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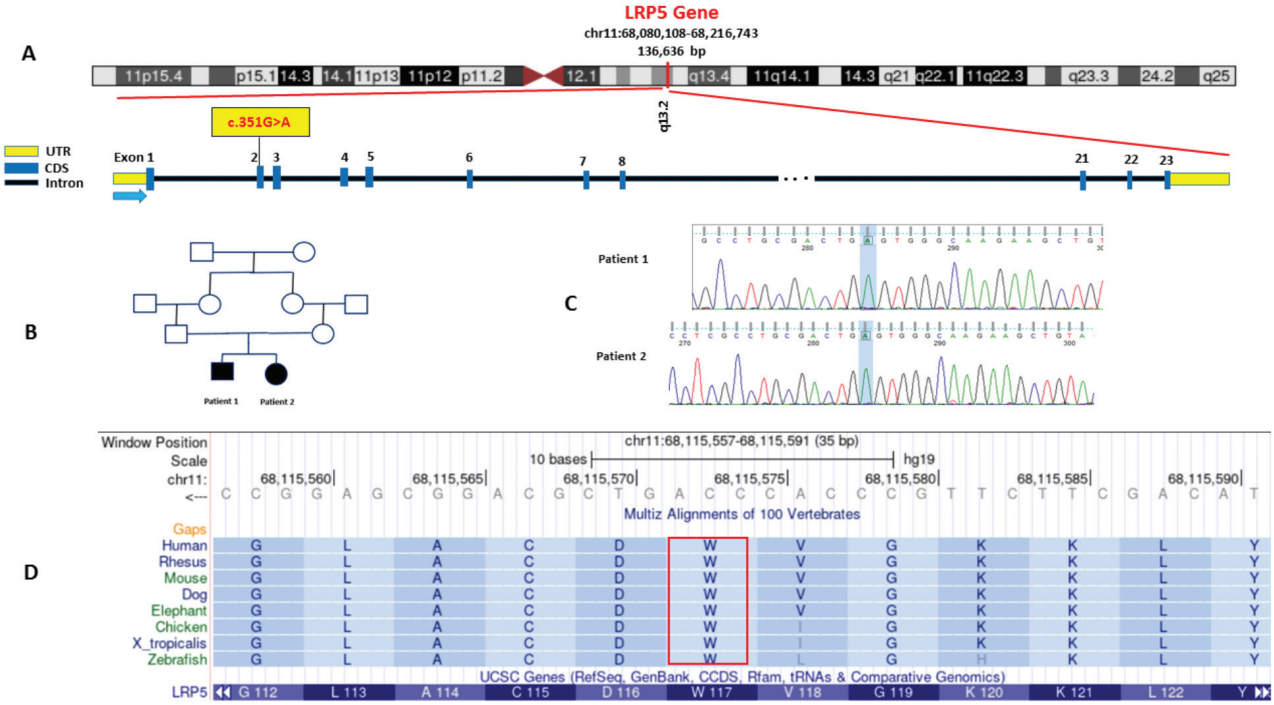

Novel Homozygous Nonsense Mutation in the *LRP5* Gene in Two Siblings with Osteoporosis-pseudoglioma Syndrome

Heidari A et al.

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ORIGINAL ARTICLE

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J Clin Res Pediatr Endocrinol 2023;15(3):230-237

Evaluation of Abnormal Uterine Bleeding in Adolescents: Single Center Experience

Sirmen Kızılcan Çetin[1], Zehra Aycan[1,2], Elif Özsu[1], Zeynep Şıklar[1], Ayşegül Ceran[1], Seda Erişen Karaca[1], Gizem Şenyazar[1], Merih Berberoğlu[1]

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What is already known on this topic?

Anormal uterine bleeding (AUB) is the most common cause of gynecological problems in adolescence. Since the adolescent age group is a transitional age group, both obstetricians and pediatricians have a role in clinical management. There is no guideline for the management of adolescent AUB. However, heavy bleeding requires urgent treatment.

What this study adds?

Diagnostic and management differences were identified between girls with and without heavy menstrual bleeding. Despite excluding patients with a previously diagnosed bleeding disorder, the frequency of factor VII deficiency was around 4%. Although *MTHFR* mutation frequency was 50% in girls with a history suggestive of thrombosis, it did not appear to increase the risk of bleeding/thrombosis and we suggest routine evaluation is unjustified.

Abstract

Objective: Abnormal uterine bleeding (AUB) is the most common gynecologic complaint in adolescent girls. The aim of this study was to identify the diagnostic and management differences between those with/without heavy menstrual bleeding.

Methods: Retrospective data was collected from adolescents aged 10-19 years, diagnosed with AUB. Adolescents with known bleeding disorders at admission were excluded. All girls were classified according to the degree of anemia; group 1 had heavy bleeding [hemoglobin (Hb) <10 g/dL] and group 2 had moderate or mild bleeding (Hb >10 g/dL). Admission and follow-up characteristics were compared between the two groups.

Results: The cohort consisted of 79 girls with a mean age of 14.3 ± 1.8 years and mean age of menarche of 11.9 ± 1.4 years, with 85% experiencing menstrual irregularity in the two years after menarche, rising to 95.3% in group 1 ($p<0.01$). Anovulation was evident in 80% of the cohort. Of these 79 girls, 13 (16.5%) had polycystic ovary syndrome and two (2.5%) had structural anomalies (uterus didelphys). Three girls (group 1, $n=2$) had previously undiagnosed clotting factor VII deficiency; no other clotting deficiencies were diagnosed. Nineteen of 34 (56%) with personal ($n=2$)/family history of thrombosis had *MTHFR* mutation. None had venous thromboembolism during follow-up of >6 months.

Conclusion: The majority of AUB (85%) occurred in the first two years after menarche. A small proportion (3.8%) had undiagnosed clotting factor deficiency. The frequency of *MTHFR* mutation was 50% in girls with history of thrombosis; however this did not increase the risk of bleeding/thrombosis and so routine evaluation does not appear to be justified.

Keywords: Abnormal uterine bleeding, adolescents, anovulatory bleeding, heavy menstrual bleeding

Introduction

Abnormal uterine bleeding (AUB) is defined as bleeding from the uterine corpus that is abnormal in duration, volume, frequency and/or regularity. AUB affects 3-20% of women of reproductive age and is more common in adolescence (1). Thus, AUB is one of the most common gynecological presentations encountered in the adolescent age group

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(2,3,4). In 2011, the International Federation of Obstetrics and Gynecology (FIGO) defined AUB as menstrual bleedings that has a uterine origin, is unrelated to pregnancy, has been abnormal in the last six months in terms of amount, frequency, and/or duration), and is either irregular or regular but persists for more than eight days (3). The term "dysfunctional uterine bleeding" has been removed because the different terminologies were considered confusing and FIGO suggests only using the label AUB (5,6).

Each menstrual cycle of healthy adolescents usually has a bleeding duration every 21-45 days, lasting from 2-7 days, and the volume is about 30-40 mL (3-6 pads) per day (2,7,8). Menarche usually occurs between 12-13 years of age (2,9). Menstrual cycles are every 21-34 days by the third year after menarche, and is similar to adults in 60-80 % of adolescents (2). A change of pad/tampon in less than a few hours, use of double pads, frequent soiling of laundry or linens, and clots more than 2.5 cm in diameter are signs of AUB (10). FIGO has suggested the mnemonic abbreviation PALM-COEIN [Polyp, Adenomyosis, Leiomyoma, Malignancy & Hyperplasia (structural causes); Coagulopathy, Ovulatory dysfunction, Endometrial, Iatrogenic and Not yet classified (non-structural causes)] for the etiology of AUB (3). It should be noted that structural causes are very rare in adolescence, reported to account for around 1.3-1.7 % of AUB (11,12).

Adolescents may not be aware that their bleeding patterns are abnormal, since there may be irregularities in the menstrual cycle during the first two years following menarche and they may be unaware of their own personal regular cycle pattern. However, AUB and the factors that cause it can cause long-term health problems. AUB may affect physical, emotional, and social wellbeing and quality of life. It has been reported that AUB may possibly lead to absenteeism from full-time education and thus cause a decrease in academic achievement (13).

AUB is the most common cause of gynecological problems in adolescents and is a major source of stress for affected adolescents and their families. Although common, this situation is rarely reported. Frequent and heavy menstrual bleeding requires urgent management, and a correct treatment approach is important. Since the adolescent age group is transitional, both obstetricians and pediatricians are often involved in clinical management. However, there is no standard guideline for optimal clinical management. The aim of the present study was to determine the differences in diagnosis and management between those with and without heavy menstrual bleeding by evaluating AUB in a large case series of adolescent girls.

Methods

Study Population

This retrospective case series included all adolescents aged 10-19 years and diagnosed with AUB at our institution between 2016 and 2021. The study was approved by the Ethical Committee of the study Ankara University (approval number: I5-378-21, date: 25.06.2021).

The folowing data were retrospectively evaluated: demography, family history, complaints at presentation, characteristics of AUB, findings of examination at diagnosis and during follow-up, results of laboratory and radiological evaluation, treatment regimen, and responses to treatments. Adolescents with typical menstrual characteristics (frequency, duration, and bleeding intensity) and with previously diagnosed bleeding disorders were excluded.

Criteria for AUB are cycles which are: frequent (interval shorter than 21 days); or rare (longer than 45 days); or prolonged (longer than seven days); or heavy (more than 80 mL bleed). Heavy bleeding was defined as using more than six pads per day for more than seven days or bleeding that affected daily activity (1). We used the parameters shown in Table 1 to define AUB.

The initial evaluation was standardized since the approach used at the single management center follows a single protocol. Laboratory tests were evaluated to determine the severity of bleeding and the potential etiologies of heavy menstrual bleeding in all cases. The minimum laboratory evaluation included complete blood count, peripheral smear, ferritin level, prothrombin time, activated partial thromboplastin time, and fibrinogen values. Based on the family history and the results of the initial tests, other factor levels were investigated with priority.

In cases with small amounts of prolonged bleeding, human chorionic gonadotropin and pelvic ultrasound (USG) were performed to exclude pregnancy.

Findings of anemia were investigated, together with the duration of anemia by enquiring about history and through physical examination, hemogram, peripheral smear, and patients' previous blood examination results. Laboratory tests, such as serum iron, total iron binding capacity, and serum ferritin value were assessed. If the serum iron was decreased and the total iron binding capacity increased, a diagnosis of iron deficiency was made, and the other causes of microcytic anemia were excluded.

Anovulation was defined using the following criteria: 1) adolescents who had menstrual bleeding occurring more frequently than every 21 days or was excessive; 2) serum

progesterone under <0.5 ng/mL at diagnosis and/or 3) exclusion of other known causes of AUB. All subjects were also classified in terms of the degree of anemia. Group 1 consisted of adolescents with heavy and severe bleeding [hemoglobin (Hb) <10 g/dL]. The rest, with moderate and mild bleeding (Hb >10 g/dL) were included in group 2. Admission and follow-up characteristics between the two groups were compared.

AUB management was performed by following the the standard protocol. According to this protocol, the main clinical aim of management was to provide hemodynamic stability by correcting anemia until the etiology was identified. Anemia was classified as: mild Hb >12 g/dL; moderate 10-12 g/dL; heavy 8-10 g/dL; and severe Hb <8 g/dL. Patients with mild/moderate menstrual bleeding were treated with non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and sodium naproxen, to decrease bleeding flow. If the Hb was 10-12 g/dL, iron supplementation with 60 mg elemental iron per day was given. For adolescents with heavy bleeding (Hb 8-10 g/dL) and severe bleeding (Hb <8 g/dL), which means active bleeding, combined oral contraceptives (COCs) containing at least 30 mcg of ethinyl estradiol (E2) were given, starting with one pill every 8-12 hours until the menstrual bleeding ceased. Then COCs were continued, giving one pill daily until the Hb level increased to more than 10 g/dL for 3-6 months. Patients with Hb <7 g/dL and patients with Hb <10 g/dL who had active heavy bleeding and hemodynamic instability were consistently hospitalized. Treatment for this group consisted of erythrocyte suspension transfusion and COCs with 30-50 mcg ethinyl E2 once every six hours for 2-4 days, then once every eight hours for three days, and then the same amount every 12 hours for the next two days. When Hb level reached 10 g/dL, COCs were given in a cyclic pattern for three to six months until Hb level reached more than 10 g/dL. Supplementation of 60-120 mg elemental iron was given to all with Hb ≤10 g/dL. Progestin-only hormone therapy (medroxyprogesterone at 10 mg/day) was given any patient with a contraindication for estrogen therapy, such as thromboembolic disease, migraine with aura, or hepatic dysfunction. Additional antiemetic treatment was used when necessary. After initial management, patients were treated according to the underlying etiology of AUB.

Table 1. Definition of abnormal uterine bleeding*

Parameter	Definition
Frequency	
Infrequent menstrual bleeding	One or two episodes in a 90-day period
Frequent menstrual bleeding	More than four episodes in a 90-day period
Regularity	
Irregular menstrual bleeding	Variation more than 20 days over a period of a year
Amenorrhea	No bleeding in a 90-day period
Amount of flow	
Heavy menstrual bleeding	Excessive blood loss which effects the woman's quality of life
Heavy and prolonged menstrual bleeding	Excessive blood loss exceeding eight days
Light menstrual bleeding	Bleeding less than 5 mL in a period
Duration of flow	
Prolonged menstrual bleeding	Menstrual periods that exceed eight days on a regular basis
Shortened menstrual bleeding	Menstrual bleeding lasting less than two days
Density of menstrual bleeding according to Hb level	
Severe (dense menstrual bleeding)	≤8 g/dL
Heavy (dense menstrual bleeding)	8-10 g/dL
Moderate	10-12 g/dL
Mild	≥12 g/dL

*The International Federation of Gynecology and Obstetrics Classification System (2,3).
Hb: hemoglobin

Statistical Analysis

All statistical analyses were performed using Statistical Package for the Social Sciences, version 21.0 (IBM Corp., Armonk, NY, USA). Data were expressed as the mean ± standard deviation (SD), median (minimum; maximum). The Kolmogorov-Smirnov test was used to evaluate the normality of variables. Descriptive analyses are presented using means ± SD for normally distributed variables. The Student's t-test was used to compare the mean values of continuous variables. The Mann-Whitney U test with appropriate confidence intervals compared nonparametric measurements. A p value <0.05 was assumed to indicate statistically significant result in all analyses.

Results

This study included 79 adolescents. The overall mean age was 14.3 ± 1.8 (10.7-17.9) years, and the age at menarche was 11.9 ± 1.4 (9.5-15.9) years. Group 1 was older than group 2 (p = 0.02). While 64.6% had normal weight, 22.8% were obese, and 11.4% were underweight. Menstrual irregularity in the first two years after menarche was reported by 85%, while this proportion increased to 95% in group 1 had irregular bleeding in the two years (p < 0.01). The most common presenting symptoms were

dizziness (n = 21, 27%) and syncope (n = 11, 14%). There was no history of anorexia and bulimia nervosa in any girl. In terms of severity of bleeding based on systemic Hb concentration, 32% had dense bleeding, and all had Hb < 10 g/dL. While 48% had frequent bleeding of whom 32% were in group 1, and the rest were in group 2. Taking both groups together, anovulation (80%) was the main reason for AUB. While 13 girls (16%) were diagnosed with polycystic ovary syndrome (PCOS), two adolescents (2%) had structural anomalies (uterus didelphys). No adolescents had hypothyroidism or hyperprolactinemia. Two girls were taking antiepileptics, and eight were on vitamin supplements (iron, vitamin D, vitamin B12). None caused bleeding.

In group 1, 25 (33%) had frequent bleeding, and 25 (33%) had dense bleeding, which caused low Hb (< 10 g/dL). Anovulation was the most common etiology. Other etiologies were PCOS (16%), and factor VII deficiency (5%) while 34% had a family history of PCOS and 6% had a family history of AUB (Table 2).

Overall, 28 girls were under close follow-up and assessment for hematological diseases. Three (10.7%) were diagnosed with factor VII deficiency and two of these were in group 1 with the other in group 2. None had von Willebrand disease (vWD), other factor deficiency or platelet structural/functional disorders. Thirty-four subjects with a suspected family history of thrombosis (history of immobilization,

Table 2. Clinical and laboratory features of cases with abnormal uterine bleeding

		All subjects (n = 79)	Group 1 (n = 43)	Group 2 (n = 36)	p (Group 1 vs Group 2)
	Age (year)	14.3 ± 1.8	14.7 ± 1.7	13.8 ± 1.7	0.02*
	Menarche age (year)	11.9 ± 1.4	12.3 ± 1.4	11.5 ± 1.2	0.01
	BMI (%)	109.3 ± 25.6	106.3 ± 21.7	112.5 ± 28.2	0.27
	Underweight (< 90%)	19	11	8	0.73
	Normal (90-110%)	29	17	12	0.57
	Overweight (110-12%)	10	4	6	0.33
	Obese (> 120%)	21	11	10	0.83
	Irregularity after menarche (n, %)				
Time	**< 2 years**	67	41	26	< 0.01
Time	**> 2 years**	12	2	10	< 0.01
Bleeding pattern	**Frequent (n)**	38	25	13	0.05
Bleeding pattern	**Dense (n)**	25	25	0	< 0.01
Bleeding pattern	**Infrequent (n)**	6	3	3	0.82
Hb (gr/dL)	**Severe (≤8 g/dL) (n)**	26	26	0	< 0.01
Hb (gr/dL)	**Heavy (8-10 g/dL) (n)**	17	17	0	< 0.01
Hb (gr/dL)	**Moderate (10-12 g/dL) (n)**	14	0	14	< 0.01
Hb (gr/dL)	**Mild (≥12 g/dL) (n)**	22	0	22	< 0.01
Diagnostic distribution	**Presence of hematological disease (n)**	3	2	1	0.66
Diagnostic distribution	Factor VII deficiency (n)	3/28	2/16	1/12	0.72
Diagnostic distribution	vWD or other Factor deficiency (n)	0/28	0	0	-
Diagnostic distribution	Platelet structural/functional disorders (n)	0	0	0	-
Diagnostic distribution	*MTHFR* heterozygous	15/36	11/25	4/11	0.67
Diagnostic distribution	*MTHFR* homozygous	4/36	3/25	1/11	0.8
Diagnostic distribution	*FVL (0/34)*	0	0	0	-
Diagnostic distribution	*PHTRT (0/34)*	0	0	0	-
Diagnostic distribution	**Anovulation(n)**	63	34	29	0.55
Diagnostic distribution	**PCOS (n)**	13	7	6	0.96
Diagnostic distribution	**Structural anomaly (n)**	2	1	1	0.9
Diagnostic distribution	**Hypothyroidism/hyperprolactinemia (n, %)**	0	0	0	-

Student's t-test.
BMI: body mass index, PCOS: polycystic ovary syndrome, vWD: von Willebrand disease, Hb: hemoglobin

pregnancy, hormone therapy) and two with a proven thrombosis history (history of hospitalization due to venous thromboembolism), underwent genetic examination. Of these, 50% had an *MTHFR* mutation (homozygous n=4, heterozygous n=15) with no hyperhomocysteinemia. Prothrombin mutation or factor V Leiden mutation was not detected. None had venous thromboembolism during at least six months of follow-up.

