated insulin levels, suggesting that NE and E are necessary for glucose homeostasis. Isolated deficiency in counter-regulatory hormones, such as growth hormone or cortisol is sufficient to expose the patient to hypoglycemia. Consequently, hypoglycemia in AADC deficiency is probably only the consequence of the altered synthesis of dopamine-derived catecholamines

AADC: aromatic L-amino acid decarboxylase

enzyme for synthesizing the monoamine neurotransmitters, serotonin and dopamine. Dopamine is the precursor of epinephrine (E) and norepinephrine (NE) (Figure 1). AADC deficiency is an autosomal recessive inherited metabolic disease that causes a deficiency of serotonin, dopamine, E, and NE. AADC deficiency usually manifests with neurologic symptoms, such as developmental delay, dystonia, oculogyric crisis, hypotonia, and autonomic findings in the early stages of life. The phenotypic spectrum of the disease is broad. Based on the clinical description, AADC may be classified as mild, moderate, or severe according to the severity of neurological symptoms. The mild phenotype may present with autonomic symptoms without significant movement disorders (3).

AADC deficiency leads to reduced dopamine, NE, and E levels. Catecholamines have essential counter-regulating functions for hypoglycemia, such as stimulaton of gluconeogenesis and lipolysis (Figure 1). Hypoglycemia may not be expected as the primary finding in AADC deficiency, where the indicative findings are usually neurological symptoms. In addition to neurological findings, hypoglycemia in intercurrent diseases has been reported in a small number of cases (4,5,6,7,8,9,10,11,12,13). Hypoglycemia is thought to develop from the deficiency of catecholamines, which are contra-insulin hormones. This report describes a case of AADC deficiency diagnosed with hypoglycemia without neurological findings.

Case Report

A 4-year-old girl presented with four episodes of hypoglycemia. The patient was born at 38 weeks, by C-section at 2700 grams. Prenatal history was unremarkable. Due to meconium staining, she was hospitalized for two days in the neonatal intensive care unit. She experienced jaundice in the neonatal period but did not need phototherapy. She received breast milk for 18 months. Her developmental stages were as expected and in line with her age; she sat unsupported at seven months, speech at 12 months, and walked unsupported at 18 months. There was a history of third-degree cousin marriage between the parents.

On physical examination, body weight was -1.4 standard daviation score (SDS), height was -1.79 SDS, and head circumference was -0.43 SDS. There was no dysmorphism, no organomegaly, and a detailed neurological examination was normal. She had normal muscle bulk, tone, and power. Deep tendon reflexes were normal. She had no movement disorder findings, such as hypokinesia, dystonia, or oculogyric crises. The patient had a history of hypoglycemia accompanied by a seizure after diarrhea, with the first episode at the age of 3.5 years. She had four episodes of hypoglycemia in total prior to presentation. Hypoglycemia was usually observed after about 10 hours of fasting and diarrhea. The fasting test for the etiology of hypoglycemia was performed with metabolic and endocrinological

sampling at 35 mg/dL glucose level at the 12th hour of fasting. Insulin, adrenocorticotropic hormone, and cortisol levels were normal. Urine ketones were negative, and the lactate level was in a normal range. The patient was unresponsive to glucagon administration. After intravenous glucose infusion at the time of hypoglycemia, blood glucose was recorded as 230 mg/dL. In the sample taken at the time of hypoglycemia, there was elevated C8:1 carnitine of 1.0 µmol/L (normal < 0.47) in the acylcarnitine profile; tiglylglycine excretion in urine organic acid screen and plasma amino acid profiles were average. Complete blood count with differential, liver, and kidney function tests, lipid profile, ammonia, and lactate levels were as expected in the laboratory analysis. Eye examination and hearing test were unremarkable. Abdominal ultrasonography and echocardiography were normal. Cranial magnetic resonance imaging and electromyography were normal.

Hypoketotic hypoglycemia, high levels of C8:1, and low insulin levels were compatible with fatty acid oxidation defects. However, the clinical exome sequencing panel revealed a homozygous p.Asp15Gly variant in the *DDC* gene. This variant was classified as a variant of uncertain significance [VUS (PM2-PP3)], according to the American College of Medical Genetics criteria. Upon this result, a lumbar puncture was performed, and low cerebrospinal fluid (CSF) levels of 5-hydroxyindole-3-acetic acid (5-HIAA) and homovanillic acid (HVA) and high levels of 3-O-methyl-dopa and 5-methyltetrahydrofolate (5-MTHF) were identified, consistent with AADC deficiency. Systemic AADC activity was low. The laboratory values of the patient are presented in Table 1. The patient was diagnosed with AADC deficiency, confirmed by enzymatic and genetic analysis. Treatment was begun with 100 mg/day of pyridoxine. The episodes of hypoglycemia were treated with raw cornstarch (1 g/kg).

Discussion

This report demonstrates the initial finding of hypoglycemia with no neurological signs in a young girl diagnosed with mild AADC deficiency. AADC deficiency is an extremely rare, inherited metabolic disease characterized by reduced activity of AADC, the key enzyme for neurotransmitter (dopamine and serotonin) synthesis. Only around 150 patients have been reported. Children with this condition are usually diagnosed in their first year of life. The cardinal sign of AADC deficiency is neurological symptoms, mainly hypotonia and oculogyric crises. In addition, autonomic nervous system dysfunction may cause extra neurological findings, such as gastrointestinal problems (diarrhea, constipation), feeding difficulties, nasal congestion, unstable body temperature, low blood pressure, and hypoglycemia (3).

Although hypoglycemia is not a cardinal finding of this disease, it was reported in five of the 82 patients in the Pediatric Neurotransmitter Diseases at BioPKU.org database. Hypoglycemia has been associated with E deficiency in AADC deficiency (10,14). Review of the literature showed that episodes of hypoglycemia are not always present in patients with AADC deficiency and have been documented only in patients with a severe phenotype (7,13). We have

Table 1. Laboratory characteristics of the patient

	Patient	Normal range
Blood (plasma/serum)		
Glucose, mg/dL	35	> 55
Insulin (during hypoglycemia), µU/mL	0.4	< 1
C-peptide (during hypoglycemia), ng/mL	0.07	< 0.30
Cortisol (during hypoglycemia), µg/dL	30.9	> 20
Growth hormone (during hypoglycemia), ng/mL	15.2	
Prolactin, µg/L	25	4.70-23.3
Plasma activity of AADC, pmol/min/mL	4	33-79
CSF		
CSF homovanillic acid, nmol/L	144.9	233-928
CSF 5-hydroxyindolacetic acid, nmol/L	39	74-345
CSF 3-O-methyl-dopa, nmol/L	415.2	< 150
CSF 5-hydroxytryptophan, nmol/L	34.2	< 10
Urine organic acid analysis		
Tiglylglycine mmol/mol creatinine	4	ND
Vanil lactic acid	ND	ND