In terms of a treatment procedures, since group 1 had patients with Hb <10 g/dL, they were on 3-4 tablets of COC daily until the bleeding ceased. In group 1, 18 (22.8%) needed erythrocyte transfusion. Half of the patients had heavy bleeding during every menstrual period since menarche. The most longest period between menarche and the onset of heavy bleeding was four years. Three of these patients required hospitalization in the intensive care unit. The bleeding ceased at a mean of 2.77±0.86 (range 1-5) days, following start of treatment with COCs. In the first month and a half, Hb increased above 10 g/dL in all groups. Oral iron supplementation was initiated in all patients. Four (5%) required rehospitalization and all four had a history of maternal AUB, but none had a family history of bleeding disorders.

In group 2, NSAIDs decreased the duration and amount of bleeding in subjects with mild uterine bleeding (n=22) and subjects with normal Hb. In adolescents with moderate uterine bleeding (n=14), iron supplementation with 60 mg of elemental iron per day and additional COCs were begun. In addition, 5 mg medroxyprogesterone acetate was started in two girls due to a proven family history of thromboembolism. Other patients were on 1-2 COC tablets with 0.15 mg desogestrel and 30 mcg ethinyl E2 per day. Except for eight of these patients, they were followed closely with outpatient treatment. In group 2, one patient had family history of AUB, 17 patients had family history of PCOS, two had family history of a bleeding disorder, and two had family history of thromboembolism. During follow-up, 20 of 79 (25.3%) did not comply with the recommended treatment. Non-compliance was observed in all patients (n=7, 8%) who presented with rebleeding. Six of these patients required rehospitalization. Three of them had bleeding disorders. The clinical and laboratory characteristics of the whole cohort and both groups are shown in Table 2. Nausea was the only COC-related short-term adverse effect (n=16; 20%). Treatment with iron supplementation or COCs was successful in the short term in all groups.

Discussion

The regular menstrual cycle is an essential indicator of female adolescent health. During the first two years of menarche, approximately half of the menstrual cycle is anovulatory but by the fifth year after menarche 75% of the cycles will be ovulatory (2). A deficiency in progesterone secretion from the ovarian follicles and excessive E2 production causes this early anovulation. Endometrial proliferation is also a cause of unpredictable menstrual bleeding (2). The results of the present study were in keeping with the published evidence that the most common cause of AUB in adolescents is anovulation (14,15,16). There was no history of excessive exercise in any of the cases included in the present study. It has been reported that obesity, anorexia, weight loss, PCOS, hyperprolactinemia, drugs such as steroids, phenothiazines, tricyclic antidepressants (3), and hypothyroidism can all cause AUB (17,18,19). All girls in the present study were evaluated for endocrinopathies and no endocrinopathies, except PCOS, were detected. Due to the study's retrospective nature, we could not assess the psychological status of girls at presentation.

The second most common cause of AUB is reported to be coagulopathies, with a 5-28% frequency (11,20,21,22). Studies have reported that vWD was the most common etiology of coagulopathy in women, with a frequency of 5-48%. Other causes of coagulopathy in descending order of reported prevalence are: platelet dysfunction (2-44%); thrombocytopenia (13-20%); coagulation factor deficiencies (8-9%); and then smaller proportions of leukemia, hypersplenism, and hereditary bleeding disorder (11,20,21,22). Earlier studies in this field have included cases previously diagnosed with a bleeding disorder. Since we did not include them, the proportion of patients diagnosed with a bleeding disorders was low. However, three girls had previously undiagnosed factor VII deficiency, and their first presenting symptom was AUB. In addition, two were in group 1 while one was in group 2, showing a phenotypical variability in this bleeding disorder. Given that this amounted to nearly 4% of our cohort, we caution colleagues that undiagnosed bleeding disorder should be considered in adolescents presenting with any degree of AUB, especially after exclusion of oither, more common etiologies.

No pregnancy was observed in this cohort. Invasive imaging methods were not needed because a structural abnormality as an etiology is not common in adolescents. Incidental structural anomaly (uterus didelphys) was detected by pelvic

USG in only two girls. We could not explain the relationship between this anomaly and AUB. These cases were also anovulatory.

Severe AUB affects 10-20% of adult women and 37% of adolescents. A study of approximately 1,000 healthy adolescents showed that 40% had heavy menstrual bleeding (23,24). About half of our patient group consisted of heavy and severe AUB. Although it was a retrospective study, the approach procedure was the same for all patients since it was a single center. Providing hemodynamic stability, resolution of anemia, and maintaining regular cycles in all patients were our main goals in managing severe AUB. Three patients in the severe group required intensive care unit follow-up. All girls with heavy bleeding were admitted to the inpatient unit. Earlier studies have emphasized that the basic approach in treatment is to stop the bleeding, treat the anemia, ensure a regular menstrual cycle, and increase the quality of life of the adolescent (25,26).

Treatment options include iron supplementation, COCs, progesterone, NSAIDs, antifibrinolytics, desmopressin, and gonadotropin releasing hormone (GnRH) analogs (27). Supportive treatment options include antiemetics, iron, NSAIDs, antifibrinolytic (tranexamic acid, aminocaproic acid), and a range of other drugs such as danazol, ulipristal acetate, and desmopressin acetate (28). The management is mainly based on the severity of bleeding and anemia. All were on COCs, NSAIDs, and iron supplementation in our follow-up. A side-effect associated with COCs was nausea. As girls who were previously diagnosed with bleeding disorders and malignancies were excluded, there is no description of our experience with GnRH analogs and antifibrinolytic treatments in this report.

The heterozygous frequency of *MTHFR* mutation in Turkey is 40-50%, while the homozygous frequency is 3-6%. Detection of hyperhomocysteinemia in the presence of homozygous mutation causes a tendency to thrombosis. This mutation alone (without homocysteinemia) does not create a significant risk factor for thrombosis (29). While there is no difference in thrombosis risk in patients with heterozygous *MTHFR* mutation compared to the average population, it has been reported that the risk of thrombosis is very low with the use of COCs in cases with homozygous mutations (30). We found the frequency of *MTHFR* mutation (50%) to be consistent with the reported frequency for a Turkish population. However, as previously suggested, this mutation did not increase the risk of bleeding/thrombosis, and we suggest that routine evaluation is unnecessary.

Menstrual problems are significant in the adolescent age group and may disrupt many aspects of daily life. A study from Hong Kong determined that approximately one-third of adolescents had restricted daily activities during menstruation, and 12% could not attend school during this period. Almost 70% of the girls described dysmenorrhea, and 17.9% were hospitalized with heavy menstrual bleeding (31). A study of 1,000 healthy adolescents stated that 73% had menstrual irregularities. In addition, 37% had heavy menstrual bleeding, and 22% were stopped with medication. Furthermore, 38% had a family history of heavy menstruation, and half of them had heavy menstruation in their children (23). Those with heavy menstrual bleeding were more tired than adolescents with normal bleeding. Their ability to participate in physical education and sports was reduced. They could not go to school at least one day a month, and their hobbies and leisure activities were disrupted (32). In 60% of adolescents, heavy menstrual bleeding seriously affected their social activities (33). However, we were unable to directly investigate quality of life in the present study but more than half of the patients were hospitalized, and this indirectly confirms a disruption of normal living. Some of the patients had also reported that AUB caused them to avoid a range of daily activities when medical history was being investigated during diagnosis.

Study Limitations

The limitations of our study include the retrospective nature of the study and that participants were all from a single center, notwithstanding that all patients diagnosed in our center over five years were eligible for inclusion. Multicenter national studies would yield more generalizable results.

Conclusion

Most adolescent girls with AUB were anovulatory in the first two years. A third had intense bleeding, and nearly a quarter needed erythrocyte transfusion. In addition, in the girls with suspected hematological disease, making up more than a third of the cohort, the frequency of undiagnosed hematological disease, in this case factor VII deficiency was 10.7%. Although *MTHFR* mutation frequency was 50%, this was consistent with frequency in the general population, did not increase the risk of bleeding/thrombosis, and thus we recommend that routine evaluation is unnecessary in Turkish adolescents with AUB.

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Ethics

Ethics Committee Approval: The study was approved by the Ankara University of Local Ethics Committee (decision no: I5-378-21, date: 25.06.2021).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Sirmen Kızılcan Çetin, Zehra Aycan, Elif Özsu, Zeynep Şıklar, Ayşegül Ceran, Seda Erişen Karaca, Gizem Şenyazar, Merih Berberoğlu, Concept: Sirmen Kızılcan Çetin, Zehra Aycan, Merih Berberoğlu, Design: Zehra Aycan, Elif Özsu, Merih Berberoğlu, Data Collection or Processing: Sirmen Kızılcan Çetin, Zeynep Şıklar, Ayşegül Ceran, Seda Erişen Karaca, Gizem Şenyazar, Analysis or Interpretation: Sirmen Kızılcan Çetin, Zeynep Şıklar, Ayşegül Ceran, Literature Search: Sirmen Kızılcan Çetin, Elif Özsu, Seda Erişen Karaca, Gizem Şenyazar, Writing: Sirmen Kızılcan Çetin, Zehra Aycan, Merih Berberoğlu.

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Fear of Hypoglycemia and Longer Disease Duration Associated with Physical Activity Avoidance in Children and Adolescents with Type 1 Diabetes Mellitus

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What is already known on this topic?

Children and adolescents with type 1 diabetes mellitus have lower levels of physical activity (PA) than their peers.

What this study adds?

This study highlights the times in school life and leisure time when PA is avoided. Moreover, the socioeconomic determinants of this avoidance are discussed. Unraveling the inequalities embedded in this behavior may help with intervention efforts.

Abstract

Objective: To determine physical activity (PA) avoidance and its associated factors among children with type 1 diabetes in four situations: leisure time (LT) PA out of school, LT PA at school during breaks, attendance at physical education (PE) classes and activity during PE classes.

Methods: Cross-sectional study. The cohort consisted of 137 children, aged 9-18 years, with type 1 diabetes registered at a tertiary center between August 2019 and February 2020, 92 of whom attended for face-to-face interview. Responses were rated on a 5-point-Likert scale for PA in the four situations. Never/rarely/occasionally responses were defined as avoidance. Chi-square, parametric/non-parametric comparison and multivariate logistic regression analysis were used to detect and confirm variables associated with each avoidance situation.

Results: Among the children 46.7% avoided PA during LT out of school and 52.2% during breaks, 15.2% avoided PE classes and 25.0% avoided active play during PE classes. Older children (14-18 year-olds) avoided PE classes [odds ratio (OR) = 6.49, 95% confidence interval (CI) = 1.10-38.13] and PA during breaks [OR = 2.85, 95% CI = 1.05-7.72] and girls avoided PA out of school (OR = 3.18, 95% CI = 1.18-8.06) and during breaks (OR = 4.12, 95% CI = 1.49-11.40). Those who had a sibling (OR = 4.50, 95% CI = 1.04-19.40) or had a poorly-educated mother (OR = 3.63, 95% CI = 1.15-11.46) avoided PA during breaks and those from low-income households avoided PE classes (OR = 14.93, 95% CI = 2.23-99.67). As the duration of disease prolonged, avoiding PA during LT out of school increased (4-9 years; OR = 4.21, 95% CI = 1.14-15.52 and ≥10 years; OR = 5.94, 95% CI = 1.20-29.36).

Conclusion: Adolescence, gender, and socioeconomic inequalities deserve greater focus for better PA behavior among young people with type 1 diabetes. As the disease duration prolongs, revising and strengthening intervention to encourage PA may be needed.

Keywords: Type 1 diabetes, physical activity, child, adolescent, socioeconomic inequalities

Introduction

Regular physical activity (PA) is known to improve the quality of life and health of those with type 1 diabetes and to reduce their risk of complications related to the disease. However, children and adolescents with type 1 diabetes have lower levels of PA than their peers without diabetes (1,2,3). Reasons given for PA avoidance include concerns

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about hypoglycemia, syllabus, weather conditions, loss of diabetes control and low fitness levels. Other causes of low PA levels in children or adolescents with type 1 diabetes stem from their families, school personnel or even physicians discouraging them from PA (4,5,6). School-age children with type 1 diabetes who lack adequate knowledge about exercise management also have trouble participating in PA at school (7). Furthermore, children with type 1 diabetes who are aware of the physiological and psychosocial benefits of regular PA may have difficulty in taking action and thus need support (8). In particular, family support plays a key role in reducing barriers preventing children with diabetes from having an active lifestyle (5). Socioeconomic inequalities have been reported to negatively affect the knowledge of healthy children about PA or their PA-related behaviors (9). However, to our knowledge, these inequalities for PA behavior have not been investigated among type 1 diabetes children. Unraveling the relationship between socioeconomic characteristics and PA may contribute to efforts aimed at increasing PA levels during the on-going management this disease in affected children and adolescents.

The aim of this study was to investigate PA avoidance behavior among children with type 1 diabetes in four situations: Leisure time (LT) PA out of school; LT PA in school during breaks; attendance in physical education (PE) classes; and activity during PE classes.

Methods

Study Population

This cross-sectional study was executed between August 2019 and February 2020 among children with type 1 diabetes aged 9-18 years, who were registered at Ege University Pediatric Endocrinology and Diabetes Outpatient Clinic, İzmir, Turkey. Of the 137 children registered, those who were diagnosed with type 1 diabetes for at least six months and those who did not have any other chronic illnesses were included in the study. For data collection, a 31-item study specific questionnaire, which is more explained in the variables title with its subheadings in the 5th and 6th page, was administered through face-to-face interviews in the diabetes education room; interviews lasted approximately 15 minutes. The interviews were carried out after routine visits to the Outpatient Clinic. The information about 11 parameters concerning their disease was retrieved from their medical records. Before the study was conducted, ethical approval was obtained from Ege University Ethical Committee (decision no: 19-7T/75, date: 7/31/2019), and before the interviews were conducted, signed, informed consent was given by the children and their parents. Data collection ceased in March 2020 when the Coronavirus disease-2019 pandemic reached Turkey.

Variables

Dependent variables: taking part in PA was questioned for: i) LT PA out of school (at home or outside); ii) LT PA at school during breaks; iii) attendance at PE classes (in some cases type 1 diabetes children are excused PE classes and do not attend); and iv) actual activity during PE classes. The children responded on a 5-point-Likert scale (never/rarely/occasionally/often/always). Responses including never/rarely/occasionally were classified as being PA avoidance. Each situation was analyzed separately for associations with independent variables. Those reporting PA avoidance were asked why they did this using an open-ended question.

Sociodemographic characteristics: Data was collected concerning the children and young peoples' age (9-13 or 14-18 years), sex, school type (private or public), the number of siblings, and parental education level using the Unesco International Standard Classification of Education (ISCED) 2011 (10). The ISCED groups were merged for analysis and grouped as low (ISCED ≤2: below lower secondary education), middle (ISCED = 3: upper secondary education) and high (ISCED ≥6: equal to or above bachelor degree). Perceived family income was graded on a 5-point-Likert scale and dichotomized as very high/high/middle and low/very low.

Characteristics of type 1 diabetes: Participants' weight (kg) and height (m), the time since diagnosis of type 1 diabetes, mean percentage (%) glycated hemoglobin (HbA1c) levels (excluding the first three months of diagnosis) in the previous year, hospital inpatient admissions in the previous year (number and reasons) and the number of outpatient admissions were retrieved from medical records. Optimal time in range was taken as ≥75% (11). Yearly outpatient control visits between 0 and 3 times were evaluated as "below optimal" and ≥4 or more visits were considered "optimal". The annual average HbA1c value was grouped as ≤7.5 (optimal), 7.5-9 (suboptimal), and ≥9 (high risk) (12). Body mass index-for-age Z-score cut-points were derived from Turkish standards by Neyzi et al. (13) and grouped as: underweight (< -2SD) normal (≤ + 2 and ≥-2) and obese (> + 2 SD). Diabetes duration was grouped as: 0-3 years, 4-9 years and ≥10 years.

Barriers to PA: The barriers defined in The Barriers to Physical Activity in Type 1 Diabetes (BAPAD1) scale for adults were used (2). The BAPAD1 scale has not been validated in pediatric and adolescent patients but has been used in young populations, previously (5). In the present

study two questions concerning fear of suffering a heart attack and having a low fitness level were not asked and the item regarding work schedule was changed to "school schedule". A total score was not derived, as the scale was not validated. Instead, each barrier was analyzed individually for any association with the dependent variables, a similar use of the scale as in the study of Jabbour et al. (5). Children were asked about these 10 possible barriers to PA on a scale ranging from 1 to 7 (1: extremely unlikely, to 7: extremely likely).

Knowledge of type 1 diabetes, nutrition and PA: This part included 10-items about type 1 diabetes (4 items), nutrition (3 items) and PA (3 items; see Appendix 1). The questions were developed in the light of literature. In order to prepare the question in regard to this part, "Patients' knowledge about the disease" in the form of introductory features developed by Karakurt et al. (14) was used and the questions were adapted to children with type 1 diabetes. In addition, the Diabetes Diagnosis and Treatment Guidelines of the Diabetes Foundation of Turkey were reviewed and the items that individuals with type 1 diabetes should know were reached and the questions were finalized in accordance with the guideline (15). Correct answers for these open-ended questions achieved a score of 1 and wrong answers/do not know options were scored zero.

Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences, version 23 (IBM Inc., Armonk, NY, USA). For categorical variables, their relationships with avoidance behavior in four situations were evaluated with chi-square tests. Whether continuous variables (barriers to PA and knowledge of diabetes, nutrition and PA) were normally distributed was tested using the Kolmogorov-Smirnov test. As none of the barriers to PA were normally distributed, log transformation to make data conform to normality was performed. For normally distributed data sets (inappropriateness of the syllabus and fear of hypoglycemia), the Student's t-test was performed. For comparison of non-parametric data sets, Mann-Whitney U test was used. A p value less than 0.05 was considered statistically significant. For logistic regression analysis, variables associated with PA avoidance behavior in bivariate analysis were included in the model.