AADC: aromatic L-amino acid decarboxylase, CSF: cerebrospinal fluid, ND: not detectable

Table 2. AADC deficiency patients with hypoglycemia in the published literature

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13
Reference	**Present study**	**Arnoux et al. (5), 2013**	**Pons et al. (4), 2004**	**Dai et al. (9), 2020**	**Dai et al. (9), 2020**	**Abdenur et al. (7), 2006**	**Korenke et al. (11), 1997**	**Spitz et al. (8), 2017**	**Helman et al. (6), 2014**	**Helman et al. (6), 2014**	**Lee et al. (12), 2009**	**Manegold et al. (13), 2009**	**Manegold et al. (13), 2009**
Gender	Female	Female	Male	Male	Male	Male	Female	Female	Male	Female	Female	Male	Female
Age at onset	42 months	3 years	2 months	3 months	3 months	2 days	3 months	3 months	7 years	10 months	3 months	4 months	5 years
Disease phenotype	Mild	Mild	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe
Hypoglycemia	+	+	+	+	+	+	+	+	+	+	+	+	+
Diarrhea	+	+	+	-	-	-	-	+	-	-	-	-	-
Temperature instability	-	-	-	-	+	+	+	**N/A**	-	-	-	-	+
Hyperhidrosis	-	-	+	+	+	+	+	+	+	-	+	+	-
Nasal congestion	-	+	+	+	+	-	-	+	+	-	+	-	-
Feeding problems	-	-	+	+	-	+	+	N/A	+	+	+	+	+
Failure to thrive	-	-	+	+	+	+	+	**N/A**	**N/A**	**N/A**	+	**N/A**	**N/A**
Movement disorders	-	+	+	+	+	+	+	+	+	+	+	+	+
Oculogyric crisis	-	-	+	+	+	+	+	+	+	+	+	+	+
Irritability	-	-	+	+	-	+	+	+	+	-	+	+	+
Developmental delay	-	-	+	+	+	+	+	+	+	+	+	+	+
Epileptic seizures	+	+	-	-	-	-	-	-	+	-	-	-	-
Sleep problems	-	-	+	+	+	-	-	+	**N/A**	**N/A**	-	+	-
Hyperprolactinemia	+	+	N/A	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Urine organic acids	Tiglylglycine	N/A	N/A	Normal	Normal	Vanillactic acid	Vanillactic acid	Vanillactic acid	N/A	N/A	N/A	N/A	N/A
AADC activity, pmol/min/mL (N 33-79)	4	5	< 1	N/A	N/A	< 1	2.6	5	2.6	1.5	3.9	0.2	1.6
DDC gene mutation Allele 1	c.44A > G	c.97G > T	c.1222C > A	c.714 + 4A > T	c.714 + 4A > T	N/A	N/A	c.823G > A	c.665T > C	N/A	c.714 + 4A > T	N/A	c.206C > T
DDC gene mutation Allele 2	c.44A > G	c.1385G > C	C102T (premature stop codon)	c.106G > A	c.714 + 4A > T	N/A	N/A	c.823G > A	c.665T > C	N/A	c.1234C > T	N/A	c.439A > C

N/A: not applicable, AADC: aromatic L-amino acid decarboxylase

summarized clinical and laboratory data of all these AADC deficiency patients with hypoglycemia in Table 2. Remarkably, our patient had hypoglycemic episodes with diarrhea as the primary symptom, and no neurological signs were observed except for a seizure triggered by hypoglycemia. Arnoux et al. (5) reported a similar patient with mild AADC deficiency, a 5-year-old girl with episodes of hypoglycemia and diarrhea who only had hypomimia and dyspraxia as neurological findings. Thus, to the best of our knowledge, only two patients have been reported who presented with hypoglycemia and were diagnosed with mild AADC deficiency, based on the differential diagnosis of hypoglycemia. Routine first-line metabolic investigations for hypoglycemia may not indicate AADC deficiency. Lactate, ammonia, acylcarnitine profile, and plasma amino acid analysis are normal in these patients. However, patients with AADC deficiency may be identified by elevated vanil lactic acid in urine organic acid analysis due to degradation of 3-O-methyl-1-dopa (3-OMD) to vanil lactic acid (7). In our patient, tiglylglycine excretion was found in the urine organic acid analysis due to fasting, and there was no vanil lactic acid excretion. A low ketone level at the time of hypoglycemia may suggest a diagnosis of hyperinsulinism or fatty acid oxidation deficiency. However, NE and E levels decrease in AADC deficiency, leading to an impaired glucagon response to hypoglycemia, which explains the low ketone levels. In particular, the main laboratory test to identify the diagnosis of AADC deficiency is the measurement of neurotransmitter levels. While the pterin level is standard in the CSF analysis, there are high 3-O-methyl-1-dopa and 5-hydroxytryptophan and decreased HVA and 5-HIAA values. 3-OMD is a disease-specific metabolite, and showing its elevation in plasma or dry blood may lead to early detection of patients in newborn screening programs in the future (15). Demonstrating AADC enzyme deficiency in plasma supported the molecular diagnosis.

Conclusion

The present case report highlights that AADC deficiency should be considered in the differential diagnosis of some patients presenting with hypoglycemia, even in the absence of classical neurological findings of the disease.

Ethics

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

Authorship Contributions

Surgical and Medical Practices: Merve Yoldaş Çelik, Ebru Canda, Havva Yazıcı, Fehime Erdem, Sema Kalkan Uçar, Mahmut Çoker, Concept: Merve Yoldaş Çelik, Sema Kalkan Uçar, Design: Merve Yoldaş Çelik, Sema Kalkan Uçar, Data Collection or Processing: Merve Yoldaş Çelik, Ebru Canda, Havva Yazıcı, Fehime Erdem, Ayşe Yüksel Yanbolu, Ayça Aykut, Asude Durmaz, Ahmet Anık, Sema Kalkan Uçar, Mahmut Çoker, Analysis or Interpretation: Merve Yoldaş Çelik, Sema Kalkan Uçar, Literature Search: Merve Yoldaş Çelik, Ebru Canda, Sema Kalkan Uçar, Mahmut Çoker, Writing: Merve Yoldaş Çelik, Sema Kalkan Uçar.

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CASE REPORT

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Sepsis-induced Pancytopenia in an Adolescent Girl with Thyroid Storm: A Case Report

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What is already known on this topic?

Thyroid storm is a rare but life-threatening acute complication of hyperthyroidism. Methimazole has been used as a first-line therapy for hyperthyroidism. Pancytopenia can be an extremely rare but serious side effect of antithyroid drugs, which should be immediately discontinued if the granulocyte count is less than 1000 cells/mm^3. Therefore, management of thyroid storm in the setting of pancytopenia is challenging.

What this study adds?

We present a 13-year-old girl with thyroid storm and pancytopenia, with symptoms similar to those of methimazole-induced pancytopenia. Due to close monitoring of complete blood cell count during fever, sepsis-induced pancytopenia in the setting of thyroid storm was considered, and methimazole treatment combined with methylprednisolone and meropenem was able to induce resolution of both pancytopenia and thyroid storm. This is the first pediatric case report that outlines the use of methimazole in the management of thyroid storm in the setting of pancytopenia.

Abstract

Thyroid storm is a rare but life-threatening condition mainly triggered by infection and abrupt discontinuation of antithyroid drug therapy for Graves' disease. Pancytopenia is a rare adverse reaction to antithyroid drugs. We present a 13-year-old girl with thyroid storm and pancytopenia with symptoms similar to those of methimazole-induced pancytopenia. Although in this context the use of methimazole is still under debate, due to multiple normal complete blood counts (CBC) monitored during fever, sepsis-induced pancytopenia with thyroid storm was considered, and methimazole treatment combined with methylprednisolone and meropenem was able to resolve both pancytopenia and thyroid storm. During the period of infection and antithyroid drug therapy, close monitoring of CBC may help differentiate the aetiology of pancytopenia. This is the first paediatric case report that outlines the use of methimazole in the management of thyroid storm with pancytopenia.

Keywords: Thyroid storm, pancytopenia, sepsis, antithyroid drugs

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Introduction

Thyroid storm is a rare, acute complication of hyperthyroidism, characterized by extreme manifestation of thyrotoxicosis, and it occurs in 1% to 2% of patients with hyperthyroidism. Methimazole has been used as a first-line therapy for Graves' disease in children. Agranulocytosis is a rare adverse reaction of antithyroid drugs and can be life-threatening, occurring in 0.2-0.5% of patients, generally within 90 days after initiation of antithyroid drug therapy (1). Moreover, pancytopenia has also been reported in some patients on antithyroid drug therapy, but its incidence is much lower than that of agranulocytosis (2). Therefore, it is challenging to differentiate whether patients with pancytopenia and thyroid storm is due to sepsis or methimazole. Herein, we present a case report of a 13-year-old girl with thyroid storm accompanied by sepsis-induced pancytopenia.

Case Report

A 13-year-old girl was diagnosed with Graves' disease and started methimazole (7.5 mg twice daily) five weeks earlier (day -35). Thyroid function showed improvement after two weeks of methimazole therapy. Table 1 shows laboratory results at the different evaluation points.

The patient developed fever 17 days after methimazole therapy, and complete blood cell count (CBC) and C-reactive protein (CRP) were normal on the first day of fever, so methimazole treatment was continued. The patient still had a fever five days later, and a review of laboratory results revealed improved thyroid function, normal CBC and liver function, but CRP was elevated. Therefore, methimazole therapy was continued and oral antibiotics were prescribed. However, the patient had a recurrent and intermittent fever, and she discontinued the use of methimazole on her own on day 11 of fever. Two days after methimazole withdrawal, she developed recurrent high fever, accompanied by sore throat and hoarseness. She was hospitalized with sepsis and pancytopenia in the local hospital. Due to no improvement in her symptoms, she was transferred to our paediatric intensive care unit (PICU) for suspected thyroid storm.

On arrival at the PICU, the patient was febrile (temperature 38.5 °C), tachycardic (heart rate 140 beats/min), tachypneic (respiratory rate 30/min), had a blood pressure of 126/72 mmHg and oxygen saturation of 98% on room air. Her weight was 30 kg. On physical examination, a neck mass or swelling was obvious, tender to palpation with multiple palpable lymph nodes, making it difficult to distinguish the thyroid from other structures, such as lymphatic tissue. On

Table 1. Laboratory profile from the patient at the different evaluation points

Test	Day -35	Day -21	Day 1	Day 5	Day 16	Day 17 (on admission)	Day 19	Day 22	Day 45 (on discharge)	Day 75
CBC										
WBC (4.1-11 × 10^9/L)	5.5	-	6.5	6.0	0.3	0.55	2.43	6.99	10.77	5.3
Neutrophils (1.8-8.3 × 10^9/L)	6.1	-	3.9	4.8	0.0	0.02	1.25	3.72	7	3.3
RBC (3.8-5.1 × 10^{12}/L)	4.7	-	4.38	4.44	4.28	3.42	3.39	3.52	3.37	4.13
Hemoglobin (115-150 g/L)	125	-	118	120	106	84	86	88	90	126
Platelets (125-350 × 10^9/L)	318	-	276	349	91	34	144	378	384	329
Liver function										
ALT (7-40 U/L)	26	-		17	31	11	44	35	13	10
AST (13-35 U/L)	19	-		22	23	8	16	13	16	16
Albumin (40-55 g/L)	51	-	-	47.4	23.6	23	28	39	38	50
Total bilirubin (0-21 μmol/L)	11.7	-	-	17.6	70	138.1	53.4	33.2	20.6	12.3
Direct bilirubin (0-8 μmol/L)	4.2	-	-	6.4	31	124.1	43.4	21.8	12.7	5.1
Thyroid function										
TSH (0.51-4.3 mIU/L)	< 0.01	< 0.01	-	< 0.01	< 0.01	< 0.01	< 0.01	-	6.68	2.49
Free T4 (12.6-21 ρmol/L)	64.1	41.9	-	33.2	52	55.1	24	-	8.99	14.4
Free T3 (3.93-7.7 ρmol/L)	42.0	18.7	-	11.4	5.7	8.19	3.96	-	4.92	6.31
TRAb (0-1.75 IU/mL)	17.24	-		-	13.1	-	-	-	-	-
Infection index										
CRP (0-10 mg/L)	-	-	8.49	67.9	> 200	156.7	54	13.5	6.89	0.58
PCT (< 0.046 ng/mL)	-	-	-	-	87.9	70.55	17.41	0.18	-	-

Reference ranges are given in brackets.
CBC: complete blood count, WBC: white blood cells, RBC: red blood cells, ALT: alanine aminotransferase, AST: aspartate aminotransferase, TRAb: thyrotropin receptor antibodies, CRP: C-reactive protein, PCT: procalcitonin, TSH: thyroid-stimulating hormone

examination of the oropharynx, retropharyngeal abscess and tonsillar abscess were significant. The respiratory examination indicated transmitted rhonchi from the upper airways. The abdomen and neurological examination were normal.