Results

Of the 137 children registered at the center, 116 (84.7%) met the inclusion criteria. Of these 116, 92 (79%) children were interviewed. Table 1 shows the scores obtained from all participants, and from those who did and did not avoid PA in the four situations under consideration for a range of variables as barriers to PA and for knowledge

Table 1. Sociodemographic and diabetes relevant variables and their association with leisure time physical activity at home/outside and at school during breaks

Sociodemographic variables		Total n (%)*	Leisure time physical activity			
			At home or outside		At school during breaks	
			Avoids n (%)	x^2 p	Avoids n (%)	x^2 p
Age (years)	9-13	36 (39.1)	12 (33.3)	4.27 **0.04**	12 (33.3)	8.41 **0.004**
	14-18	56 (60.9)	31 (55.3)		36 (64.3)	
Gender	Girls	57 (61.9)	33 (57.9)	7.49 **0.01**	38 (66.7)	12.6 **<0.001**
	Boys	35 (38.1)	10 (28.6)		10 (28.5)	
Type of school	Public	68 (73.9)	30 (44.1)	0.72 0.39	36 (52.9)	0.06 0.80
	Private	24 (26.1)	13 (54.2)		12 (50.0)	
Siblings	None	77 (83.7)	34 (45.9)	5.14 **0.02**	45 (58.4)	7.43 **0.006**
	≥1	15 (16.3)	9 (50.0)		3 (20.0)	
Education level of mother	Low	43 (46.7)	24 (55.8)	3.10 0.21	28 (65.1)	7.38 **0.02**
	Middle	21 (22.8)	7 (33.3)		11 (52.4)	
	High	28 (30.5)	12 (42.9)		9 (32.1)	
Education level of father	Low	38 (41.3)	19 (50.0)	0.52 0.76	24 (63.2)	3.38 0.18
	Middle	20 (21.7)	8 (40.0)		8 (40.0)	
	High	34 (37.0)	16 (47.1)		16 (47.1)	
Income	Very high, high, middle	85 (92.4)	40 (47.1)	0.04 0.83	44 (51.8)	0.07 0.78
	Low, very low	7 (7.6)	3 (42.9)		4 (57.1)	

Table 1. Continued

Sociodemographic variables		Total n (%)*	Leisure time physical activity			
			At home or outside		At school during breaks	
			Avoids n (%)	x^2 p	Avoids n (%)	x^2 p
Variables relevant to diabetes						
Time after diagnosis (years)	0-3	18 (19.6)	4 (22.3)	7.06 **0.03**	10 (55.6)	3.92 0.14
	4-7	54 (58.7)	26 (48.1)		24 (44.4)	
	≥10	20 (21.7)	13 (65.0)		14 (70.0)	
BMI for age	Healthy weight	56 (60.9)	24 (42.9)	0.81 0.36	32 (57.1)	1.41 0.23
	Unhealthy weight	36 (39.1)	19 (52.8)		16 (44.4)	
Hospitalization in the previous year	≥1	74 (80.4)	10 (55.6)	0.69 0.40	10 (55.6)	0.10 0.74
	None	18 (19.6)	33 (44.6)		38 (51.3)	
Attending appointments in the previous year	≤3	25 (27.2)	9 (37.5)	1.11 0.29	9 (37.5)	2.80 0.09
	≥4	67 (72.8)	34 (50.0)		39 (57.4)	
HbA1c (last year average, %)	Optimal	28 (30.5)	9 (32.1)	4.22 0.12	15 (53.6)	2.57 0.27
	Suboptimal	39 (42.3)	19 (48.7)		17 (43.5)	
	High risk	25 (27.2)	15 (60.0)		16 (64.0)	
Total		92 (100)	43 (46.7)		48 (52.2)	

HbA1c: glycated hemoglobin

of their disease. Sociodemographic and type 1 diabetes characteristics and the association with PA avoidance in the four situations is presented in Table 2. The mean overall age of the participants was 14.08 ± 2.63 years and mean duration since diagnosis was 6.55 ± 3.84 years. Among the causes of hospitalization in the previous year, the most common was hyperglycemia/ketoacidosis (33.3%). None of the children were hospitalized for hypoglycemia. Of the four PA avoidance situations, the most frequent one was during breaks at school (52.2%). In the open-ended question for this, the most frequent reason (91.7%) declared was "being a grown up". Avoiding PA out of school ranked second (46.7%) and among the open-ended responses, 60.5% cited "being a grown-up" while 34.9% reported "spending time for studying". Regarding PE classes, 15.2% did not attend and 25.0% avoided PE activity during the PE class. Among the causes of avoidance, "spending time for studying" was again cited by many with 78.6% of the non-attending group and 69.6% of those who avoided PE activity at PE class using this reason. A smaller proportion cited blood sugar irregularities as the reason for avoiding PE classes completely (7.1%) while 8.7% used this reason to avoid PE activity at PE class. Variables associated with avoidance of PA at Table 1 and Table 2 in at least one of the situations included being older [14-18 years, $p = 0.04$ (at home or outside)], $p = 0.004$ (at school during breaks) and $p = 0.03$ (attendance to PE), being female [$p = 0.01$ (at home or outside) and $p < 0.001$ (at school during breaks)], having a sibling [$p = 0.02$ (at home or outside) and $p = 0.06$)], having a less well educated mother [$p = 0.02$ (at school during breaks)] and being from a low-income household [$p = 0.01$ (attendance to PE) and $p = 0.04$ (active plays during PE)]. Diabetes related determinants did not have an effect, with the exception of disease duration [$p = 0.03$ (at home or outside)].

Comparisons of the scores for items which were considered barriers to PA and concerning knowledge of their diabetes for those who did and did not avoid PA for each of the four situations are presented in Table 3. School schedule had the highest score (3.04 ± 2.37) among all the barriers, followed by fear of hypoglycemia (2.72 ± 2.02). The score for fear of hypoglycemia was higher among those both avoiding PE classes and avoiding PA in PE classes. Loss of control of diabetes was reported to be the biggest reasons by those who avoided LT PA both outside school and during school during breaks. Fear of being tired was higher among those who avoided LT PA out of school or at school during breaks and those who avoided activity during PE class. Fear of hurting oneself was higher among those who avoided LT PA at school during breaks and those who avoided attendance at PE classes. In terms of knowledge about diabetes the three highest scores (0.96 ± 0.21) concerned signs and management of low blood sugar and number of meals a day that should be consumed. Notably, the lowest score concerned PA and asked about the minimum minutes per week of PA (0.54 ± 0.50).

The results of the logistic regression analysis of PA avoidance in the four situations among young people with

type 1 diabetes are presented in Table 4. The older group (14-18 years) avoided PE classes [odds ratio (OR) = 6.49, 95% confidence interval (CI) = 1.10-38.13] and PA during breaks (OR = 2.85, 95% CI = 1.05-7.72) and girls avoided PA out of school (OR = 3.18, 95% CI = 1.18-8.06) and during breaks (OR = 4.12, 95% CI = 1.48-11.40). Children having a sibling (OR = 4.50, 95% CI = 1.04-19.40) and those having poorly educated mothers (OR = 3.63, 95% CI = 1.15-11.46) avoided PA during breaks and children from low-income households avoided PE classes (OR = 14.93, 95% CI = 2.23-99.67). As the duration since diagnosis got longer, participants were more likely to avoid PA during LT and out of school (duration 4-9 years; OR = 4.21, 95% CI = 1.14-15.52 versus duration ≥10 years; OR = 5.94, 95% CI = 1.20-29.36).

Discussion

The present study found that in children and adolescents with type 1 diabetes, older patients (14-18 years), girls, those with siblings, and those with less well educated mothers or from low-income households were more likely to avoid PA in one or more of the situations investigated. A general finding was that as the disease duration increased, PA avoidance became more likely.

Table 2. Sociodemographic and diabetes relevant variables and their association with attendance to PE classes and taking part in active plays during PE classes

Sociodemographic variables		Total n (%)*	Physical education classes at school			
			Attendance to		Active plays during	
			Avoids n (%)	x^2 p	Avoids n (%)	x^2 p
Age (years)	9-13	36 (39.1)	2 (5.5)	4.27 **0.03**	6 (16.7)	2.19 0.13
	14-18	56 (60.9)	12 (21.4)		17 (30.4)	
Gender	Girls	57 (61.9)	10 (17.5)	0.62 0.42	17 (29.8)	1.86 0.17
	Boys	35 (38.1)	4 (11.4)		6 (17.1)	
Type of school	Public	68 (73.9)	8 (11.8)	2.40 0.12	16 (23.5)	0.30 0.58
	Private	24 (26.1)	6 (25.0)		7 (29.2)	
Siblings	None	77 (83.7)	12 (15.6)	0.05 0.82	19 (24.7)	0.02 0.87
	≥1	15 (16.3)	2 (13.3)		4 (26.7)	
Education level of mother	Low	43 (46.7)	8 (18.6)	0.83 0.65	11 (25.6)	0.34 0.84
	Middle	21 (22.8)	3 (14.3)		6 (28.6)	
	High	28 (30.5)	3 (10.7)		6 (21.4)	
Education level of father	Low	38 (41.3)	6 (15.8)	0.58 0.74	10 (26.3)	0.34 0.84
	Middle	20 (21.7)	2 (10.0)		4 (20.0)	
	High	34 (37.0)	6 (17.7)		9 (26.5)	
Income	Very high, high, middle	85 (92.4)	10 (11.7)	10.32 **0.01**	19 (22.4)	4.17 **0.04**
	Low, very low	7 (7.6)	4 (57.1)		4 (57.1)	
Variables relevant to diabetes						
Time after diagnosis (years)	0-3	18 (19.6)	1 (5.6)	2.79 0.24	5 (27.8)	1.75 0.41
	4-7	54 (58.7)	8 (14.8)		11 (20.3)	
	≥10	20 (21.7)	5 (25.0)		7 (35.0)	
BMI for age	Healthy weight	56 (60.9)	7 (12.5)	1.12 0.56	14 (25.0)	0.78 0.67
	Unhealthy weight	36 (39.1)	7 (19.4)		1 (0.03)	
Hospitalization in the previous year	≥1	74 (80.4)	4 (22.2)	0.85 0.35	6 (33.3)	0.82 0.36
	None	18 (19.6)	10 (13.6)		17 (23.0)	
Attending appointments in the previous year	≤3	25 (27.2)	3 (12.5)	0.18 0.66	5 (20.8)	0.30 0.58
	≥4	67 (72.8)	11 (16.2)		18 (26.5)	
HbA1c (last year average, %)	Optimal	28 (30.5)	5 (17.8)	0.33 0.84	7 (25.0)	0.02 0.98
	Suboptimal	39 (42.3)	5 (12.8)		10 (25.6)	
	High risk	25 (27.2)	4 (16.0)		6 (24.0)	
Total		92 (100)	14 (15.2)		23 (25.0)	

PE: physical education, HbA1c: glycated hemoglobin

Table 3. The comparison of the scores on items for barriers for physical activity and knowledge for diabetes between groups avoiding and those not avoiding physical activity at four situations

		Leisure time physical activity						Physical education classes at school					
	Total (mean ± SD)	At home or outside (mean ± SD)			At school during breaks (mean ± SD)			Attendance to (mean ± SD)			Active plays during (mean ± SD)		
Barriers for physical activity		Avoids	Does not avoid		Avoids	Does not avoid		Avoids	Does not avoid		Avoids	Does not avoid	
School schedule	3.04 ± 2.37	3.40 ± 2.47	2.73 ± 2.26	p = 0.16	3.43 ± 2.46	2.61 ± 2.22	p = 0.21	3.64 ± 2.23	2.93 ± 2.39	p = 0.41	3.96 ± 2.40	2.74 ± 2.30	p = 0.86
Fear of hypoglycemia	2.72 ± 2.02	2.90 ± 1.87	2.55 ± 2.16	p = 0.24	2.98 ± 2.19	2.43 ± 1.81	p = 0.06	1.92 ± 1.20	2.85 ± 2.11	p = 0.01	2.26 ± 1.63	2.87 ± 2.13	p = 0.047
Weather conditions	2.66 ± 2.11	2.23 ± 3.04	1.86 ± 2.25	p = 0.07[u]	2.62 ± 2.03	2.70 ± 2.20	p = 0.93[u]	3.35 ± 2.09	2.53 ± 2.09	p = 0.07[u]	3.47 ± 2.35	2.39 ± 1.96	p = **0.03**[u]
Risk of hyperglycemia	2.25 ± 1.88	2.32 ± 2.18	1.70 ± 2.03	p = 0.2[u]	2.29 ± 1.95	2.20 ± 1.81	p = 0.90[u]	1.78 ± 1.47	2.33 ± 1.93	p = 0.30[u]	2.08 ± 1.78	2.30 ± 1.91	p = 0.55[u]
Location of a gym	2.20 ± 2.09	2.09 ± 2.28	1.93 2.22	p = 0.87[u]	2.43 ± 2.31	1.93 ± 1.79	p = 0.40[u]	1.85 ± 1.87	2.25 ± 2.12	p = 0.45[u]	2.13 ± 2.15	2.21 ± 2.07	p = 0.68[u]
Loss of control for diabetes	2.09 ± 1.89	2.27 ± 1.79	1.91 1.97	p = **0.04**[u]	2.43 ± 2.04	1.70 ± 1.65	p = **0.02**[u]	2.07 ± 1.43	2.08 ± 1.96	p = 0.53[u]	2.26 ± 1.94	2.03 ± 1.89	p = 0.47[u]
Fear of being tired	2.04 ± 1.93	2.30 ± 1.81	2.00 ± 1.85	p = **0.03**[u]	2.43 ± 2.13	1.61 ± 1.60	p = **0.02**[u]	2.50 ± 2.40	1.96 ± 1.84	p = 0.57[u]	2.74 ± 2.28	1.81 ± 1.75	p = **0.02**[u]
Fact that you have diabetes	2.02 ± 1.72	1.74 ± 2.26	1.25 ± 2.01	p = 0.66[u]	1.91 ± 1.59	2.13 ± 1.85	p = 0.91[u]	1.85 ± 1.29	2.05 ± 0.78	p = 0.91[u]	1.95 ± 1.58	2.04 ± 1.76	p = 0.86[u]
Actual physical health, excluding your diabetes	1.82 ± 1.83	1.65 ± 1.95	1.60 ± 2.02	p = 0.73[u]	1.89 ± 1.99	1.72 ± 1.66	p = 0.73[u]	2.07 ± 2.01	1.76 ± 1.80	p = 0.51[u]	2.13 ± 2.13	1.71 ± 1.72	p = 0.50[u]
Fear of hurting yourself	1.52 ± 1.37	1.67 ± 1.38	1.49 ± 1.25	p = 0.12[u]	1.97 ± 1.78	1.02 ± 0.15	p = **0.001**[u]	2.14 ± 1.87	1.94 ± 1.24	p = **0.04**[u]	1.95 ± 1.89	1.37 ± 1.25	p = 0.12[u]
Knowledge for diabetes													
Name of hormone causing diabetes	0.63 ± 49	0.70 ± 0.46	0.57 ± 0.50	p = **0.02**	0.73 ± 0.45	0.52 ± 0.51	p = 0.09	0.64 ± 0.50	0.63 ± 0.49	p = 0.83	0.61 ± 0.50	0.64 ± 0.48	p = 0.64
Organ responsible for diabetes	0.82 ± 0.39	0.88 ± 0.32	0.76 ± 0.43	p = **0.001**	0.90 ± 0.31	0.73 ± 0.45	p = 0.19	0.71 ± 0.47	0.83 ± 0.38	p = 0.07	0.78 ± 0.42	0.83 ± 0.38	p = 0.37
One sign of low blood sugar	0.96 ± 0.21	0.95 ± 0.21	0.96 ± 0.20	p = 0.89[u]	0.96 ± 0.20	0.95 ± 0.21	p = 0.93[u]	1.00 ± 0.00	0.95 ± 0.22	p = 0.39[u]	1.00 ± 0.00	0.94 ± 0.24	p = 0.24[u]
What to do when blood sugar decreases	0.96 ± 0.21	0.98 ± 0.15	0.94 ± 0.24	p = 0.37[u]	0.96 ± 0.20	0.95 ± 0.21	p = 0.93[u]	0.93 ± 0.27	0.96 ± 0.19	p = 0.58[u]	0.96 ± 0.21	0.96 ± 0.21	p = 1.00[u]
How many meals a day	0.96 ± 0.21	0.98 ± 0.15	0.94 ± 0.24	p = 0.38[u]	1.00 ± 0.00	0.91 ± 0.29	p = **0.03**[u]	0.93 ± 0.27	0.96 ± 0.19	p = 0.58[u]	0.96 ± 0.21	0.96 ± 0.21	p = 1.00[u]

Table 3. Continued

		Leisure time physical activity						Physical education classes at school					
	Total (mean ± SD)	At home or outside (mean ± SD)			At school during breaks (mean ± SD)			Attendance to (mean ± SD)			Active plays during (mean ± SD)		
Most important food group in type 1 diabetes	0.71 ± 0.46	0.74 ± 0.44	0.67 ± 0.47	p = 0.14	0.77 ± 0.42	0.64 ± 0.49	p = 0.06	0.64 ± 0.49	0.72 ± 0.45	p = 0.33	0.74 ± 0.45	0.70 ± 0.46	p = 0.41
A food containing 15 grams of carbohydrates	0.72 ± 0.45	0.86 ± 0.35	0.59 ± 0.50	p = **0.05**[u]	0.77 ± 0.42	0.66 ± 0.48	p = 0.24[u]	0.88 ± 0.36	0.69 ± 0.46	p = 0.21[u]	0.78 ± 0.42	0.70 ± 0.46	p = 0.42[u]
Adjusting the insulin dose for exercise	0.84 ± 0.37	0.88 ± 0.32	0.80 ± 0.41	p = **0.02**	0.81 ± 0.39	0.86 ± 0.35	p = 0.54	0.79 ± 0.43	0.85 ± 0.36	p = 0.29	0.78 ± 0.42	0.86 ± 0.35	p = 0.12
PA-At least how many days per week	0.65 ± 0.48	0.67 ± 0.47	0.63 ± 0.49	p = 0.41	0.69 ± 0.47	0.61 ± 0.49	p = 0.66	0.57 ± 0.51	0.67 ± 0.47	p = 0.29	0.70 ± 0.47	0.64 ± 0.48	p = 0.28
Minimum physical activity minutes per week	0.54 ± 0.50	0.56 ± 0.50	0.53 ± 0.50	p = 0.61	0.54 ± 0.50	0.55 ± 0.50	p = **0.001**	0.50 ± 0.52	0.55 ± 0.50	p = 0.70	0.52 ± 0.51	0.55 ± 0.50	p = 0.70

[u]p value for Mann-Whitney U test statistics.
SD: standard deviation, PA: physical activity

The results showed that the risk of not attending PE classes and avoiding activity during breaks increased by 6.49 times and 2.85 times respectively, in the 14-18 age group. This may be due to behavioral changes associated with adolescence. El Achhab et al. (16) conducted a study of with 346 healthy adolescents and 58.8% displayed sedentary behavior. Sleeping less than seven hours, increased screen time and excessive use of smartphones, compared to younger children, were some of the reasons for the high rate of physical inactivity (16,17). In addition, because those in the ≥14-year-old group get prepared for high school and university entrance exams, they allocate less time to sports, leading to their being more inactive (18). The results of the present study appear to show that these trends of healthy adolescents are found amongst adolescent patients with type 1 diabetes, too. Given the frequent access to healthcare and health education in children with diabetes, there seems to be an opportunity to modify adolescent attitudes towards PA in these patients. Healthcare providers should increase the emphasis on PA to improve the quality of life and health of patients with type 1 diabetes and this message needs to be repeated regularly. Furthermore, the context and methods to promote PA during transition to adult services, which coincides with the adolescent period of the patients, should probably be modified in order to change the attitude and behavior of adolescent patients with type 1 diabetes towards PA.