Further initial laboratory assessment revealed a decrease in white blood cell count (0.55×10^9/L, normal range 4.1-11 $\times 10^9$/L) with only 0.02×10^9/L (normal range 1.8-8.3 $\times 10^9$/L) neutrophil count, as well as decreased haemoglobin concentration (84 g/L, normal range 115-150 g/L) and platelet count (34×10^9/L, normal range 125-350 $\times 10^9$/L). A peripheral blood smear confirmed pancytopenia with lymphocytosis (lymphocytes 80%). Thyroid function evaluation showed severe thyrotoxicosis (thyroid-stimulating hormone < 0.01 mIU/L, normal range 0.51-4.3 mIU/L; fT3 8.19 ρmol/L, normal range 3.93-7.7 ρmol/L; and fT4 55.1 ρmol/L, normal range 12.6-21 ρmol/L). Liver function evaluation showed normal alanine aminotransferase and aspartate aminotransferase but hypoalbuminemia (23 g/L, normal range 40-55 g/L) and markedly increased total bilirubin (138.1 μmol/L, normal range 0-21 μmol/L), in which direct bilirubin corresponded to 124.1 μmol/L (normal range 0-8 μmol/L). The infection index indicated significant increases in CRP (156.7 mg/L, normal range 0-10 mg/L) and procalcitonin (70.55 ng/mL, normal range < 0.046 ng/mL). Renal function tests, coagulation function, and electrolytes were normal. A pharyngeal throat swab was positive for *Aeromonas caviae*. Blood culture, serum cytomegalovirus DNA, Epstein-Barr virus DNA, mycoplasma pneumoniae antibody, and antibody profiles for autoimmune diseases were negative. An electrocardiogram revealed sinus tachycardia. Normal echocardiographic values were recorded with an ejection fraction of 64%. Chest X-ray was normal. Magnetic resonance imaging of the neck revealed suppurative cervical lymphadenitis with abscess formation (measuring 34 × 23 × 38 mm), accompanied by inflammatory infiltrates in the bilateral parapharyngeal, retropharyngeal and cervical fascial space.

Following transfer to the PICU, the patient was treated with intravenous meropenem (0.6 g every 8 hours), oral metoprolol (10 mg every 8 hours), oral acetaminophen (0.3 g when body temperature higher than 38.5 degrees Celsius), and supportive care in the form of fluid and electrolyte replacement. In addition, an evaluation by a multidisciplinary team involving endocrinology, haematology, infectious diseases, and surgery was requested. The initial diagnosis was thyroid storm. Pancytopenia due to sepsis was first considered and discussed from a multidisciplinary standpoint. As a result methimazole treatment was restarted (7.5 mg twice daily). To rule out haematological system diseases, bone marrow aspiration was performed and a high-dose corticosteroid was used (methylprednisolone 50 mg intravenous every 8 hours). During the first three days of methimazole therapy in the PICU, CBC was monitored daily. The clinical status of the patient improved rapidly, she became afebrile as early as 24 hours after initial therapy, and her CBC count elevated after 48 hours. Neck abscess was operated on for incision and drainage, and approximately 50 mL of purulent blood material were drained from the right side and 10 mL from the left. Bone marrow tests revealed decreased granulocytes with maturation disorder and clearly showed histiocytes and phagocytes with occasional haemophagocytic cells. Further immunophenotyping was performed with normal results. Both granulocyte and platelet counts were normal after five days of therapy (Figure 1). Figure 1 depicts variation of CBC before and after thyroid storm. Methylprednisolone therapy was tapered over the course of one week. Bone marrow aspiration was reviewed two weeks later and showed obvious hypercellular marrow and granulocytes, of which 64% had toxic granulations. The patient remained afebrile throughout her hospital stay, and she was discharged after four weeks of hospitalization and prescribed 7.5 mg methimazole per day. Thyroid function, CBC, and liver function tests were checked 10 days after discharge, and methimazole was weaned to 6.6 mg daily. These indicators were then rechecked every 2 weeks thereafter, and methimazole was weaned to 5 mg daily about 1 month after discharge, CBC and liver function tests were all within the normal range.

Discussion

Thyroid storm is a rare but life-threatening endocrine emergency with a mortality rate of up to 22% (3), which is mainly triggered by precipitating factors such as discontinuation of antithyroid drug therapy for Graves' disease and infection (4). In the presented case, uncontrolled thyrotoxicosis appears to have been precipitated by infection characterized by intermittent fever and elevated CRP, subsequently followed by abrupt withdrawal of methimazole therapy. Therefore, the onset of thyroid storm was not a surprise. Similarly, in the case of thyroid storm with extreme metabolic disorder, the infection cannot be controlled and progresses to sepsis. Early diagnosis of thyroid storm is challenging due to the lack of a global "gold standard" diagnostic test and because the associated multisystem involvement can mimic many other conditions. The diagnosis is largely based on clinical assessment. In the last 20 years, the most commonly used diagnostic criterion has been the Burch-Wartofsky Point Scale (BWPS), in which

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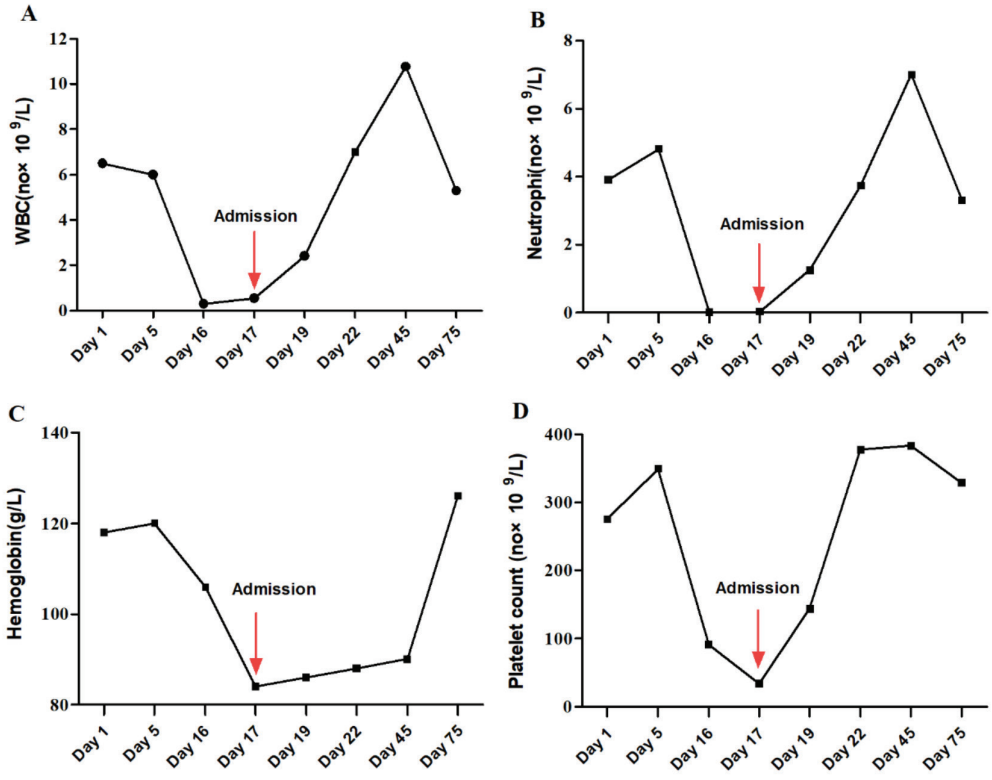

Figure 1. Chronological variation of CBC count during the period of fever. White blood cells (A), neutrophils (B), haemoglobin (C), and platelets (D)

CBC: complete blood count, WBC: white blood cells

a score of 45 or more is highly suggestive of thyroid storm. New peer-reviewed diagnostic criteria for thyroid storm were proposed by the Japan Thyroid Association (JTA) in 2012 (5), in which the grade of TS1 indicates definite thyroid storm. Although it is not specific to paediatrics, the BWPS or the JTA criteria for thyroid storm can be used (6). The presented patient met the criterion for definite thyroid storm under both diagnostic schemes. The BWPS score was 90 (temperature ≥39.5 °C, agitation, jaundice, heart rate ≥140 bpm, positive precipitant history), and the grade of TS1 was determined by the JTA criteria (thyrotoxicosis, central nervous system manifestation of agitation, fever, tachycardia, total bilirubin level ≥3.0 mg/dL).

Pancytopenia is a rare but severe complication of Graves' disease (7) and is an extremely rare adverse reaction to antithyroid drug therapy (2). Based on the results of previous studies, pancytopenia in patients with Graves' disease has been reported to be caused either by Graves' disease itself or by methimazole therapy (7,8,9,10), but the therapeutic principles are totally different. If patients with Graves' disease have pancytopenia before methimazole treatment, then pancytopenia is considered to be due to Graves' disease itself, and antithyroid drugs can be used to treat both pancytopenia and Graves' hyperthyroidism with close monitoring of CBC counts (7,11). In contrast, if patients with Graves' disease present with pancytopenia during methimazole treatment, then pancytopenia is highly suspected to be due to the antithyroid drugs, and methimazole treatment must be discontinued immediately because sepsis induced by methimazole therapy with pancytopenia is often fatal. Alternatively, treatments such as total thyroidectomy or radioactive iodine therapy should be considered as first-line therapy (12).

Similar to the symptoms in a previously reported case (8), the presented patient was diagnosed with methimazole-induced agranulocytosis and had neck abscess. Therefore, methimazole-induced pancytopenia is a possible diagnosis in our case as well, and the diagnosis of sepsis-induced pancytopenia in our patient can be challenged. Moreover, according to the American Thyroid Association guidelines, antithyroid drugs should be immediately discontinued if the granulocyte count is less than 1000 cells/mm^3 (13). Therefore, the question of whether to start therapy with methimazole was debatable. Pancytopenia is a relatively common phenomenon encountered in clinical practice, and numerous aetiologies may determine pancytopenia (14).

Identifying the true pathogenesis is crucial for implementing an appropriate therapy. By reviewing the entire case, we found that the CBC level in our patient was checked multiple times before pancytopenia occurred, with two normal CBC results during the period of fever before the thyroid storm. If methimazole-induced pancytopenia was the diagnosis, the neutrophil count must have been decreased whenever fever occurs. Consequently, combined with a healthy past medical history and normal bone marrow results, the diagnosis of sepsis-induced pancytopenia with thyroid storm can be confirmed.

Conclusion

Our case serves to remind physicians that close monitoring of CBC counts may help avoid mistaking sepsis-induced pancytopenia for the side effects of antithyroid drugs. This is the first paediatric case report that outlines the use of methimazole in the management of thyroid storm with pancytopenia.

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Ethics

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

Authorship Contributions

Surgical and Medical Practices: Qing-Xian Fu, Chao-Chun Zou, Hui Liu, Concept: Qing Zhou, Design: Qing Zhou, Qing-Xian Fu, Chao-Chun Zou, Hui Liu, Data Collection or Processing: Qing Zhou, Li-Yong Zhang, Analysis or Interpretation: Qing Zhou, Li-Yong Zhang, Literature Search: Qing Zhou, Hui Liu, Writing: Qing Zhou, Qing Zhou, Qing-Xian Fu, Chao-Chun Zou, Hui Liu.

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CASE REPORT

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Clinical Presentation and Genetic Analysis of Neonatal 11β-Hydroxylase Deficiency Induced by a Chimeric *CYP11B2*/*CYP11B1* Gene

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What is already known on this topic?

11β-hydroxylase deficiency (11β-OHD) is an autosomal recessive disorder caused by genetic variations in the *CYP11B1* gene. Most cases of 11β-OHD are caused by single nucleotide variations or small insertions/deletions in the *CYP11B1* gene, but cases resulting from chimeric *CYP11B2/CYP11B1* genes are rare.

What this study adds?

This study presents a rare case of neonatal 11β-OHD induced by a chimeric *CYP11B2/CYP11B1* gene. This case highlights the importance of considering gene fusion variants in the diagnosis of 11β-OHD, particularly in neonatal and early infantile cases.

Abstract

In terms of prevalence, 11β-hydroxylase deficiency (11β-OHD), a common form of congenital adrenal hyperplasia, closely follows 21-hydroxylase deficiency. 11β-OHD has been attributed to diminished enzymatic activity owing to *CYP11B1* gene variants, mainly encompassing single nucleotide variations and insertions-deletions. The involvement of chimeric *CYP11B2/CYP11B1* genes in 11β-OHD has rarely been reported. We conducted a genetic investigation on a male infant with generalized pigmentation and abnormal steroid hormone levels. Whole-exome sequencing revealed a heterozygous variant in *CYP11B1* inherited from the mother (NM_000497.4: c.1391_1393dup [p.Leu464dup]). Long-range polymerase chain reaction revealed an additional allele, a chimeric *CYP11B2/CYP11B1* gene, inherited from the father. The current case report highlights the need to consider the occurrence of gene fusion variants in the diagnosis of neonatal or early infantile 11β-OHD.

Keywords: 11β-hydroxylase deficiency, 11β-OHD, *CYP11B1*, chimeric gene

Introduction

11β-hydroxylase deficiency (11β-OHD) is an autosomal recessive hereditary disorder caused by genetic variations in the *CYP11B1* gene, accounting for approximately 5-8% of congenital adrenal hyperplasia (CAH) cases (1). After 21-hydroxylase deficiency, 11β-OHD is the second leading cause of CAH (2). The primary features of 11β-OHD include reduced synthesis of metabolic end products and accumulation of precursor substances. Patients commonly exhibit symptoms, such as low-renin hypertension, hypokalemia, masculinized early puberty due to elevated

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androgen levels, and pseudohermaphroditism in female patients (3).