Girls were more likely to avoid LT activities, both out of school (OR = 3.18) and at school during breaks (OR = 4.12) in comparison to boys. According to a study conducted with children/young people with type 1 diabetes in the 6-17 years age group, the proportion taking part in licensed sports (professional athletes who have sports licencing) was lower in girls (30.4%) than in boys (69.6%) (19). Girls also participated less than boys (62.8% vs. 37.2%) in PA during the week. These gender differences has been previously attributed to boys' interest in fitness and improved masculine body image, while girls' interest was in self-care activities. Other factors affecting this gender difference were school environment, family support and relations with neighbors. Society's expectations of girls may be very different from the expectations placed on boys (20,21,22). The similar findings for adolescent girls in the present study may not, therefore, be surprising. To eliminate these gender inequalities in young patients with type 1 diabetes, given the benefit of PA for these patients, should become a target for healthcare professionals dealing with this disease.

Having a sibling was identified as a risk factor for avoidance of PA in this study. It has been suggested that older siblings

Table 4. The results of the logistic regression analysis for avoiding physical activity at four settings among children with type 1 diabetes

		Avoiding leisure time physical activity		Avoiding physical education classes at school	
		At home or outside OR (95% CI)	At school during breaks OR (95% CI)	Avoids attendance to OR (95% CI)	Avoids active plays during OR (95% CI)
Age (Ref: 9-13 years old)	14-18 years old	1.44 (0.51-4.09)	2.85* (1.05-7.72)	6.49* (1.10-38.13)	
Gender *(Ref: Boys)*	Girls	3.18* (1.18-8.06)	4.12* (1.49-11.40)		
Siblings *(Ref: None)*	≥1 sibling	4.08 (0.99-16.74)	4.50* (1.04-19.40)		
Education level of mother *(Ref: High)*	Low Middle		3.63* (1.15-11.46) 2.16 (0.57-8.13)		
Income *(Ref: Very high, high, middle)*	Low, very low			14.93* (2.23-99.67)	4.63 (0.95-22.52)
Time after diagnosis *(Ref: ≤3 years)*	4-9 years ≥10 years	4.21* (1.14-15.52) 5.94* (1.20-29.36)			

OR: odds ratio, CI: confidence interval

may be involved with the care of the younger sibling, especially for older girls, and thus having less time for participating in PA (23,24). The findings in McMinn's study showed that European children showed a significant positive association between number of siblings and PA whereas an inverse relation existed for South Asian children (25). Thus, the meaning of being a child, and the expected roles and responsibilities may differ between populations; these responsibilities also apply to children with diabetes. However, in the present study, only 15 children were only-children and nearly all (14/15) came from middle/high income families. Being an only-child may be an indicator of better-off living conditions and opportunities. These children may benefit from interpersonal, social and environmental factors that affect their PA participation positively (24).

As the education level of the mother decreased, the avoidance of LT PA increased during breaks in the school day. These mothers may be less aware of the benefits of PA and less supportive or even discouraging about PA, regarding diabetes. Moreover, for these mothers, allocating more time for school lessons may also be prioritized and emphasized for the child. Doing PA can be interpreted as avoiding studying. Civil (19) reported that more than half of the participants whose mothers had a postgraduate degree, were licensed for sports activities. High educated mothers may be more aware of the benefits of PA, more encouraging and more confident about disease management during PA. Raising the awareness of less well educated mothers about the benefits of PA in diabetes may have a positive impact for their children.

Participants in the present study from low-income households were more likely to avoid both going to PE classes and if in a PE class, were less likely to actually take part. High socioeconomic status increased the likelihood of taking part in PA activities among healthy adolescents (26). The better the income, the more the family encourages children to participate in PA. However, in low-income households the child may be expected to study more and perform better at educational activities than recreational ones. This was a common reason given in the present study for children from poorer families to avoid school-based PA, possibly because of a perception of PA as lost studying time.

Exercise is important for glycemic control and better blood sugar profile in patients with type 1 diabetes, and there is a significant relationship between exercise and glycemic control (27). In contrast, Civil (19) reported no significant correlation between HbA1c values and the frequency of PA. In order to explain this contradiction, planning long-term and prospective studies can affect the outcome of the relationship.

Having a longer duration since diagnosis increased the trend towards PA avoidance. If the period since diagnosis was 4-9 years or ≥10 years, they were more likely to avoid LT PA activities at home/outside than children with a diagnosis within the last three years. Children with type 1 diabetes are reported to be shy of their peers or teachers, and may be anxious about being different from others (28). We did not investigate if the participants in this study hid their disease from their peers. However, this research could lead to investigate further researches on shyness of diabetes and PA behavior. This psychology may have discouraged PA outside of school or participating in LT activities. However, the participants reported fear of tiredness and losing control of their diabetes as the main reasons for not taking part in PA. Once again, the benefits of taking part in PA do not appear to have been emphasized enough by the healthcare providers that the study participants have contact with. This indicates an important need to strengthen pro-PA interventions, probably during the whole course of the disease.

Jabbour et al. (5) also reported fear of hypoglycemia, pressures of school schedule, and weather conditions as strong barriers for PA participation in children with type 1 diabetes. Further evidence suggests that fear of hypoglycemia will reduce the frequency of PA participation and, because of anxiety of patients about hypoglycemia, children are discouraged from participating in PA (4,19,29).

Interestingly, children who avoided PA in one of the situations scored better on knowledge of diabetes, including how to make insulin adjustments for exercise, than participants who took part in PA. This may show that better understanding of the disease and the possible effect of PA brought about more hesitancy or even fear of taking part in PA. This is parallel to the finding on fear of hypoglycemia. Finally, it was shown that children with poorer knowledge about the recommended minimum PA minutes per week tended to avoid PA. This appears to be more evidence that the message about the benefits of PA in type 1 diabetes are simply not as strong as the patient or family anxiety concerning hypoglycemia, loss of control and the perception of exercise as a waste of (studying) time or even just not suitable for adolescent girls in some societies.

Study Limitations

This was a cross sectional study, thus causal interpretations should be interpreted with care. The study was limited to the type 1 diabetes population of one pediatric clinic and to those who attended the clinic during data collection. The relatively small sample size and the possible selection bias may have influenced the final findings. Those who attend regularly could be those who were more worried about PA (may result in overestimated frequencies of avoidance) or who were more educated/aware of the benefits of PA (may result in underestimated frequencies for avoidance).

Conclusion

Adolescence, gender and socioeconomic inequalities deserve special management when seeking to improve levels of PA among children with type 1 diabetes. Many of the findings in this cohort with type 1 diabetes parallel the findings in their healthy peers. However, given the frequent opportunities to access healthcare and health education in this population, it appears that more emphasis is required on the benefits of PA in type 1 diabetes to overcome the negative effects of adolescence, patient and parental anxiety, and gender differences. In terms of the effect of socioeconomic inequality and poor parental education, these are wider societal problems that will require changes beyond the scope of individual healthcare services. The strong association between disease duration and poorer participation in PA indicate an urgent need to strengthen interventions designed to promote PA during the whole course of the disease, starting with parental education at diagnosis.

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Ethics

Ethics Committee Approval: The study was approved by the Ege University Ethical Committee (decision no: 19-7T/75, date: 7/31/2019).

Informed Consent: Before the interviews were conducted, signed, informed consent was given by the children and their parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Büşra Donat Ergin, Işıl Ergin, Damla Gökşen, Design: Büşra Donat Ergin, Işıl Ergin, Damla Gökşen, Data Collection or Processing: Büşra Donat Ergin, Analysis or Interpretation: Büşra Donat Ergin, Işıl Ergin, Damla Gökşen, Literature Search: Büşra Donat Ergin, Writing: Büşra Donat Ergin, Işıl Ergin, Damla Gökşen.

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Click for Appendix 1. Knowledge on type 1 diabetes, nutrition, and PA

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ORIGINAL ARTICLE

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Adverse Events Associated with COVID-19 Vaccination in Adolescents with Endocrinological Disorders: A Cross-Sectional Study

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What is already known on this topic?

Adverse events associated with Coronavirus disease-2019 (COVID-19) vaccination in healthy adolescents are mostly similar to those in adults. There is a need for knowledge about the safety and adverse events of COVID-19 vaccines in special patient groups.

What this study adds?

We showed that the incidence and severity of adverse event associated with COVID-19 vaccination in adolescents with endocrinological disorders were similar to healthy subjects. Autoimmune and non-autoimmune endocrine disorders had similar side effect profiles after COVID-19 vaccines. Treatment agents for endocrinological diseases did not cause a difference in the incidence of adverse events.

Abstract

Objective: The aim was to evaluate the adverse events seen after Coronavirus disease-2019 (COVID-19) vaccination in pediatric patients with diagnosed endocrinological problems and to compare them with healthy controls.

Methods: In this cross-sectional study, patients aged 12-18 years who attended a single department between January and May 2022 and were followed up for at least six months due to endocrine diseases, and healthy subjects in the same age group, all of whom had received a COVID-19 vaccine [BNT162b2 mRNA or inactivated vaccine] were included. Adverse events experienced after the vaccination were evaluated by questionnaire.

Results: A total of 160 subjects (85 patients, 75 healthy controls) with a median (25-75p) age of 15.5 (14.1-16.9) years were included. The frequency of adverse events was higher in those vaccinated with the mRNA vaccine compared to the inactivated one after the first dose ($p = 0.015$). The incidence of adverse events observed after the first and second doses of both COVID-19 vaccines was similar in the patient and control groups ($p = 0.879$ and $p = 0.495$, respectively), with local reactions being the most common. The frequency of adverse events was similar among the patients who did or did not receive any endocrinological treatment ($p > 0.05$). The incidence and severity of systemic reactions were similar to those in healthy subjects for both vaccine doses, regardless of the underlying diagnosis, autoimmunity state, or treatment regimen used in patients with endocrine diseases.

Conclusion: The incidence and severity of adverse events associated with COVID-19 vaccinations in adolescents with endocrinological disorders were similar to healthy subjects, in the early post-vaccination period.

Keywords: Coronavirus, vaccination, endocrine, pandemic, pediatrics

Introduction

The whole world has been impacted by the Coronavirus disease-2019 (COVID-19) pandemic, which has lasted more than three years at the time of writing, caused by the newly identified member of the coronavirus family, Severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) (1). Since there is no effective treatment, mass national vaccination

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programs have been used widely in an attempt to control the pandemic. Therefore, vaccine studies have come to the fore and vaccines that are reported to be effective against the disease have begun to be used (2,3).

Although COVID-19 generally has a milder clinical course in the pediatric population, it may have a severe course in any age group and serious complications, such as multi-system inflammatory syndrome in children, have been observed in this age group (4,5). Therefore, the necessity of initiating an effective vaccination program in children arose, and after studies focusing on this subject, the use of COVID-19 vaccines in children aged 12 years and older was initially approved. Adverse events seen with these vaccines in adolescents are mostly similar to those in adults, though several serious events, including myocarditis, were reported and raised concerns about the use of these vaccines in this age group (6,7,8). Furthermore, these findings were largely reported in patients who were healthy prior to COVID-19, and the side effect profile of these vaccines in children and adolescents with chronic diseases is not yet known.

Given the systemic function of hormones, questions were raised about the effects and side effects of COVID-19 vaccines in patients with endocrine disorders and several reports commented on this topic (9,10,11,12). In addition, there were concerns that autoimmunity, which may be the etiology in a number of endocrine diseases, may also change the response to COVID-19 and the vaccines. However, vaccination recommendations similar to those made for the healthy population were made for patients who were not on conventional immunosuppressants, high-dose glucocorticoids, or immunomodulatory treatments, such as biological agents, for a systemic autoimmune disease (13). Unfortunately, the evidence to support these recommendations are mainly based on adult studies and there is no data regarding the safety and adverse events of COVID-19 vaccines in children and adolescents with endocrine diseases or who were under treatment for these diseases.

In this study, the aim was to investigate the adverse events observed after COVID-19 vaccination with either inactive SARS-CoV-2 or BNT162b2 messenger RNA (mRNA) COVID-19 in use in our country in adolescents with endocrinological disorders and to compare these results with healthy subjects.

Methods

This cross-sectional study was conducted in a single center between January and May 2022. Patients aged 12-18 years who attended the pediatric endocrinology department and who had been followed up for at least six months for an endocrinological disorder were included. Patients with any other underlying diseases, syndromes, or a history of drug use for non-endocrinological disorders were excluded. Obesity was defined in patients with exogenous obesity by a body mass index above the 95th percentile, and those with any underlying non-endocrinological causes of obesity were also excluded. The control group consisted of healthy adolescents in the same age group without any acute or chronic disease, who attended outpatient clinics for routine check-ups. The main inclusion criterion for both groups was to be vaccinated against SARS-CoV-2. The medical history of the patients was confirmed by medical records and the vaccination status of all participants was verified by national registries. Individuals whose verification failed or whose medical records could not be accessed were not included in the study.

All patients and healthy controls were vaccinated with at least one dose of one of the COVID-19 vaccines. Information about the type of vaccine and the adverse events after vaccinations were gathered using a questionnaire, that was answered during a routine outpatient visit. In our country, two types of vaccines against SARS-CoV-2, namely inactivated vaccine and BNT162b2 mRNA, became available for all children older than 12 years in September 2021. Individuals were free to choose their vaccine types and both vaccines were administered in two doses with a one-month interval.

Adverse events after vaccinations were evaluated by the Pediatric Infectious Disease department, based on the recommendations made by the Vaccine Adverse Event Reporting System administered by the Food and Drug Administration and Centers for Disease Control and Prevention (14). Criteria include, redness, warmth, pain, or tenderness at the injection site that was defined as a local reaction. Events causing permanent sequelae, life-threatening issues, or requiring hospitalization were classified as serious adverse events. Other remaining reactions, such as fever, rash, and/or joint pain, were classified as non-serious systemic adverse events.

Ethics

The Dokuz Eylül University Local Ethics Committee of the host institute approved the study (ethics approval number: 2022/05-08, date: 09.02.2022), and the study was performed in accordance with the principles of the Declaration of Helsinki. Informed written consent was provided by each patient and his/her parents before participating in the study.

Statistical Analysis

All statistical analyses were performed using Statistical Package for the Social Sciences for Windows, version 24.0

(IBM Inc., Armonk, NY, USA). The distribution of data was evaluated with the Kolmogorov-Smirnov test and the data are presented as number (%) for categorical variables, and median (25^{th}-75^{th} percentile) for numerical variables. Comparisons were performed using the Pearson chi-square test and the Fisher exact for categorical variables and the Student's t-test or the Mann-Whitney U test for continuous variables, as appropriate. The McNemar test was used to compare the categorical data of two related groups. A p value of < 0.05 was considered statistically significant.

Results

A total of 160 subjects (85 patients, 75 healthy controls) were included in the study, 56.3% of whom were girls. The median age of patients was 15.5 (14.1-16.9) years. Groups were similar in terms of age and gender (p = 0.185 and p = 0.563, respectively). Subjects were included after a median of 5 (5-6) months from the first dose of vaccination. Endocrinological diagnoses in the patient group were: type 1 diabetes (41.1%, n = 35); obesity (18.8%, n = 16); hypothyroidism (17.6%, n = 15); hypopituitarism (5.9%, n = 5); polycystic ovarian syndrome (4.7%, n = 4); autoimmune polyglandular syndrome type 2 (3.5%, n = 3); hyperthyroidism (2.4%, n = 2); pubertal disorders (2.4%, n = 2); congenital adrenal hyperplasia (2.4%, n = 2); and prolactinoma (1.2%, n = 1). In total, 48 patients (56.5%) had an autoimmune disease. Sixty-three (74.1%) of the patients were receiving treatment for endocrine disorders. The most commonly used treatments were insulin (43.5%), levothyroxine (20%), and hydrocortisone (4.7%).

One hundred and fifty individuals (93.8%) were vaccinated with BNT162b2 mRNA (77 patients, 73 controls), and the remainder (6.2%, n = 10) with the inactivated vaccine (eight patients, two controls) for the first dose. The frequency of adverse events was higher in those vaccinated with the mRNA vaccine compared to the inactivated vaccine (p = 0.015), but it was similar among the patient and control groups regardless of vaccine type (p = 0.879; Table 1). Both groups experienced local events as the most common side effect of the first dose of COVID-19 vaccines (p = 0.634). In terms of systemic symptoms, fatigue, myalgia, and fever were the most common findings and the rate of all systemic events was not different between the two groups (p = 0.763) (Table 1). Sixteen subjects in the patient and control groups (30.2% and 35.6%, respectively) suffered both local and systemic symptoms (p = 0.692). Three individuals (one patient with hypothyroidism, one with obesity, and a healthy control) needed admission to hospital, classified as a severe reaction, because of symptoms after the first dose. All of them had fever and the one with obesity also had vomiting.

One hundred and forty-six individuals (77 patients, 69 healthy controls) completed the vaccination schedule with a second dose. The BNT162b2 mRNA vaccine was given to 138 subjects (70 patients, 68 controls) and the inactivated vaccine was administered to eight (seven patients, one control). Although there was a higher incidence of side effects with the second dose of the mRNA vaccine than with the inactivated vaccine, this was not significant (p = 0.053). The frequency of adverse events was again similar after the second dose of vaccine in both adolescents with endocrinological problems and healthy subjects (p = 0.495). As with the first doses, the most common complaint was local reactions after the second dose (p = 0.919). The rate of systemic events did not significantly differ between the two groups (p = 0.958) (Table 2). Eighteen patients (36.7%) and 15 (33.3%) controls experienced both systemic and local reactions (p = 0.813). One patient with obesity (because of myalgia and a severe local reaction) and two healthy adolescents (because of fever, myalgia, and vomiting) were admitted to hospital after the second dose. There was no difference in the incidence of adverse events between the patient and control groups for each dose of each vaccine type (p > 0.05) (Figure 1). The distribution of these events observed after each vaccination is given in Table 3.