11β-Hydroxylase belongs to the cytochrome P450 enzyme family, with two isoforms encoded by *CYP11B1* and *CYP11B2* in humans. Given the high sequence homology between *CYP11B1* and *CYP11B2*, with approximately 95 and 97% identity in their coding and non-coding regions, respectively, there is a risk of recombination and fusion during mitosis (4,5). The chimeric *CYP11B2*/*CYP11B1* gene has been associated with familial hyperaldosteronism type I (HALD1, OMIM#103900), also known as glucocorticoid-remediable aldosteronism, predominantly characterized by clinical features of hypertension and hypokalemia (6). However, cases of 11β-OHD resulting from a chimeric *CYP11B2*/*CYP11B1* gene remain rare. Typically, patients with 11β-OHD exhibit single nucleotide variations (SNVs) and insertions-deletions (indels) in *CYP11B1*. The primary variant types are missense and loss-of-function variants, both of which can lead to reduced 11β-hydroxylase enzyme activity and disease onset (7,8).

Herein, we report an intriguing case of an infant who presented with widespread skin pigmentation shortly after birth, particularly prominent around the penis and nipples. Genetic testing revealed a frameshift variant in one allele of *CYP11B1*, with the other allele harboring a chimeric *CYP11B2*/*CYP11B1* gene. This case report suggests that the possibility of the chimeric *CYP11B2*/*CYP11B1* gene should be considered as the underlying cause of CAH when clinically suspected.

The patient was from a non-consanguineous family. Informed consent was obtained from the infant's parents, and all research procedures were approved by the Medical Ethics Committee of the Anhui Children's Hospital (ID: EYLL-2018-020).

Whole-exome Sequencing (WES) and Sanger Sequencing

Genomic DNA was extracted from blood samples using a blood DNA extraction kit (TianGen, Beijing, China). DNA quality was assessed for optimal 260/280 ratios (1.6-2.0) and a total yield exceeding 1 μg. Targeted exonic sequences were captured using the xGen Exome Research Panel (Integrated DNA Technologies, Rockville, MD, USA), followed by high-throughput sequencing on an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA). Raw sequencing data underwent quality control, alignment to the human genome reference build 19 via BWA software, and identification of SNVs and indels using the Genome Analysis Toolkit. Suspected variants were assessed for population frequency in gnomAD_ALL/EAS, with their pathogenicity predicted using various bioinformatics algorithms (SIFT, Polyphen2, MutationTaster). Variant pathogenicity was assessed following American College of Medical Genetics and Genomics (ACMG) guidelines (9). Suspected variants were validated by designing primers from Ensembl data (Table 1) and conducting Sanger sequencing on an ABI 3500XL Genetic Analyzer (Applied Biosystems, CA, USA).

Long-range Polymerase Chain Reaction (L-PCR) and Fusion Variant Verification

L-PCR was used to detect fusion genes in the patient and his parents, with normal samples serving as negative controls. The LA Taq Hot-Start Version kit (#RR042B, TaKaRa, Kusatsu, Japan) was used for PCR. Gel electrophoresis bands were examined to ascertain the formation of the chimeric *CYP11B2*/*CYP11B1* gene resulting from homologous recombination. Each reaction contained 100 ng of genomic DNA. Following PCR, the products were subjected to 1% agarose gel electrophoresis. Analysis was conducted on a gel imaging system (#1600; Tanon, Shanghai, China).

Validation was performed using a human *CYP11B1*/*CYP11B2* gene detection kit (WeHealth BioMedical, Shanghai, China). Initially, target libraries of *CYP11B1* and *CYP11B2* gene fragments were constructed from sample DNA. Following successful library quality assessment, the NovaSeq 6000 sequencing platform (Illumina) was employed. Subsequently, the relative depth of the differential sites on *CYP11B1* and *CYP11B2* was computed to discern *CYP11B1*/*CYP11B2* gene fusion outcomes.

Case Report

This case report involves a 2-month-old male infant (46, XY karyotype). He was the firstborn of his mother and was delivered vaginally at 37^{+2} weeks of gestation, with a birth weight of 2900 g and an unremarkable pregnancy history. Shortly after birth, the infant developed generalized skin pigmentation, prominent around the areola and penis. His blood pressure was 104/67 mmHg (reference values for individuals under 3 years of age not exceeding 100/60 mmHg). Auxiliary examinations revealed an elevated aldosterone level of 175.90 pg/mL (reference range: 12.00-170.80) and higher-than-normal levels of both supine angiotensin and renin activity. Measurement of steroid hormones revealed elevated levels of 11-deoxycortisol, dehydroepiandrosterone, and androstenedione, which

Table 1. Primer sequences for Sanger sequencing

Variant*	Primers
Forward	5'-CTCTACTCTCTGGGTCGCAACC-3'
Reverse	5'-CAGGACCTACACAGCCTCAACC-3'

**CYP11B1* NM_000497.4: exon8:c.1391_1393dup

exceeded reference ranges (Table 2). The patient's 24-year-old father presented with facial hemiparesis and a history of hypertension during adolescence, albeit without treatment. At the time of examination, his blood pressure was 165/110 mmHg, without additional auxiliary investigations. The patient's mother did not exhibit notable anomalies. The couple denied a consanguineous relationship.

WES Results

A total of 54.9 million clean reads were obtained in the WES testing, with an average sequencing depth of 127.2X. The average coverage of target regions with a depth greater than 20X was 99.20%. Based on the WES analysis results, the patient had a heterozygous variation in *CYP11B1*; this variant was identified as NM_000497.4: c.1391_1393dup (p.Leu464dup). The presence of this variant in the patient's mother was confirmed using Sanger sequencing (Figure 1). The distribution frequency of this variant in gnomAD_ALL/EAS was 0.000007/0, indicating an extremely low occurrence rate. Moreover, the identified variant site has been previously reported to be deleterious to enzymatic activity (10,11). Following the ACMG guidelines, this variant was classified as likely pathogenic based on the following criteria: PM3 + PS3 + PM2 + PP4. Within the target sequence, no SNV/Indel variations on the other allele of *CYP11B1* were identified. Additionally, we did not identify any pathogenic variants in other genes associated with adrenal insufficiency.

Table 2. Auxiliary examination data of the proband

Parameter	Measurement	Reference
Aldosterone	175.90 pg/mL	12.00-170.80 pg/mL
Angiotensin 1	6.47 ng/mL	11.00-88.00 ng/L
Angiotensin 2 (recumbent)	72.40 pg/mL	25.00-60.00 pg/mL
Renin activity (supine)	5.03 ng/(mL*h)	0.15-2.33 ng/(mL*h)
11-deoxycorticosterone	3.164 ng/mL	0.070-0.570 ng/mL
Dehydroepiandrosterone	6.020 ng/mL	<2.900 ng/mL
Androstenedione	1.937 ng/mL	0.060-0.780 ng/mL
Serum potassium	5.80 mmol/L	3.50-5.50 mmol/L
Serum sodium	137.6 mmol/L	135.0-145.0 mmol/L

CYP11B1 and *CYP11B2* Gene Fusion Variant

The L-PCR results unequivocally confirmed the occurrence of a chimeric *CYP11B2*/*CYP11B1* gene through homologous recombination between *CYP11B1* and *CYP11B2*, constituting a paternally inherited pathogenic variation. Using the *CYP11B1*/*CYP11B2* gene detection kit, we performed a copy number analysis of the *CYP11B2*/*CYP11B1* genes within the sample DNA. Subsequently, we found that the proband exhibited a relative copy number of 1 for exons 1-6 of *CYP11B1*, indicative of a heterozygous deletion. Similarly, a relative copy number of 1 was observed for exons 7-9 of *CYP11B2*, indicating a heterozygous deletion. The proband's father exhibited the same copy number of deletion variants. Conversely, the mother's copy numbers of both *CYP11B1* and *CYP11B2* were within normal limits (Figure 2).

Discussion

Herein, we report the case of a neonate with 11β-OHD, primarily presenting with genital skin pigmentation and

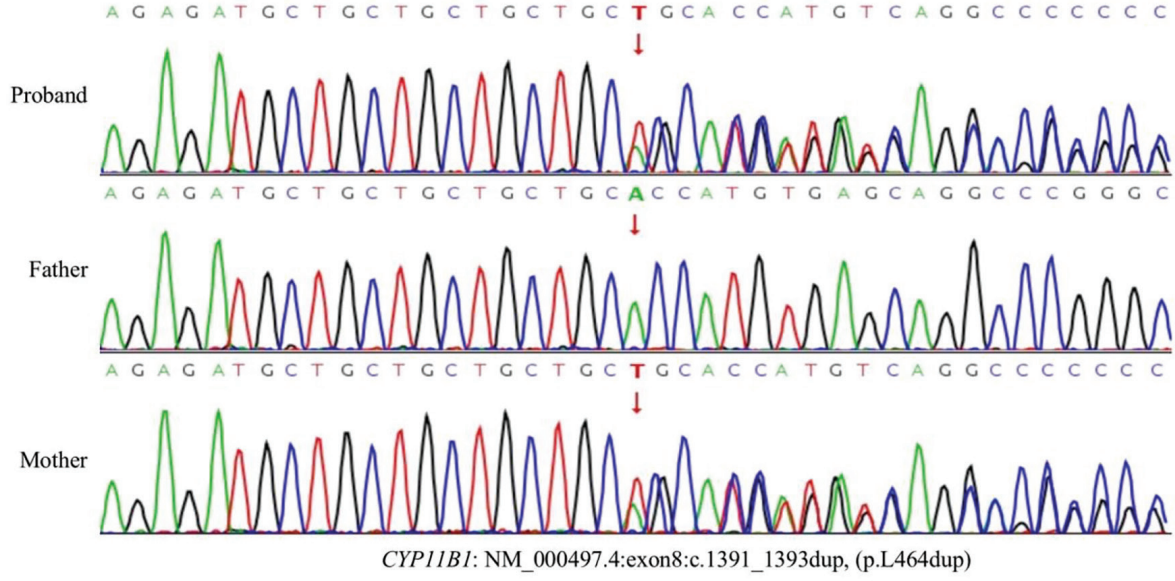

Figure 1. Sanger sequencing results showing a heterozygous variation in the patient's *CYP11B1* (NM_000497.4: c.1391_1393dup [p.Leu464dup]), which was inherited from his mother

hypertension phenotype. WES analysis revealed only one heterozygous variation in *CYP11B1* inherited from the mother, identified as c.1391_1393dup (p.Leu464dup). The other allele originated from the father and constituted a chimeric *CYP11B2/CYP11B1* gene, resulting in a compound heterozygous variation in *CYP11B1*. The diagnosis of 11β-OHD was established based on the patient's clinical presentation. Although routine next-generation sequencing can be effectively employed to identify SNVs/indels in the field of pediatric genetics, the detection of copy number variations or fusion variants in *CYP11B1* and *CYP11B2* presents a substantial challenge (12).

Distinct approaches have been employed to recognize chimeric *CYP11B2/CYP11B1* genes differently. Methods include customized probes for multiplex ligation-dependent