Table 1. The comparison of the two groups regarding the findings of the first dose administration of Coronavirus disease-2019 vaccines

	Patient group (n = 85)	Control group (n = 75)	p
Age (years)	15.1 (13.9-17.0)	16.0 (14.7-16.8)	0.182
Female [n (%)]	46 (54.1%)	44 (58.7%)	0.563
BNT162b2 mRNA vaccine [n (%)]	77 (51.3%)	73 (48.7%)	0.105
Adverse event incidence [n (%)]	52 (61.2%)	45 (60.0%)	0.879
Local reactions [n (%)]	32 (37.6%)	31 (41.3%)	0.634
Systemic reactions [n (%)]	36 (42.4%)	30 (40.0%)	0.763
Both local and systemic reactions [n (%)]	16 (30.2%)	16 (35.6%)	0.692
Severe adverse events [n (%)]	2 (2.4%)	1 (1.3%)	1.000

Table 2. Comparison of patient and healthy control groups after the second dose of Coronavirus disease-2019 vaccines

	Patient group (n = 77)	Control group (n = 69)	p
Age (years)	15.1 (13.8-17.0)	16.0 (14.7-16.8)	0.132
Female [n (%)]	43 (55.8%)	38 (55.1%)	0.925
BNT162b2 mRNA vaccine [n (%)]	70 (90.9%)	68 (98.6%)	0.066
Adverse event incidence [n (%)]	46 (59.7%)	45 (65.2%)	0.495
Local reactions [n (%)]	33 (42.9%)	29 (42.0%)	0.919
Systemic reactions [n (%)]	34 (44.2%)	31 (44.9%)	0.925
Both local and systemic reactions [n (%)]	18 (23.4%)	15 (21.7%)	0.813
Severe adverse events [n (%)]	1 (1.3%)	2 (2.9%)	0.603

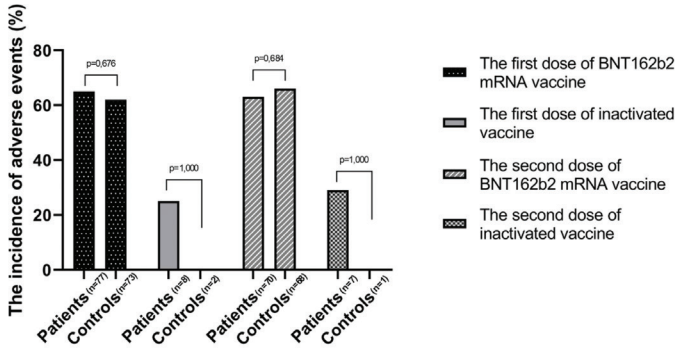

Figure 1. The incidence of adverse events after each type and dose of Coronavirus disease-2019 vaccine in the patient and control groups

Table 3. The distribution of adverse events observed after Coronavirus disease-2019 vaccines

Adverse events	The first dose of the BNT162b2 mRNA vaccine		The first dose of the inactivated vaccine		The second dose of the BNT162b2 mRNA vaccine		The second dose of the inactivated vaccine	
	Patients (n = 77)	Controls (n = 73)	Patients (n = 8)	Controls (n = 2)	Patients (n = 70)	Controls (n = 68)	Patients (n = 7)	Controls (n = 1)
Local reactions	31 (40.3%)	31 (42.5%)	1 (12.5%)	0 (0%)	32 (45.7%)	29 (42.6%)	1 (14.3%)	0 (0%)
Fever	13 (16.9%)	8 (11.0%)	1 (12.5%)	0 (0%)	13 (18.6%)	11 (16.2%)	1 (14.3%)	0 (0%)
Myalgia	14 (18.2%)	15 (20.5%)	1 (12.5%)	0 (0%)	11 (15.7%)	15 (22.1%)	2 (28.6%)	0 (0%)
Fatigue	16 (20.8%)	15 (20.8%)	2 (25.0%)	0 (0%)	14 (20.0%)	20 (29.4%)	0 (0%)	0 (0%)
Sore throat	2 (2.6%)	3 (4.1%)	0 (0%)	0 (0%)	3 (4.3%)	3 (4.4%)	0 (0%)	0 (0%)
Headache	7 (9.1%)	4 (5.5%)	0 (0%)	0 (0%)	1 (1.4%)	4 (5.9%)	0 (0%)	0 (0%)
Arthralgia	5 (6.5%)	4 (5.5%)	1 (12.5%)	0 (0%)	2 (2.9%)	2 (2.9%)	1 (14.3%)	0 (0%)
Cough	3 (3.9%)	2 (2.7%)	0 (0%)	0 (0%)	3 (4.3%)	1 (1.5%)	0 (0%)	0 (0%)
Vomiting	3 (3.9%)	1 (1.4%)	0 (0%)	0 (0%)	2 (2.9%)	2 (2.9%)	0 (0%)	0 (0%)
Abdominal pain	1 (1.3%)	0 (0%)	0 (0%)	0 (0%)	1 (1.4%)	0 (0%)	0 (0%)	0 (0%)
Diarrhea	1 (1.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Rash	0 (0%)	2 (2.7%)	0 (0%)	0 (0%)	0 (0%)	1 (1.5%)	0 (0%)	0 (0%)
Hyperglycemia	7 (9.1%)	0 (0%)	1 (12.5%)	0 (0%)	4 (5.7%)	0 (0%)	0 (0%)	0 (0%)
Hypoglycemia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.4%)	0 (0%)	0 (0%)	0 (0%)
Menstrual irregularity	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.5%)	0 (0%)	0 (0%)
Chest pain	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.4%)	0 (0%)	0 (0%)	0 (0%)

When the patients were grouped by the presence of autoimmunity as part of their disease or not, the incidence of side effects did not differ between these patient subgroups for each dose of the vaccine (Table 4). Also, the frequency of adverse events was similar after either the first or second doses of vaccines between patients with and without treatment for the underlying endocrine diagnosis ($p > 0.05$). When the patients with or without autoimmune disorders and who only received the mRNA vaccine ($n = 77$) were compared, the incidence of adverse events was similar after both doses ($p = 0.896$ and $p = 0.955$ for the first and second doses, respectively). In addition, the frequency of local and systemic reactions was similar between these two groups after either dose of the mRNA vaccine ($p > 0.05$).

In children with endocrinological problems, 70.5% of those who experienced side effects after the first dose also reported adverse events after the second dose ($p = 0.851$). Eight patients with type 1 diabetes experienced hyperglycemia after the first dose, four experienced hyperglycemia after the second dose, and one patient had hypoglycemia after the second dose. Detailed information on symptoms after each dose of COVID-19 vaccine in the patient group according to underlying diagnosis is presented in Figure 2. The incidence of side effects was similar after either vaccine dose, regardless of the treatment agents used in patients with endocrine disorders ($p > 0.05$).

Discussion

Several types of COVID-19 vaccines were produced rapidly, with mRNA and inactivated types as the most commonly used vaccines. These vaccines were administered in adults initially and, after safety approvals, vaccination schedules for adolescents and children were launched (2,3). While the most frequently reported adverse event was local reactions at the vaccination site, systemic symptoms including fatigue, headache, myalgia, and fever were also commonly reported. Moreover, the incidence of side effects after vaccination was reported to be higher with mRNA vaccines than with the inactivated type (15,16). The results of the present study confirm that the most common adverse event was a local reaction in both patient and control groups after COVID-19 vaccines. Thus, there was a higher rate of complaints after the first dose of the mRNA vaccine than with the inactivated type. It should be noted that there were very few subjects in the inactivated vaccine group, which may have skewed our results. Published evidence in this field is largely derived from studies in adult age groups and initial results of vaccination studies in adolescents supported the safety of COVID-19 vaccines (6). However, more serious complications, such as myocarditis and pericarditis, have also been reported in adolescents (7,8). The frequency of adverse events in the early post-vaccination period was reported to be as high as 60%, although most were local events in pediatric studies (17,18,19). All these data were obtained from previously healthy children, and the side-effect profile of these vaccines

Table 4. Comparison of patients with endocrine disorders stratified by the presence of an autoimmune mechanism in the condition or not

		Autoimmune diseases (n = 48)	**Non-autoimmune diseases (n = 37)**	**p**
Age (years)		14.9 (13.9 – 17.0)	15.2 (13.8 – 17.1)	0.940
Female [n (%)]		28 (58.3%)	18 (48.2%)	0.374
The first dose (n = 85)	BNT162b2 mRNA vaccine [n (%)]	42 (87.5%)	35 (94.6%)	0.457
	Adverse event incidence [n (%)]	29 (60.4%)	23 (62.2%)	0.870
	Local reactions [n (%)]	16 (33.3%)	16 (43.2%)	0.350
	Systemic reactions [n (%)]	22 (45.8%)	14 (37.8%)	0.460
	Both local and systemic reactions [n (%)]	9 (18.8%)	7 (18.9%)	0.984
	Severe adverse events [n (%)]	0 (0%)	2 (5.4%)	0.187
		Autoimmune diseases (n = 43)	**Non-autoimmune diseases (n = 34)**	**p**
The second dose (n = 77)	BNT162b2 mRNA vaccine [n (%)]	38 (88.4%)	32 (94.1%)	0.455
	Adverse event incidence [n (%)]	25 (58.1%)	21 (61.8%)	0.747
	Local reactions [n (%)]	17 (39.5%)	16 (47.1%)	0.508
	Systemic reactions [n (%)]	20 (46.5%)	14 (41.2%)	0.640
	Both local and systemic reactions [n (%)]	9 (20.9%)	9 (26.5%)	0.568
	Severe adverse events [n (%)]	0 (0%)	1 (2.9%)	0.442

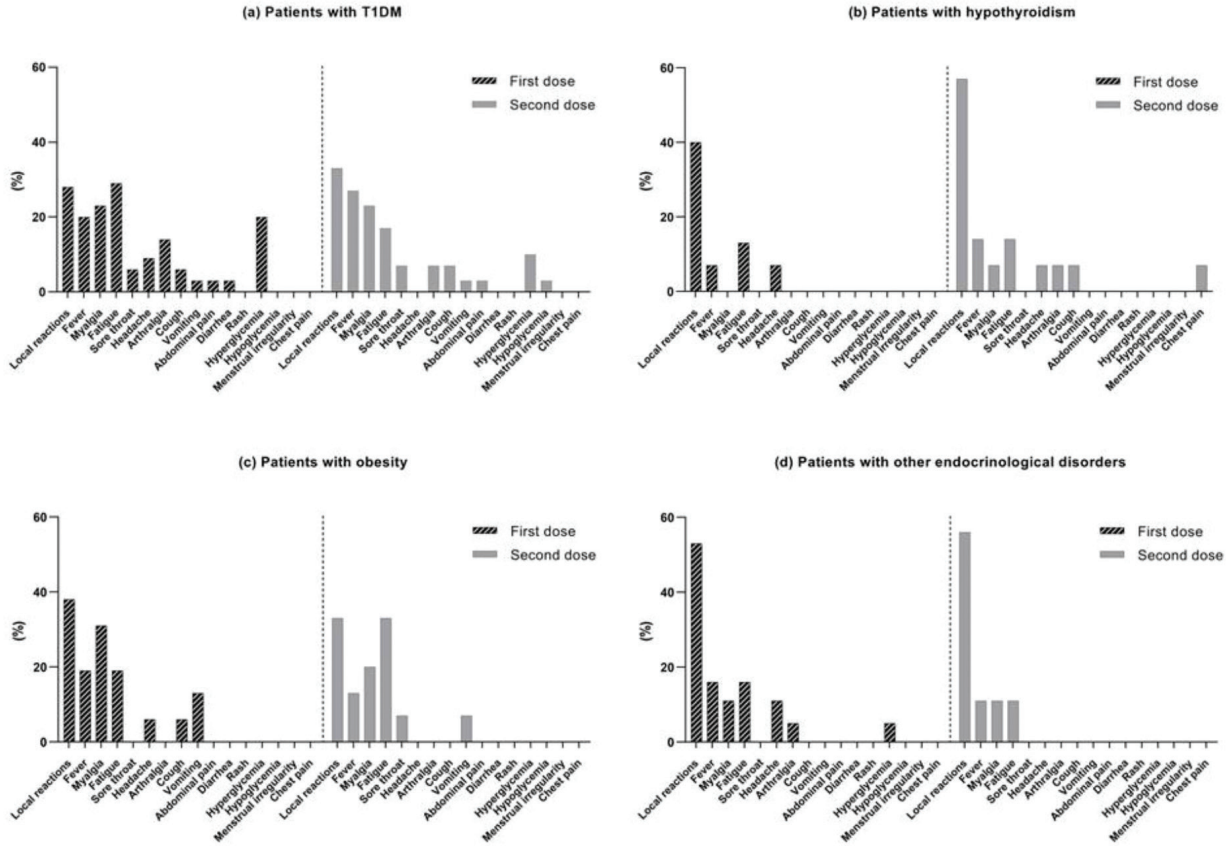

Figure 2. The distribution of adverse events after each dose of Coronavirus disease-2019 vaccine in the patient group, stratified by underlying diagnosis; (a) patients with T1DM, (b) patients with hypothyroidism, (c) patients with obesity, (d) patients with other endocrinological disorders

T1DM: type 1 diabetes mellitus

in pediatric patients with chronic diseases is unclear. The results of the present study are comparable with these earlier reports, showing a similar adverse event incidence after COVID-19 vaccines in patient and control groups. It also appears that experiencing an adverse events after the first dose did not affect the frequency of experiencing an event after the second dose of COVID-19 vaccines in patients with endocrinological disorders.

The endocrine system was thought to be affected by COVID-19 vaccines (11,12). However, the overall benefits of COVID-19 immunization were accepted to outweigh the risk of adverse events. Nevertheless, questions are still unresolved regarding the frequency of COVID-19 vaccine-related side effects in patients with an endocrinopathy (12). In addition to the adverse events seen in the general population, further complications, such as interstitial lung disease, have been reported in several patients with autoimmune diseases, particularly after the inactivated vaccine. In addition, exacerbation of the underlying disease has been an important consequence of COVID-19 vaccination (13). In light of this information, concerns arose about whether COVID-19 vaccines cause different and severe adverse events in children and adolescents with chronic diseases. In a recent study, Haslak et al. (20) found a similar safety and side-effect profile of COVID-19 vaccines in adolescents with rheumatological diseases, including autoimmune ones, compared to healthy controls. They reported a rate of adverse events of approximately 40%, which were observed more frequently with the mRNA-type vaccine. Their results showed that adverse events in adolescents with chronic diseases were commonly mild, such as fatigue, myalgia, and reaction at the vaccine site, regardless of the underlying disease or treatment regimens (20). The present study found similar results with a similar adverse event profile and severity in adolescents with endocrinological disorders. The patient group did not have a different frequency of side effects in the early post-vaccination period compared with healthy controls. In addition, an autoimmune element of the endocrinopathy had no effect on the incidence of events after the first or second dose of vaccines. This finding suggests that autoimmune disorders as part of one of the

endocrinopathies included in this study and not treated by immunomodulatory drugs, result in similar immune reactions as seen in non-autoimmune conditions. However, further molecular and clinical studies should focus on this issue, investigating the possible effects of these vaccines on autoimmunity and autoimmune diseases. The distribution of complaints varied among patients regarding underlying diagnoses, such as glycemic changes in some diabetic individuals. This may be due to the fact that inflammatory responses influence insulin sensitivity, causing a fluctuation in blood glucose levels, but also by the individual variabilities in glycemic controls of patients with diabetes.

Despite the generally good safety record of vaccines, a small number of reported severe complications, such as acute respiratory failure, sensorineural hearing loss, myocarditis, or thromboembolic events after COVID-19 vaccines, especially with the mRNA type, are reported to have increased patient hesitancy regarding immunization programs (21,22,23,24). However, we found that endocrine diseases did not increase the incidence of severe reactions requiring hospitalization in either vaccine type or at first or second dose. This finding was similar to a previous report showing severe reactions in only two of 223 adolescents with rheumatological problems who received the mRNA vaccine (20). Hence, the results of the present study add to the evidence suggesting a good safety profile for COVID-19 vaccines in adolescents with endocrinological disorders. It should be noted that the adverse event profile was only relevant for the early period after vaccination and there is a need for studies to evaluate the long-term side effects, and also to show the efficacy of these vaccines by investigating immune response or antibody levels in specific patient groups.

Routine childhood vaccines are generally regarded as being safe in children with endocrine disorders (25,26). In particular, steroids are known to alter the immune system and immunological reactions, and conflicting results about adverse events of these vaccines under steroid treatment were reported recently (27,28). Some authors reported a history of steroid intake as a predictor of adverse reactions, but short-term and low-dose corticosteroid use was shown to reduce reactogenicity after COVID-19 vaccines in another study (29,30). Yet, there is no evidence that hormone replacement therapies reduce the efficacy of COVID-19 vaccines or increase adverse side effects. The present study showed that adverse events did not differ among treated or untreated patients with endocrinological disorders. Furthermore, none of the patients on hydrocortisone replacement for adrenal insufficiency had a severe reaction. This may be due to the use of physiological dosing for replacement of endogenous hormones in these patients. It was suggested that patients with endocrinological problems should be included in the routine vaccination programs, but they should be followed closely in terms of complications that may develop specific to the disease (10). Some suggestions were made for these patients, such as close blood glucose monitoring in diabetic individuals, additional insulin dose administration when necessary, or increasing the steroid replacement dose in patients with adrenal insufficiency when fever and serious side effects develop. All these recommendations were based on data from adult patients, and the present study in adolescents supports these reports. For example, some of our patients with diabetes had hyperglycemia or hypoglycemia within the early period after COVID-19 vaccination. Both the results of the present study and published evidence indicate the importance of a patient-based, individualized approach after the administration of COVID-19 vaccines in patients with endocrinological problems.

Study Limitations

The main strength of our study was that this is the first study to investigate early adverse events after COVID-19 vaccination in adolescents with endocrinological disorders. Nevertheless, there are notable limitations. First of all, data were collected by questionnaire for past events, which is subject to patient/family recall bias. However, most of the studies into drug/vaccine adverse events use similar methods. Another limitation was that our patient group was not homogenous and we could not evaluate the duration or dosage of the treatment regimens for endocrine diseases. Also, the history of COVID-19 infection before vaccination was unknown but the regulations in our country did not permit COVID-19 vaccination within six months of a known infection. Finally, the low number of individuals in subgroup analyses lessen the reliability of these results. In this regard there was a notably small group size for the inactivated vaccine group, especially for healthy controls, which may influence any comparison with this subgroup. Therefore, there is a need for prospective studies in this field with larger cohorts to clearly elucidate the potential complications of COVID-19 vaccines in such special patient groups.

Conclusion

The incidence and severity of adverse events after either inactivated or mRNA COVID-19 vaccines in our country were similar between adolescents with endocrinological disorders and healthy subjects. Adverse events were more frequent after the mRNA vaccine compared to the inactivated one, but the most common complaint was local

reactions with both vaccine types. Endocrine disorders with or without an autoimmune element had similar side effect profiles. Treatment agents for endocrine diseases did not appear to cause a difference in the incidence of adverse events. However, patients should be informed and followed closely for disease-specific complications.

Ethics

Ethics Committee Approval: The Dokuz Eylül University Local Ethics Committee of the host institute approved the study (ethics approval number: 2022/05-08, date: 09.02.2022).

Informed Consent: Informed written consent was provided by each patient and his/her parents before participating in the study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: İbrahim Mert Erbaş, İrem Ceren Erbaş, Gözde Akın Kağızmanlı, Kübra Yüksek Acinikli, Özge Besci, Korcan Demir, Ece Böber, Nurşen Belet, Ayhan Abacı, Concept: İbrahim Mert Erbaş, İrem Ceren Erbaş, Ayhan Abacı, Design: İbrahim Mert Erbaş, İrem Ceren Erbaş, Ayhan Abacı, Data Collection or Processing: İbrahim Mert Erbaş, İrem Ceren Erbaş, Gözde Akın Kağızmanlı, Kübra Yüksek Acinikli, Özge Besci, Korcan Demir, Ece Böber, Nurşen Belet, Ayhan Abacı, Analysis or Interpretation: İbrahim Mert Erbaş, İrem Ceren Erbaş, Gözde Akın Kağızmanlı, Kübra Yüksek Acinikli, Özge Besci, Korcan Demir, Ece Böber, Nurşen Belet, Ayhan Abacı, Literature Search: İbrahim Mert Erbaş, İrem Ceren Erbaş, Gözde Akın Kağızmanlı, Kübra Yüksek Acinikli, Özge Besci, Korcan Demir, Ece Böber, Nurşen Belet, Ayhan Abacı, Writing: İbrahim Mert Erbaş, İrem Ceren Erbaş, Ayhan Abacı.

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Differentiated Thyroid Cancer in Adolescents: Single Center Experience and Considerations for Surgical Management and Radioiodine Treatment

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What is already known on this topic?

Although most studies of pediatric differentiated thyroid cancer include all patients aged less than 18 years, the "adolescent" group, defined by World Health Organization as patients between 10 and 19 years of age, seem to present a less aggressive course than in children under 10 years old. Compared to patients older than 19 years, they also seem to have higher cure rates, despite higher rates of cervical lymph node and pulmonary metastasis.

What this study adds?

This study focused on adolescents aged between 10 and 19 years and treated at a single center. There was a higher rate of follicular variant papillary thyroid cancer than other previous pediatric studies but also a high cure rate at a median of 60.7 months after surgery and following first-line 2-3.7 GBq radioiodine treatment (RAIT) administered postoperatively. Adjuvant RAIT may thus obviate the postoperative morbidity of prophylactic lymphadenectomy for these young patients.

Abstract

Objective: Differentiated thyroid cancer (DTC) in adolescents rare but with a favorable outcome, despite higher rates of cervical lymph node and pulmonary metastasis compared to adults. The aim of this study was to critically evaluate treatment of adolescents with DTC at a single center.

Methods: Patients receiving postoperative radioiodine treatment (RAIT) for DTC between 2005 and 2020 at our institution were screened to identify adolescents according to the World Health Organization definition (10-19 years of age). Demographics, clinico-pathological characteristics, treatment and outcome were analyzed.

Results: Among 1,897 DTC patients, 23 (1.3%) were adolescents with a median (range) age of 16 (10-18) years. The female to male ratio was 3.6:1. Sixty percent had classic papillary thyroid cancer, with follicular variant in 40%, which was higher than previously reported (15-25%) for this age group. pT-status was pT1 in 9 (39.2%), pT2 in 8 (34.8%), pT3 in 3 (13%) and pT4 in 3 (13%) patients. In 19 (82.6%) patients, central lymphadenectomy was performed and metastasis was seen in 57%. All patients received RAIT with initial activities of 1.2 (n=1, 4.3%), 2 (n=12, 52.2%) or 3.7 GBq (n=10, 43.5%). Eighteen (78.2%) patients were free of biochemical and radiologic disease at a median follow-up of 60.7 months. Second-line surgery for lymph node relapse was necessary in 3 (13%) cases. There was one disease-associated death.

Conclusion: Despite high rates of metastasis, most patients were cured, and second-line surgery was rarely required. Further prospective studies are needed to determine whether less aggressive surgical management or omitting adjuvant RAIT are feasible in patients with limited stages at diagnosis.

Keywords: Differentiated thyroid cancer, adolescents, prophylactic lymphadenectomy

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Introduction

Differentiated thyroid cancer (DTC) is rare in the population younger than 19 years of age (1). The currently available published evidence about this topic is limited due to the paucity of cases but suggests some interesting differences in comparison to adult DTC. For example, reported rates of cervical lymph node involvement are higher (40-80% vs. 20-50% in adults) (2,3). Even pulmonary metastases are more common (9-30% vs. 2-9%) (2). Nevertheless, the long-term outcome has been found to be generally favorable, with low mortality rates for non-radiation induced DTC (2). In a retrospective Italian analysis of 250 patients with a mean age of 14.2 years (range 4-18 years), the overall survival was 100% (4). Data from the US on patients with a median age of 17.7 years at diagnosis reported overall survival at 20 and 30 years of 100% and 94.4%, respectively. The progression free survival rates at 10, 20, and 30 years were 71%, 62%, and 55%, respectively (5). Compared to both adults and patients younger than 10 years of age, the course of disease in the 10-19 years age group seems to be less aggressive (3), suggesting that pediatric and adolescent DTC should be considered as separate entities.

In Germany, there are no specific guidelines for children and adolescents with DTC, so they are treated according to protocols for adult patients (6). The German Pediatric Oncology Hematology-Malignant Endocrine Tumor (GPOH-MET) protocol from 1995 recommended total thyroidectomy including routine central neck dissection for all children and adolescents with DTC, as well as postoperative treatment with ^{131}Iodine (^{131}I) radioiodine treatment (RAIT) (3). The American Thyroid Association (ATA) pediatric guidelines from 2015 recommend "future studies to assess if total thyroidectomy with prophylactic central node dissection will lead to reduced reliance on ^{131}I treatment, re-operative procedures, and improved diseases free survival" (7), but these studies are still pending.

Some retrospective analyses have reported recurrence rates as high as 35.7% in children and adolescents (5), generally occurring within the first five years after treatment in patients who underwent immediate postoperative RAIT (3,4,5); but there is also evidence of late events, more than 10 years after primary treatment, particularly in those patients who did not receive RAIT (5).

In order to optimize clinical management of adolescents with DTC, demographics, clinico-pathological characteristics, treatments and outcomes of adolescents treated at a single center were evaluated. Furthermore, a review of the current literature on this subject was undertaken.

Methods

Patients and Tumors

Patients who underwent postoperative RAIT for DTC between 01.01.2005 and 31.07.2020 at the University Hospital of Cologne, Germany were screened. From these patients, the adolescent group, defined as patients between 10 and 19 years of age according to the current World Health Organization (WHO) definition, was identified. Patients receiving surgical treatment at our institution as well as those only referred to our center for RAIT and oncologic care after external surgery were included in the present study.

Histologic classification was made according to the current International Agency for Research on Cancer WHO classification of tumors of endocrine organs at the time of diagnosis and staging was performed using the Union for International Cancer Control TNM classification of malignant tumors (8). The current American Joint Committee on Cancer 8th edition (9) classifies papillary thyroid cancer (PTC) as follows: pT1, tumor ≤2 cm in greatest dimension limited to the thyroid (pT1a, tumor ≤1 cm in greatest dimension limited to the thyroid; pT1b, tumor >1 cm but ≤2 cm in greatest dimension limited to the thyroid); pT2, tumor >2 cm but ≤4 cm in greatest dimension limited to the thyroid; pT3, tumor >4 cm limited to the thyroid or gross extrathyroidal extension invading only strap muscles (pT3a, tumor >4 cm limited to the thyroid; pT3b, gross extrathyroidal extension invading only strap muscles (sternohyoid, sternothyroid, thyrohyoid or omohyoid muscles) from a tumor of any size); pT4, gross extrathyroidal extension into major neck structures (pT4a, gross extrathyroidal extension invading subcutaneous soft tissues, larynx, trachea, esophagus or recurrent laryngeal nerve from a tumor of any size; pT4b, gross extrathyroidal extension invading prevertebral fascia or encasing carotid artery or mediastinal vessels from a tumor of any size).

Primary Treatment

In Germany the primary surgical management of DTC in patients <19 years is the same as in adults, according to the current guidelines (6): for PTC >1 cm diagnosed preoperatively by fine needle biopsy or intraoperatively by frozen section, the guidelines recommend total thyroidectomy and, depending on the experience of the operating surgeon, prophylactic central lymphadenectomy. Therapeutic lymphadenectomy due to proven or suspicious lymph nodes is always recommended. In case of diagnosis of follicular thyroid cancer (FTC), which is generally postoperative, the need for thyroidectomy depends on the

presence of angio-invasion. Thus, hemithyroidectomy is followed by contralateral hemithyroidectomy and RAIT, if the final histopathology report diagnoses widely invasive FTC (WIFTC) (6).

RAIT is recommended in all patients with PTC > 1 cm, PTC < 1 cm with lymph node metastases or WIFTC.

Diagnostic whole-body scintigraphy scan (DWBS) is regularly performed three months after RAIT and represents the only staging routinely performed for DTC, beside comprehensive cervical sonography.

Evaluation of Response

Evaluation of response at our institution is routinely based on physical examination, thyroglobulin blood levels (Tg) and imaging studies, including cervical sonography, DWBS with 185-370 MBq of radioiodine (^{131}I) and ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (PET/CT), if necessary. Complete response is defined as Tg ≤0.2 ng/mL, negative neck ultrasound and negative DWBS six to nine months after RAIT.

During follow-up, significant elevation of basal and stimulated serum Tg compared to the nadir value, as well as all values > 1 ng/mL measured with an ultrasensitive assay (incomplete biochemical response) again led to DWBS with 185-370 MBq of radioiodine followed by ^{18}F-FDG PET/CT if radioiodine uptake was low or absent. If DWBS was positive, patients received a therapeutic activity of 3.7 GBq of radioiodine ^{131}I. If DWBS was negative, but ^{18}F-FDG PET/CT confirmed structural recurrence, surgery was considered. If the imaging studies did not indicate recurrence, serum Tg levels were monitored, as described above (10).

In case of Tg antibodies, which are also routinely tested during follow-up, Tg cannot be used to diagnose biochemical recurrence and follow-up is mainly based on radiologic studies, including complete neck ultrasound, DWBS and/or ^{18}F-FDG PET/CT, in case of suspicion.

Surgery in Cases of Recurrence

Indication for surgery is always initiated by the multidisciplinary tumor board (MTB) at our institution. The MTB includes experts in surgery, nuclear medicine, endocrinology, histopathology and radiation therapy. Surgery for recurrence consists in resection of isolated soft tissue tumors in the perithyroidal/paratracheal space or systematic lymphadenectomy, in case structural recurrence does not appear iodine-avid on DWBS or is deemed too large for repeated ^{131}I therapy. Surgery in these cases was always performed by specialized endocrine surgeons with intraoperative frozen section and neuromonitoring.

Follow-up

Follow-up examinations took place every six months for five years after initial diagnosis and thereafter once every year in the nuclear medicine department and included physical examinations, Tg level, Tg antibodies test, and cervical ultrasound. DWBS and/or magnetic resonance imaging (MRI) and ^{18}F-FDG PET/CT were performed only if clinically indicated. Median follow-up was 60.7 months (range 12-177) after thyroidectomy. All follow-up examinations performed up to December 2021 were included in this study. Response was regularly re-evaluated according to Dietlein et al. (11).

The study was performed according to the rules and regulations for retrospective analysis of the Ethical Committee of the University Hospital Cologne (decision no: 22-1100, date: 02.03.2022).

Statistical Analysis

Data from electronic and paper records of patients identified as eligible for this study were retrospectively collected and analyzed. Data were analyzed using IBM Statistical Package for the Social Sciences statistics for Windows, version 25.0. (IBM Inc., Armonk, NY, USA).

Results

Patient Characteristics

During the review period, 1,897 patients with a median (range) age of 49 (7-87) years received RAIT for DTC. Of these 1,897, 25 (1.3%) met the study definition of adolescent at the time of diagnosis and so were included in this study. In two cases, documentation was incomplete, and these cases were, therefore, excluded. The median (range) age of adolescents with DTC undergoing RAIT was 16 (10-18) years (Figure 1).

There was a female predominance (3.6:1), with no significant (p = 0.22) age difference between male and female patients with median (range) ages of 16 (11-17) and 16.5 (10-18) years, respectively. Only one 17-year-old girl had FTC while all other cases were diagnosed with PTC, thirteen (59.1%) with classic and 9 (40.9%) with follicular variants.

Tumor Stages and Metastatic Disease at the Time of Diagnosis

One patient (4.3%) had pT1a, eight (34.7%) patients had pT1b tumors, eight (34.7%) had pT2 while there were three (13%) of each of pT3 and pT4 tumors.

Central lymphadenectomy was performed in 19 (82.6%) patients. In 11 (57.9%) cases lymph node metastases were found with a median rate of positive versus collected lymph nodes of 0.48 (range: 0.12-1). Extracapsular extension (ECE)

of metastasis was not documented regularly in pathology reports.

Distant metastases were present in 2 (8.7%) patients, both with pT4a tumors.

Radioiodine Treatment

Patients initially received RAIT with an activity of 1.2 (n = 1, 4.3%), 2.0 (n = 12, 52.2%) or 3.7 GBq (n = 10, 43.5%). One 16-year-old female patient with a pT4a pN1 (29/39) cM1 classic variant PTC (patient 23, Table 1) received a mean cumulative activity of 16.4 GBq for lymph node and lung metastases at the time of diagnosis.

Repeated Cervical Surgery

Second-line surgery for relapse after thyroidectomy was performed in 3 (13%) of these patients: patients 19, 22 and 16 (Table 1). One 15-year-old female patient (patient 19) with a pT2m pN1b (4/31) cM0 tumor developed an

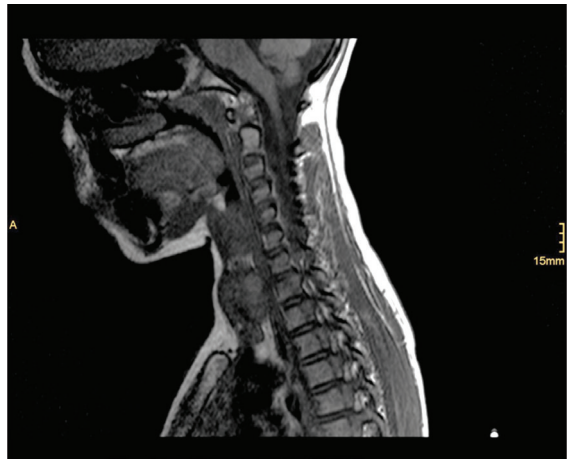

Figure 1. pT2 papillary thyroid carcinoma in a young patient included in the present study (patient 1). This MRI was performed after a fall, in order to rule out spine injury. The thyroid nodule was diagnosed incidentally

MRI: magnetic resonance imaging

Table 1. Three (13%) patients underwent repeat surgery consisting of lateral node dissection in one case and resection of paratracheal recurrences in the others. Patient 21 was diagnosed when metastatic disease was already present and subsequently deceased, despite systemic treatment. In contrast, patient 23 was free of biochemical and radiologic disease at last follow-up

Pt number	Gender, age (years)	pT	Variant	pN	cM	Cumulative RAIT activity (GBq)	Repeat surgery	Last recorded response
1.	**M, 11**	pT2	Foll. PTC	0	0	1.2	-	CR
2.	**F, 10**	pT1b	Class. PTC	1	0	2.0	-	CR
3.	**F, 17**	pT1b	Foll. PTC	0	0	2.0	-	CR
4.	**F, 14**	pT2	Foll. PTC	cN0	0	2.0	-	CR
5.	**F, 17**	pT2	Class. PTC	0	0	2.0	-	CR
6.	**F, 14**	pT3	Foll. PTC	0	0	2.0	-	CR
7.	**F, 16**	pT2	Class. PTC	0	0	2.0	-	CR
8.	**F, 17**	pT1b	Class. PTC	0	0	2.0	-	CR
9.	**F, 17**	pT1b	Class. PTC	cN0	0	2.0	-	CR
10.	**F, 17**	pT2	Class. PTC	1	0	2.0	-	CR
11.	**M, 14**	pT3	Class. PTC	1	0	2.0	-	CR
12.	**M, 17**	pT1b	Foll. PTC	cN0	0	2.0	-	CR
13.	**M, 16**	pT2	Foll. PTC	cN0	0	2.0	-	CR
14.	**F, 15**	pT3b	Class. PTC	1	0	3.0	-	CR
15.	**F, 17**	pT1b	FTC	0	0	3.5	-	CR
16.	**F, 18**	pT4a	Class. PTC	1	0	3.7	Paratracheal LN recurrence	Not available yet
17.	**F, 17**	pT2m	Class. PTC	1	0	3.7	-	CR
18.	**F, 14**	pT1m	Class. PTC	0	0	3.7	-	CR
19.	**F, 15**	pT2	Class. PTC	1	0	3.7	Paratracheal LN recurrence	IBR
20.	**F, 16**	pT1m	Foll. PTC	1	0	3.7	-	CR
21.	**F, 17**	pT4a	Class. PTC	1	1	3.7	-	Deceased
22.	**M, 17**	pT1a	Foll. PTC	1	0	3.7	Lateral LN recurrence	IBR
23.	**F, 16**	pT4a	Class. PTC	1	1	16.4	-	CR

RAIT: radioiodine therapy; M: male; F: female; Class. PTC: classic variant papillary thyroid cancer; Foll. PTC: follicular variant papillary thyroid cancer; LN: lymph node; CR: complete response; IBR: incomplete biochemical response; FTC: follicular thyroid cancer

enlarged retroclavicular and paratracheal located lymph node measuring 1.9x1.2x2.6 cm one year after initial surgery, diagnosed by sonography, MRI and positive DWBS. This was surgically removed, since it was too large for successful treatment with RAIT. Another 17-year-old male patient with a pT1a pN1 (2/2) cM0 follicular variant PTC was diagnosed 6 months after initial surgery with non-radioiodine-avid, ^{18}F-FDG-PET positive lymph nodes in the lateral compartment and underwent lateral lymphadenectomy, delivering four lymph node metastases (maximal size 1.8 cm) without ECE in 12 harvested nodes. Patient 16, initially diagnosed with a pT4a (4.2 cm), L0, V0, pN1b (12/25), M0, R0 classic PTC developed non-radioiodine-avid, ^{18}F-FDG-PET positive lymph nodes in the ipsilateral central lymph node compartment 52 months after primary treatment with small pulmonary metastases and underwent cervical surgery shortly before completion of the present study.