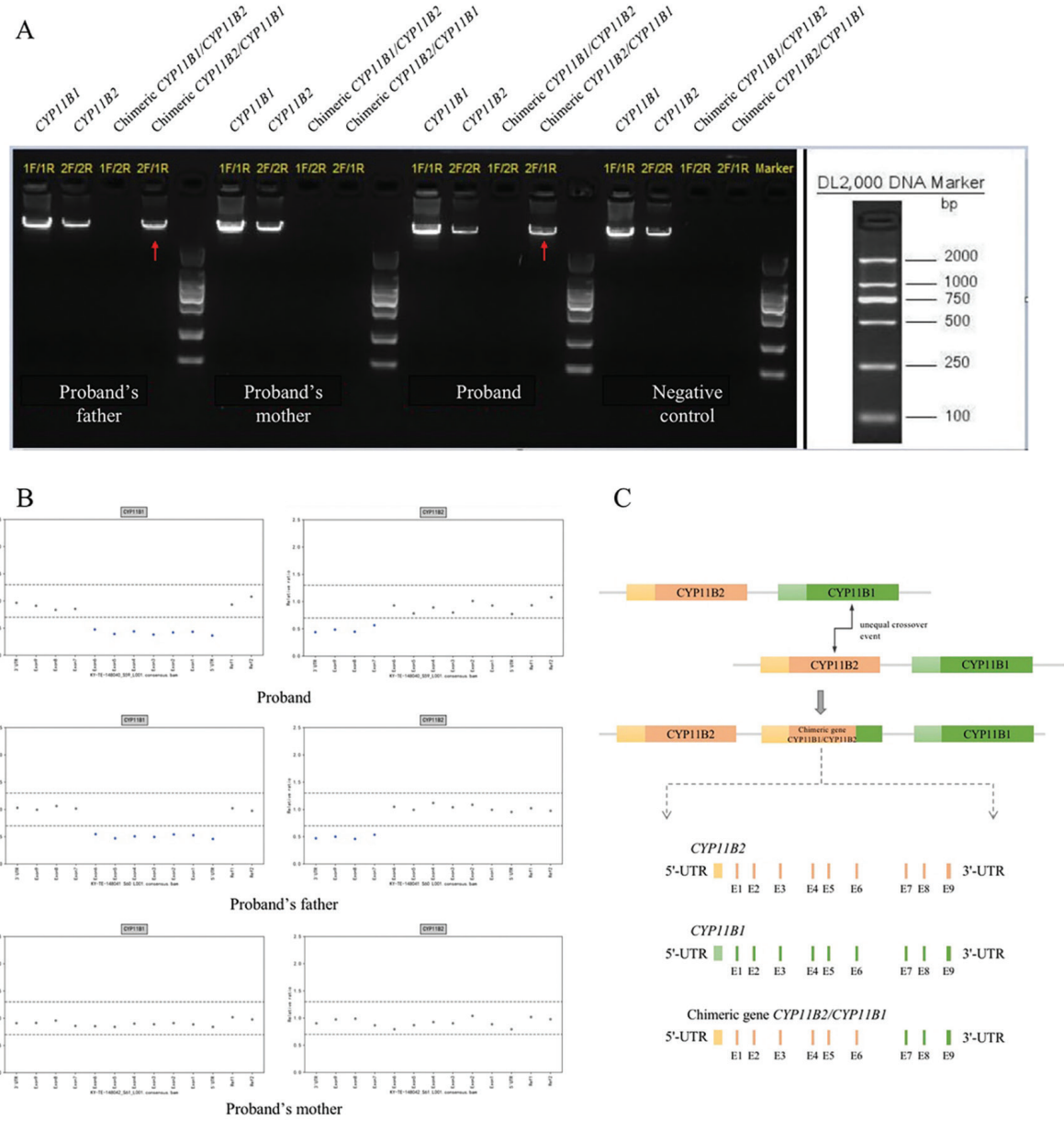

Figure 2. Validation of the chimeric *CYP11B2/CYP11B1* gene. A) Long-range polymerase chain reaction results showing homologous recombination between *CYP11B1* and *CYP11B2* in the patient and his father, generating a chimeric *CYP11B2/CYP11B1* gene (indicated by red arrows). B) The patient exhibits heterozygous deletions in exons 1-6 of *CYP11B1* (blue dots) and in exons 7-9 of *CYP11B2* (blue dots). The patient's father carries the same copy number variation as the patient, while the mother shows no variations. Dashed lines represent the upper (1.3) and lower (0.7) limits of normal copy numbers. Copy numbers near 1 denote normal copy numbers (gray dots), while copy numbers near 0.5 indicate heterozygous deletion variations (blue dots). C) Schematic representation of the chimeric *CYP11B2/CYP11B1* gene occurrence in the patient

probe amplification by Menabò et al. (13), specific real-time PCR by MacKenzie et al. (14), and optimized targeted sequencing algorithms by Xie et al. (11), and all aimed at enhancing the efficiency of chimeric *CYP11B2*/*CYP11B1* gene identification. However, these methods lack broad applicability. In the current case, the causative variant was identified using L-PCR and a dedicated kit. This could be partly attributed to the precise phenotypic assessment of the patient in a clinical setting. Given the strong clinical suspicion of CAH and recognition of a high sequence homology between *CYP11B1* and *CYP11B2*, additional verification of the chimeric *CYP11B2*/*CYP11B1* gene was performed, thereby establishing the patient's etiological diagnosis.

CYP11B1 variations are complex, with over 150 variants documented in the Human Gene Mutation Database (https://www.hgmd.cf.ac.uk/). These variants include missense, non-sense, splicing, and small indel variants. However, reports on chimeric *CYP11B2*/*CYP11B1* genes are relatively scarce. To date, only 11 cases of this variation have been reported (4,5,11,15,16,17,18), with the *CYP11B1* exon 7-9/*CYP11B2* exon 1-6 configuration commonly documented (4,5,11,15,16,17,18). Notably, Xie et al. (11) have identified four unrelated Chinese patients who shared the same breakpoint (*CYP11B2* g.9559-9742) in their chimeric *CYP11B2*/*CYP11B1* genes, thereby suggesting a potential founder effect in the Chinese population, with a possible frequency of 1 in 10,000. Thus, the distribution frequency of the chimeric *CYP11B2*/*CYP11B1* gene in the Chinese population might be higher than that predicted. Nevertheless, chimeric *CYP11B2*/*CYP11B1* genes remain underreported, possibly reflecting an oversight in their verification.

CYP11B1 is located in the chromosomal region 8q22 and comprises nine exons, spanning a length of 6.03 kb and encoding 503 amino acids. Within the zona fasciculata of the adrenal cortex, 11β-hydroxylase, encoded by *CYP11B1*, converts 11-deoxycortisol and 11-deoxycorticosterone into cortisol and corticosterone, respectively (1). In patients with 11β-OHD, reduced cortisol levels lead to feedback elevation of adrenocorticotropic hormone levels, resulting in increased adrenal androgen production; this can manifest as peripheral precocious puberty in males and virilization in females. Additionally, enhanced synthesis of corticosterone, a weak mineralocorticoid, can induce clinical manifestations, such as hypokalemia and hypertension (19). Classical phenotypes frequently include virilization in females and enlarged genitalia in males. However, genital and hypertensive features might not be prominent in some infantile patients, potentially resulting in a misdiagnosis (20). The current case report revealed certain clinical indications, primarily suggesting a potential disease risk related to pigmentation surrounding the infant's genitalia. Notably, the patient's aldosterone and renin levels were slightly above the upper limits of the reference range, which may be related to varying degrees of elevated levels in newborns or during the early stages of infants (21,22). Therefore, for patients in the early stages of life, an increased awareness of molecular diagnostic methods is crucial, highlighting the importance of selecting an appropriate molecular diagnostic approach.

Patients with 11β-OHD mainly require lifelong glucocorticoid replacement therapy. Importantly, early and accurate diagnosis, followed by glucocorticoid treatment, can prevent an Addisonian crisis and hypertension symptoms (23). Dexamethasone therapy should be avoided in the neonatal or pediatric period despite its stronger sodium-retaining properties than those of hydrocortisone. Dexamethasone potently suppresses the pituitary-adrenal axis, which can lead to growth retardation in neonates and children (24). In affected children, low-dose hydrocortisone could help achieve biochemical control within the normal range. During long-term treatment, combining low-dose hydrocortisone with gonadotropin-releasing hormone agonists and growth hormone may be considered to optimize final adult height recovery (25,26). However, the intricate nature of 11β-OHD demands a refinement of standard treatment protocols for pediatric patients to mitigate potential glucocorticoid-related morbidity.

Conclusion

In conclusion, we report a case of a male infant who exhibited generalized pigmentation centered on the genitals and nipples since birth. Using WES, we initially identified a heterozygous variation in *CYP11B1* inherited from the mother, subsequently verifying the presence of a chimeric *CYP11B2*/*CYP11B1* gene originating from the father. The current case report highlights the potential for atypical presentation and misdiagnosis of infantile 11β-OHD, underscoring the critical role of careful molecular diagnosis in such cases.

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Ethics

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

Authorship Contributions

Surgical and Medical Practices: Wenjuan Cai, Jian Gao, Concept: Wenjuan Cai, Jian Gao, Qian Deng, Yuqing Chen, Design: Wenjuan Cai, Dan Yu, Huihui Lin, Yuqing Chen, Data Collection or Processing: Dan Yu, Jian Gao, Qian Deng, Huihui Lin, Yuqing Chen, Analysis or Interpretation: Dan Yu, Literature Search: Qian Deng, Writing: Wenjuan Cai, Dan Yu, Huihui Lin, Yuqing Chen.

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