Overall Outcome

There was one disease-associated death in a 17-year-old girl with metastatic, classic PTC pT4 (lung, liver and bone metastasis). The patient died despite chemotherapy and tyrosine kinase inhibitors treatment with sunitinib approximately two years after thyroidectomy and RAIT (patient 21, Table 1).

Eighteen patients (78%) were cured and are free of biochemical and radiologic disease after a median follow-up of 60.7 months. These eighteen included a 16-year-old girl with a pT4a pN1 cM1 PTC (patient 23) who was last followed-up in January 2022, 120 months after first diagnosis of pulmonary involvement and 54 months after last RAIT.

Three (13%) patients required additional surgery for relapse and are currently in early follow-up. Patients 19 and 22 currently have no sign of radiologic disease but do have slightly elevated Tg levels indicating incomplete biochemical response at 107 and 12 months after surgery, respectively. Patient 16 underwent surgery for paratracheal metastatic lymph nodes diagnosed 52 months after first treatment and a further RAIT only a few weeks before completion of this manuscript. Therefore, early follow-up is not yet available.

Finally, a 15-year-old female girl with a pT2 pN1 (3/13) cM0 classic PTC, without evidence of radiologic disease had a slightly elevated Tg level (0.79 ng/L) without evidence of structural disease. None of the patients experiencing recurrence/persistence had a complete response after initial surgery and RAIT. Tg antibodies were found in only one patient (patient 16, Table 1).

Discussion

Thyroid cancer is rare in patients younger than 19 years. The recent publication of the "GPOH-MET" for the years 1995-2019 included 354 patients (3), thus there was approximately 14 patients/year in Germany. In the Netherlands, over a 43-year period, 170 patients were identified (12). Although most studies include all patients aged less than 19 years, DTC seems to be more aggressive in patients younger than 10 years of age than in adolescents (3,13,14). Thus, adolescent DTC seems to present a specific entity, which we believed warranted special focus, hence the present study.

In the present study, pT1 and pT2 status were frequent and comparable to the literature on adolescent DTC, identified in 73.9% of the cohort compared to rates of 69.6% and 58.4% previously reported by Markovina et al. (5) and Spinelli et al. (4). Similarly, rates of lymph node involvement were also comparable to those reported in similar groups, at 57.1% in the present study versus 60% reported by Spinelli et al. (4) and 65.2% reported by Markovina et al. (5). The rate of PTC follicular variant (40.9%), however, was higher in our cohort compared to these earlier studies, that reported rates of 24.8% (4) and 20.6% (5) and also when compared to a report from the USA of 15% (13). The reasons underlying these differences might be due to demographic, ethnic and/or environmental differences, but addressing this point would go beyond the scope of this study.

We observed a recurrence/persistence rate of 17.3% at a median follow-up of slightly more than five years. In contrast, Markovina et al. (5) reported recurrence in 35.7% of patients with a median follow-up duration of 18.1 years. Most recurrences observed in their study occurred within the first 10 years. One reason for the discrepancy might be because they also included some patients younger than 10 years, in whom DTC seems to be more aggressive (3,13,14). Moreover, the median follow-up of 60.7 months in our study may have missed some longer term recurrences.

The proportion of patients undergoing central lymphadenectomy in the cohort of Markovina et al. (5) was similar to ours (70%), but undergoing central lymphadenectomy did not correlate with recurrence in their study. Both our data and the currently available evidence do not allow the development of clear recommendations concerning prophylactic central lymphadenectomy for adolescent DTC. In the recent Dutch guidelines for pediatric DTC, prophylactic lymphadenectomy is not recommended in patients <18 years with a negative comprehensive ultrasound exam of all neck regions performed by a radiologist experienced in head and neck imaging (15,16).

However supporters of prophylactic central lymph node dissection underline the problems associated with decades of long-term follow-up, pleading for treatments that minimize the risk of persistence and recurrence (17).

Another possible reason for the higher recurrence rate reported by Markovina et al. (5) might be that 18% patients did not initially receive ^{131}I therapy. Routine postoperative RAIT is currently recommended in children and adolescents with tumors >1 cm in Germany and in the Netherlands (12,18). The pediatric ATA guidelines recommend ^{131}I only for nodal or other loco-regional disease that is not amenable to surgery, as well as for distant metastases that are known or presumed to be iodine-avid (7). In addition, some experts also advocate routine ^{131}I therapy for children with T3 tumors or extensive regional nodal involvement (N1a or N1b) (7). Another factor to consider is that some recent evidence has been presented suggesting that there is an increased risk of leukemia or solid cancers more than 20 years after childhood RAIT (19).

The recent data published by Redlich et al. (3) indicate age <10 years at diagnosis, ATA high-risk level, and poor response to initial therapy as significant negative prognostic factors for event free survival in pediatric DTC (5). This might help to tailor a risk-adapted individualized therapy, restricting the need for prophylactic lymphadenectomy and adjuvant RAIT for poor responders to initial treatment. Molecular pathology (20,21) and new additional diagnostic tools, such as detection of circulating tumor cells in patients' blood might also play a role for guiding treatment in the future (22).

Study Limitations

The present study cohort was not large enough to permit the formulation of robust general recommendations, especially concerning prophylactic lymphadenectomy, which was omitted in only four cases. In addition, the cohort included a high percentage of pT1 and pT2 tumors, which in general have a favorable outcome, as in adults. However, these survival rates are similar to rates reported in other studies and seem to be common in adolescents. In addition, we do not have a control group of adolescents undergoing thyroidectomy without RAIT, as RAIT is standard treatment for all patients with tumors larger than 1 cm in Germany (18). Finally, it should be remembered that age at presentation or thyroidectomy does not always represent the age at occurrence, due to the indolent biological behavior of DTC (23).

Conclusion

The need for prophylactic lymphadenectomy and adjuvant RAIT remain a matter of debate, due to the rarity of DTC in adolescents. The unique nature of these tumors presenting as more aggressive, in terms of lymph node involvement but behaving more favorably than in adults in terms of survival, deserves more attention in the future and the development of individualized treatments, as Redlich et al. (3) recently recommended. Further prospective studies are needed to determine whether less aggressive surgical management or omitting adjuvant RAIT are feasible in some patients with less severe staging at diagnosis.

Ethics

Ethics Committee Approval: The study was performed according to the rules and regulations for retrospective analysis of the Ethical Committee of the University Hospital Cologne (decision no: 22-1100, date: 02.03.2022).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Costanza Chiapponi, Boris Decarolis, Thorsten Simon, Christiane Josephine Bruns, Michael Faust, Anne Maria Schultheis, Matthias Schmidt, Hakan Alakus, Concept: Costanza Chiapponi, Matthias Schmidt, Hakan Alakus, Design: Costanza Chiapponi, Data Collection or Processing: Costanza Chiapponi, Milan Janis Michael Hartmann, Matthias Schmidt, Hakan Alakus, Analysis or Interpretation: Costanza Chiapponi, Milan Janis Michael Hartmann, Boris Decarolis, Thorsten Simon, Christiane Josephine Bruns, Michael Faust, Anne Maria Schultheis, Matthias Schmidt, Hakan Alakus, Literature Search: Costanza Chiapponi, Milan Janis Michael Hartmann, Hakan Alakus, Writing: Costanza Chiapponi, Milan Janis Michael Hartmann, Boris Decarolis, Thorsten Simon, Christiane Josephine Bruns, Michael Faust, Anne Maria Schultheis, Matthias Schmidt, Hakan Alakus.

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The Effect of the SARS-CoV-2 Pandemic on Presentation with Diabetic Ketoacidosis in Children with New Onset Type 1 Diabetes Mellitus

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What is already known on this topic?

In children with type 1 diabetes mellitus (T1DM), there may be decreased neutrophil function, T-cell response and abnormal humoral immunity, any or all of which may result in increased susceptibility to various infections. These infections may trigger acute complications of T1DM including diabetic ketoacidosis (DKA) and, on some occasions, hypoglycemia.

What this study adds?

Although there was no increase in the frequency of DKA in our study, an increase was observed in severe DKA cases. First-line clinicians should encourage information on early diagnosis, and children with new-onset T1D and/or DKA symptoms should have prompt access to experienced teams.

Abstract

Objective: Diabetic ketoacidosis (DKA) is a life-threatening, acute complication of type 1 diabetes mellitus (T1DM). Infection is the most common precipitating factor for DKA, being responsible for more than 50% of such complications. The frequency and severity of DKA in children with T1DM, before and during the coronavirus disease 2019 outbreak were evaluated and compared with pre-pandemic presentation and severity rates.

Methods: In total, 199 patients younger than 18 years were included in the study. Patients were divided into two groups: the Coronavirus disease-2019 (COVID-19) pandemic group (new onset T1DM presenting from March 2020 to March 2021; the control group included new onset T1DM from March 2016 to March 2020.

Results: The rate of DKA at presentation was similar ($p = 0.393$) during the pandemic period (58.3%) compared to the pre-pandemic years (44.8-64.3%). Although the percentage of DKA was similar, the rate of severe DKA in the COVID-19 group was higher than previous years. Although not significant, the duration of diabetes symptoms was longer in the COVID-19 period than the previous years.

Conclusion: This study suggests that the rate of severe DKA, but not the overall rate of DKA, has increased during the COVID-19 pandemic compared to the prior four years. This may be due to the behavior of the parents of sick children and the limited access to the healthcare system. Despite this limited access, parental concern may have been sufficiently high to seek medical attention for their children, avoiding an increased frequency of DKA as the first presentation of new-onset T1DM.

Keywords: Type 1 diabetes mellitus, diabetic ketoacidosis, Coronavirus disease-2019

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Introduction

Type 1 diabetes mellitus (T1DM) is characterized by chronic immune-mediated destruction of pancreatic β cells leading to partial or absolute insulin deficiency. In the majority of cases, T1DM results from autoimmune-mediated pancreatic β-cell destruction and is influenced by different factors, such as genes, age, and ethnicity (1). In children with T1DM, there may be decreased neutrophil function, T-cell response and abnormal humoral immunity, which may result in increased susceptibility to various infections (2,3). These infections may trigger acute complications of T1DM, including diabetic ketoacidosis (DKA), a potentially severe and life threatening condition, and in some occasions, hypoglycemia. There are reports about the relationship between fulminant DM and different infections (4,5,6,7), but such reports involving Severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) infection are rare (8). However, infection is often a precipitating factor for DKA and has been reported to be responsible for more than half of cases (9).

Pancreatic β cell function may deteriorate after Coronavirus disease-2019 (COVID-19), and this may trigger DKA in diabetic patients (10). The aim of this study was to determine the clinical characteristics and frequency of DKA in children diagnosed with T1DM during the COVID-19 pandemic and to compare this with similar characterisitics in patients presenting in the pre-COVID-19 years.

Methods

The study population included children less than 18 years of age presenting with new onset T1DM. The COVID-19 pandemic group comprised those presenting with new onset T1DM from March 2020 to March 2021. The control group included new onset T1DM presenting from March 2016 to March 2020.

Patients with syndromic diabetes, type 2 diabetes, maturity onset diabetes in the young, and secondary diabetes due to any cause, such as cystic fibrosis, steroid use or lipodystrophy, were excluded. Patient data were analyzed by retrospective review of medical records. All patients were diagnosed with T1DM according to the guidelines of the International Pediatric and Adolescent Diabetes Association (ISPAD). DKA was defined according to ISPAD criteria (blood glucose >11 mmol/L, venous pH <7.3 or bicarbonate <15 mmol/L, ketonemia and ketonuria), and severe DKA was categorized as pH <7.1, bicarbonate <5 mmol/L (11,12).

Ethics approval was granted by the Ege University Ethics Committee (approval number: 21-6.1T/71, date: 10.04.2016).

Statistical Analysis

All analysis were performed using Statistical Package for the Social Sciences, version 21 (IBM Inc., Chicago, IL, USA). Categorical data are described using observed frequencies and percentages, and continuous variables are summarized as mean and standard deviation. In cases of non-parametric distribution of data, median and interquartile range is used to describe the data set. Patient and control data were compared using chi-square test. A p value <0.05 was considered to be statistically significant.

Results

Between March 2016 and March 2021, 199 patients were diagnosed with T1DM, of whom 105 (52.7%) were boys. Baseline demographics and characteristics at diagnosis are given in Table 1. The mean age at the time of diagnosis of the patients was 8.4 ± 3.8 years, and ranged from 2 to 18 years.

The rate of DKA at presentation was similar in the COVID-19 group (2020 and 2021) compared to the pre-pandemic

Table 1. Demographics and clinical characteristics at diagnosis of T1DM from March 2016 to March 2020 (control period) and March 2020 to March 2021 (pandemic period)

		2020-2021	2019-2020	2018-2019	2017-2018	2016-2017
New onset diagnosed T1DM	(n)	48	47	33	29	42
Median (range) age at diagnosis (years)		9.4 (0.8-18)	7.0 (1.2-17)	9.8 (1.2-16.5)	9.2 (1.09-16)	9.8 (0.7-15.5)
Age groups n (%); median (range) age (years)	≤5 years	11 (23%); 3.1 (1.1-4.9)	14 (29.8%); 3.2 (2.8- 4.5)	6 (18.2%); 4.3 (4- 4.8)	6 (20.7%); 3.9 (3.2- 4.6)	12 (28.6%); 4.1 (1.7- 4.9)
	5-12 years	19 (39.5%); 8.7 (5.1-11.6)	21 (44.7%); 7.8 (5.2-11.7)	14 (42.4%); 10.3 (5.6-11.7)	14 (48.3%); 9.5 (5.1-11.9)	12 (28.6%); 7.9 (5.1-11.3)
	12-18 years	18 (37.5%); 13.7 (12.2-18)	12 (25.5%); 14.5 (12.1-17.5)	13 (39.4%); 15.4 (12.4-17.5)	9 (31.0%); 15.6 (12.5-18.0)	18 (42.9%); 16.2 (12.4-17.9)
Median (range) duration of symptoms (days)		30 (2-90)	10 (1-90)	10 (1-90)	8.5 (4-90)	20 (0-90)

T1DM: type 1 diabetes mellitus

control period (2016-2018) (58.3% in 2020 vs 55.3% in 2019, 45.5% in 2018, 44.8% in 2017, 64.3% in 2016, $p > 0.05$). Although the rate of DKA was similar, the rate of severe DKA in the last 2 years was higher than previous years (30.4% in 2020 and 45.7% in 2019, 24.2% in 2018, 18.5% in 2017, 17.1% in 2016, $p = 0.027$). Although statistically insignificant, the mean duration of symptoms prior to presentation during the pandemic period was longer than the previous years ($p > 0.05$) (Table 1).

Discussion

The COVID-19 pandemic has continued since first being declared in early 2019. During the pandemic period, many national health care systems have experienced great difficulties and resources have been stretched. Furthermore, given the fear and uncertainty in the population, especially in the initial period of the pandemic many people postponed doctors appointments and did not attend hospital until complaints had progressed (13,14). The study center for the present study is a tertiary level university hospital with 165,000 outpatients/year. Following the movement restrictions imposed because of the COVID-19 pandemic, services were transferred to online outpatient clinics and only emergencies were accepted.

Delayed diagnosis of T1DM may predispose to DKA and thus DKA incidence may be expected to increase during COVID-19 due to late presentation. Although there is conflicting evidence about whether or not COVID increased the frequency of DKA, it does not seem to have increased the incidence of T1DM during the first 14 months of the pandemic (15,16,17). Ho et al. (18) in a retrospective analysis of 221 new onset T1DM in a single center in Canada, showed no increase in the incidence of T1DM during 2020, compared to 2019.

Zubkiewicz-Kucharska et al. (19) analyzed the incidence of diabetes in the last 20 years from lower Silesia in 1961 new onset T1DM pediatric patients. The incidence of T1DM in children from Lower Silesia during the COVID-19 pandemic was compared with previous years, and their clinical status (incidence of DKA, the mean pH upon at admission) at the time of diagnosis was worse during the pandemic than prior to it. A study from Southeastern Brazil revealed a higher incidence and severity of new onset T1DM cases with DKA during the pandemic period (20). Lawrence et al. (21) compared new onset pediatric T1DM patients during the pandemic to those presenting in the previous 5 years and showed an increased incidence of severe DKA during pandemic period. These authors attributed this to delayed admission as a result of concerns regarding COVID-19 and the restrictions put in place to combat the COVID-19 pandemic. In the study of Kamrath et al. (22), 532 children with new onset T1DM presenting during the pandemic were examined and the frequency of DKA and severe DKA was 44.7% and 19.4% respectively, which was double that found in the pre-pandemic period (aRR 1.8-2.7 and aRR 1.4-2.1, respectively). Patients in younger age groups exhibited a higher risk of DKA (RR 2.1-2.8). In the present study, the frequency of DKA was 58.3%, while the frequency of severe DKA was 30.4%. Although Turkey imposed had intensive measures aimed at combatting the spread of SARS-CoV-2 and most outpatient clinics were suspended except for emergencies, the incidence of DKA at presentation with newly diagnosed T1DM did not change compared to previous years.

In the present study, the duration of symptoms prior to presentation during the pandemic period was 30 (2-90) days. In the study of Zubkiewicz-Kucharska et al. (19), the mean duration of symptoms was 13.1 ± 10.96 days, which was similar to previous years. In our study, although the duration of symptoms was not statistically significant, was much longer than expected and longer than the previous years (Table 1). The increased frequency of severe DKA, may be due to the delay in accessing health care due to lack of access or fear of COVID-19. Although a one-year period is a relatively short period of time to draw detailed and robust conclusions about the impact of the COVID-19 outbreak, this study showed an increased incidence of severe DKA compared with the three years prior to the pandemic and longer duration of symptoms prior to presentation.

Study Limitations

We did not have data about the presence of antibodies to SARS-CoV-2 or previous COVID-19 infection in the patients with new onset T1DM in the years 2019 and 2020. Furthermore, making estimates of changes in incidence of a relatively rare disease in a small population on a year-to-year basis is somewhat unreliable although trends of clinical significance may be identified.

Conclusion

Although COVID-19 infection does not seem to have increased the incidence of DKA, it is important to encourage knowledge among first-line clinicians about the likelihood of DKA likely to increase during the COVID-19 outbreak. Severe DKA was seen more frequently at the time of diagnosis in children with T1DM diagnosed during the pandemic period, and the duration of hyperglycemia symptoms was longer than in previous years. Delays in diagnosis are possibly due to parental behavior and poor access to healthcare.

Ethics

Ethics Committee Approval: Ethics approval was granted by the Ege University Ethics Committee (approval number: 21-6.1T/71, date: 10.04.2016).

Informed Consent: Written consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Arzu Jalilova, Damla Gökşen, Design: Şükran Darcan, Damla Gökşen, Data Collection or Processing: Arzu Jalilova, Hafize Işıklar, Analysis or Interpretation: Günay Demir, Samim Özen, Şükran Darcan, Literature Search: Arzu Jalilova, Yasemin Atik Altınok, Writing: Arzu Jalilova, Aysun Ata, Damla Gökşen.

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Growth Hormone Dosing Estimations Based on Body Weight Versus Body Surface Area

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What is already known on this topic?

Drug doses calculated based on body weight and body surface area may be different under certain circumstances.

What this study adds?

In children younger than 11 years of age with body mass index levels less than 18 kg/m^2, growth hormone dosing based on body weight may be preferable.

Abstract

Objective: Both body weight (BW)- and body surface area (BSA)-based dosing regimens have been recommended for growth hormone (rhGH) replacement. The aim was to compare the two regimens to determine if either resulted in inadequate treatment depending on anthropometric factors.

Methods: The retrospective study included children diagnosed with idiopathic isolated growth hormone deficiency. BW-based dosing in mcg/kg/day was converted to BSA in mg/m^2/day to determine the equivalent amounts of the given rhGH. Those with a BW-to-BSA ratio of more than 1 were allocated to the "relatively over-dosed group", while the remaining patients with a ratio of less than 1 were assigned to the "relatively under-dosed" group. Patients with a height gain greater than 0.5 standard deviation score (SDS) at the end of one year were classified as the height gain at goal (HAG), whereas those with a height gain of less than 0.5 SDS were assigned as the height gain not at goal (NHAG).

Results: The study included 60 patients (18 girls, 30%). Thirty-six (60%) patients were classified as HAG. The ratio of dosing based on BW-to-BSA was positively correlated both with the ages and body mass index (BMI) levels of the patients, leveling off at the age of 11 at a BMI of 18 kg/m^2. The relative dose estimations (over- and under-dosed groups) differed significantly between the patients classified as HAG or NHAG. Fifty-six percent of NHAG compared to 44% of HAG patients received relatively higher doses, while 79% of HAG compared to 21% of NHAG received relatively lower doses ($p = 0.006$). When the patients were subdivided according to their pubertal status, higher doses were administrated mostly to the pubertal patients in both the NHAG and HAG groups. In the pre-pubertal age group, 73% of NHAG compared to 27% of HAG received relatively higher doses, while 25% of NHAG compared to 75% of HAG received relatively lower doses ($p = 0.01$).

Conclusion: Dosing based on BW may be preferable in both prepubertal and pubertal children who do not show adequate growth responses. In prepubertal children, relatively lower doses calculated based on BW rather than BSA provide similar efficacy at lower costs.

Keywords: Body surface area, body weight, growth hormone, IGF-1, IGFBP-3, pharmacotherapy

Introduction

Dysfunction of the growth hormone (GH)-insulin-like growth factor-1 (IGF-1) axis may result in varying degrees of growth failure and a variety of other pathological clinical features, including central obesity, loss of lean muscle mass, osteoporosis, deterioration of metabolic profile, and decreased cardiac function (1,2,3). The diagnosis of growth

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hormone deficiency (GHD) is accomplished by combining medical history, auxological measures, biochemical markers, and radiological imaging (2,4).

The standard treatment for GHD is recombinant human growth hormone (rhGH) replacement, which is customized to each child's body weight (BW) or body surface area (BSA) (5). Both BSA- and BW-based dose regimens have been recommended for rhGH replacement, on the assumption that they are equally effective (5,6). Yet, some countries still choose one regimen over the other (7,8,9,10,11).

Nevertheless, under certain circumstances, the differences between the two dosing regimens may become apparent (4,7,8,12). For instance, it has been demonstrated that BW-based dosing considerably underestimates the necessary treatment in individuals with weights of less than 30 kg (6). In contrast, due to disparities in drug clearance, obese patients are at risk of over-exposure when BW-based dosing is used, and of underexposure when BSA-based dosing is preferred (13). Hence, alternate rhGH dosing may be utilized for certain patient groups to boost effectiveness and/or to decrease toxicity (13,14).

In general, BSA-based regimens are favored for antineoplastic medications, whereas BW-based regimens are favored for cardiovascular, central nervous system, and anti-infective treatments (13). BSA-based dosing has been considered to be more closely associated with total body water, extracellular body fluid, total clearance, liver volume, and renal function (15,16). Given that rhGH is predominantly metabolized in the liver and kidneys, and the kidneys account for around 60-90% of the clearance, BSA-based dosing seems to be a more favorable method for rhGH replacement (7,17,18). However, there is insufficient data to support one over the other (5).

There is limited data (7,8) comparing the effectiveness of different rhGH dosages with regard to BSA versus BW. Homogenous studies on BW and BSA-based dosing strategies are needed. This retrospective study was designed to compare the rhGH doses in BW versus BSA in children diagnosed with idiopathic isolated GH deficiency (IGHD) who were not obese. Moreover, based on the growth responses of the patients over the first year of treatment, it would be evaluated whether either of the two regimens would result in higher or lower treatment under different conditions of age and patient anthropomorphic characteristics.

Methods

Patients

The retrospective study included children aged 1-18 years from two different centers. Individuals with obesity, defined as a body mass index (BMI) standard deviation (SD) scores ≥2, genetic anomalies, scoliosis, chronic diseases, such as diabetes mellitus or celiac disease, a history of significant trauma, low birth weight, neoplasia, brain tumor, or intracranial radiation were excluded from the study. Children with short stature who had IGHD and were treated with rhGH for at least one year between 2017 and 2022 were included. GHD was suspected in the presence of short stature (< -2 SDS) or growth deceleration (velocity < 25% of corresponding chronological age), and diagnosed when serum peak GH concentration was less than 7 ng/mL in two different GH stimulation tests (clonidine, insulin tolerance test, and levodopa) (2,9). Isolated deficiency is defined as the presence of a solitary pituitary hormone deficiency. Each child received 25-35 mcg/kg/day of rhGH replacement (4). The changes in height velocity and height SD scores were evaluated to assess treatment efficacy while IGF-1 and insulin-like growth factor binding protein-3 (IGFBP-3) levels were monitored in order to avoid excessive dosing to ensure safety of the treatment (2,4,19,20). Clinical and laboratory data were monitored every 6-12 months to adjust rhGH doses (mg) (2,4). To exclude concomitant pathologies, each patient underwent pituitary MRI at the start of the therapy.

Data Collection

The following clinical parameters were recorded: age (years); gender; pubertal status [according to Tanner and Whitehouse (21)]; bone age [calculated according to the Greulich and Pyle (22) Atlas; height [measured with a sensitivity of 0.1 cm, using a Harpenden stadiometer, (cm)], weight [measured using a scale with a sensitivity of 0.1 kg, (kg)], BMI (kg/m^2), target height (mother's height + father's height)/2 ± 6.5, (cm)], predicted adult height [calculated according to the Roche et al. (23) method, (cm)], and the respective SD scores [calculated according to Turkish standards (24)]. The IGF-1/IGFBP-3 levels and the respective SD scores were recorded (24). The prescribed rhGH doses based on BW were also recorded. All data, which was re-evaluated every six months, was recorded.

Design of the Study

For the purpose of this paper, BW-based dosing in mcg/kg/day, which is routinely employed in our clinical practice, was converted to BSA in mg/m^2/day. Assuming that the average BW of a child with a BSA of 1 m^2 is 28 kg (7,8), all doses were separately converted to equivalent BSA formats. Hence, the routinely prescribed doses of 25, 30, and 35 mcg/kg/day were found to be equivalent to 0.7, 0.8, and 1 mg/m^2/day, respectively.

Then the BSA of each patient was calculated separately using the following empirical formulas:

i. Costeff's Formula (25): BSA (m^2) $= \frac{4\,x\,weight\,(kg) + 7}{weight\,(kg) + 90}$

ii. Mosteller's Formula (26): BSA (m^2) $= \sqrt{\frac{(weight\,(kg) \times height\,(cm))}{3600}}$

Finally, initially prescribed doses based on BW (mcg/kg/day) and the hypothetically calculated equivalent doses based on BSA (mg/m^2/day) were calculated for each patient to be given as milligrams per day (mg/day).

Stratification of the Patients

Patients were divided into two groups based on their height increase over one year. The change in height SD score was determined by subtracting the height SD score measured at the beginning of treatment from the height SD score measured after the first year of rhGH treatment. Based on Bang criteria (27), those with a height gain greater than 0.5 SD score at the end of one year were classified as height gain at goal (HAG), whereas those with a height gain of less than 0.5 SD score were classified as height gain not at goal (NHAG).

Patients were also divided into two groups based on their actual (BW-based) and estimated (BSA-based) rhGH doses in mg. Those with a BW-to-BSA ratio of more than 1 were allocated to the "relatively over-dosed group" (n = 32), while the remaining patients with a ratio of less than 1 were assigned to the "relatively under-dosed" group (n = 28).

Ethical Approval

This study was approved by the Ethical Committee of Dokuz Eylül University Faculty of Medicine (approval number: 2022/42-14, date: 28.12.2022) and performed according to the principles of the Declaration of Helsinki. Informed consent to participate in the study was obtained from all participants (or their parents or legal guardian in the case of children under 16).

Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences, version 24 for Windows (IBM Inc., Armonk, NY, USA). The homogeneity of the data obtained in the study was tested using Shapiro-Wilk and Kolmogorov-Smirnov tests. Numerical variables were not normally distributed and so non-parametric presentation was used; median [interquartile range (IQR)], unless otherwise stated. The correlation between the actual and estimated doses was assessed with Spearman's correlation test (r_s). Categorical variables were analyzed by chi-square or Fisher's exact test. All tests were two-tailed, and a p value of less than 0.05 was taken as statistically significant.

Results

The study included 60 patients with IGHD, of whom 18 (30%) were girls, and the whole cohort had a median (IQR) age of 11.9 (3.8) years. Table 1 summarizes the characteristics of all patients and the comparison of patients with HAG vs NHAG. Of the 60 patients, 36 (60%) were classified as HAG after one year of treatment. Overall, the median (IQR) dose administered per kg BW (mcg/kg/day) was reduced significantly over 1-year period [30 (4) and 27.6 (7) mcg/kg/day; p = 0.007]. While the doses were similar in the prepubertal group [30 (5) and 28 (7), p = 0.29, respectively], they were significantly reduced in the pubertal group [30 (1.5) and 26 (7), p = 0.002, respectively]. The two groups classified as HAG and NHAG were not significantly different in terms of sex, ages at the start of treatment, puberty status, rhGH doses, target height, predicted adult height, and SD scores for weight, BMI, IGF-1, and IGFBP-3 (Table 1). The follow-up of the SD scores for weight and height, along with IGF-1 and IGFBP-3 levels are presented in Figure 1. The SD scores for IGF-1 levels were in the reference ranges at the end of the first year of treatment. IGF-1 levels were not correlated with the prescribed doses ($r_s = 0.164$, $p = 0.22$; $r_s = 0.14$, $p = 0.3$; $r_s = 0.14$, $p = 0.3$, according to BW and BSA calculated either by Costeff's and Mosteller's formulas, respectively).

The estimated daily doses calculated for BSA using Costeff's and Mosteller's formulas were strongly correlated [(r_s) = 0.974, $p < 0.001$]. The actual daily doses given, based on BW and the estimated doses calculated according to BSA were also strongly correlated (Spearman's correlation (r_s) = 0.990, $p < 0.001$; (r_s) = 0.977, $p < 0.001$, BSA calculated with Costeff's and Mosteller's formulas, respectively). The median BW-to-BSA was 1 (0.2), with a full range of 0.65 to 1.34, while BSA-to-BW ratio ranged from 0.75 to 1.53. The ratio of the dose given based on BW and the dose calculated according to BSA were positively correlated both with the ages and BMI of the patients for both Costeff's formula, (r) = 0.814, $p < 0.001$, (r) = 0.776, $p < 0.001$ and Mosteller's formula (r) = 0.747, $p < 0.001$, (r) = 0.797, $p < 0.001$) (Figure 2). As shown in Figure 2a, b, the ratio of BW-to-BSA was equal to 1 at the age of approximately 11 years with a BMI of 18 kg/m^2. The slopes and the intercepts calculated for the best-fit lines for both Costeff's and Mosteller's formulas yielded similar results (age: Costeff's formula: Y = 0.03331*X + 0.6254; Mosteller's formula: Y = 0.03587*X + 0.6009; BMI: Costeff's formula: Y = 0.03250*X + 0.4222; Mosteller's formula: Y = 0.03939*X + 0.3043).

Table 1. The clinical and laboratory characteristics of the patients

Clinical and laboratory characteristics	All patients, (n = 60)	Height gain at goal, (n = 36)	Height gain not at goal, (n = 24)	p[a]
At GH start				
Age, years	11.9 (3.8)	11.9 (5.3)	12 (2.8)	0.39
Prepubertal (%)	35 (58%)	21/36 (58%)	14/24 (58%)	1.00[b]
Weight, SDS	-1.9 (1.4)	-2.2 (1.8)	-1.7 (1)	0.12
BMI, SDS	-0.5 (1.5)	-0.6 (1.7)	-0.4 (0.9)	0.83
Height, SDS	-2.8 (1)	-3.1 (1.1)	-2.5 (0.6)	**0.02**
Bone age, years	9 (5)	7.8 (6.8)	9.8 (3.4)	0.12
Target height	165.5 (11.8)	166 (12)	166 (9)	0.71
Target height, SDS	-1.2 (1.1)	-1 (1.4)	-1.6 (1.6)	0.13
Predicted adult height	162.6 (13.7)	161 (15)	163 (10)	0.15
Predicted adult height, SDS	-1.5 (1.4)	-1.8 (1.7)	-1.5 (1.2)	0.9
IGF-1 at the start, SDS	-1.2 (1.2)	-1.3 (1.4)	-1.0 (1.3)	0.24
IGFBP-3 at start, SDS	-0.6 (1.6)	-0.7 (1.9)	-0.5 (1.3)	0.73
Peak GH responses, ng/mL	4.3 (4)	4.3 (3.4)	4.1 (4)	0.37
GH doses				
mcg/kg/day	30 (4)	30 (3.5)	30 (4)	0.78
mg/m^2/day (Costeff)	0.8 (0.4)	0.8 (0.4)	0.8 (0.3)	0.84
mg/m^2/day (Mosteller)	0.8 (0.4)	0.8 (0.4)	0.8 (0.3)	1
At 1st year of GH treatment				
Height, SDS	-2.2 (1)	-2 (1.4)	-2.2 (0.8)	0.14
Weight, SDS	-1.7 (1.4)	-1.7 (1.6)	-1.2 (1)	0.82
BMI, SDS	-0.4 (1.3)	-0.5 (1.6)	-0.2 (1)	0.88
Bone age, years	11 (4.6)	10.5 (7.5)	11 (2.5)	0.40
GH dose, mcg/kg/day	27.6 (7)	28 (7.5)	27 (8.2)	0.64
Predicted adult height	167.3 (15.7)	167 (15)	170 (13)	0.5
IGF-1, SDS	0.6 (1.2)	0.5 (1.7)	0.8 (1.3)	0.47
IGFBP-3, SDS	0.4 (1.5)	0.4 (1.7)	0.7 (1)	0.10
Annual Δ Height SDS	0.6 (0.5)	0.9 (0.6)	0.3 (0.3)	**0.02**

Data are given as median (interquartile range). [a]Mann-Whitney U test. [b]Chi-squared test.
GH: growth hormone, SDS: standard deviation scores, BMI: body mass index, IGF-1: insulin-like growth factor-1, IGFBP-3: insulin-like growth factor binding protein-3, Annual Δ height: height at 1st year of treatment - height at start of treatment

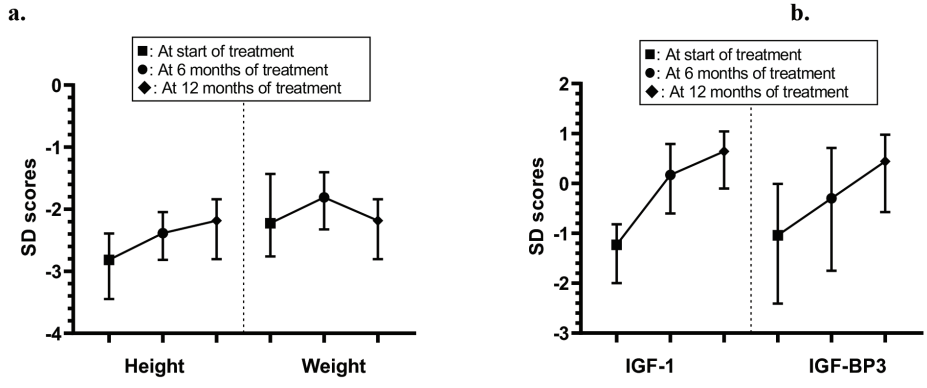

Figure 1. The standard deviation scores for a) auxological measurements for height and weight and b) laboratory tests for IGF-1 and IGFBP-3 levels. The symbols represent median values and the vertical bars indicate the interquartile range

SD: standard deviation, IGF-1: insulin-like growth factor-1, IGFBP-3: insulin-like growth factor binding protein-3

Table 2 shows the number and percentages of patients according to growth responses (HAG, NHAG) and relative dose estimations (relatively over- and under-dosed groups). The relative dose estimations (relative over- and under-dosed groups) differed significantly between the patients classified as HAG or NHAG. Fifty-six percent of patients in the NHAG group compared to 44% of patients in the HAG group received relatively higher doses, while 79% of patients classified as HAG compared to 21% of patients classified as NHAG received relatively lower doses (p = 0.006). When the patients were subdivided according to their pubertal status, the results showed that higher doses were administered mostly to the pubertal patients in both NHAG and HAG groups (10/18; 56% and 11/14; 79%, respectively). In the pre-pubertal age group, 73% of patients classified as NHAG compared to 27% of patients in the HAG group received relatively higher doses, while 25% of patients classified as NHAG compared to 75% of patients classified as HAG received relatively lower doses (p = 0.01). In the pubertal groups, patients in the NHAG and HAG groups received comparable doses (p = 0.125) (Table 2).

Discussion

In this study, differences between the BW- and BSA-based dosing methods have emerged in children. We have shown that rhGH dosage calculations based on BW compared to BSA may result in the administration of relatively higher or lower doses, depending on the ages and BMIs of the patients, which may be particularly important in patients with good growth responses.

The notion that BW- and BSA-based dosages are equivalent has been examined by various studies in several disciplines, and the potential risks of over and undertreatment have

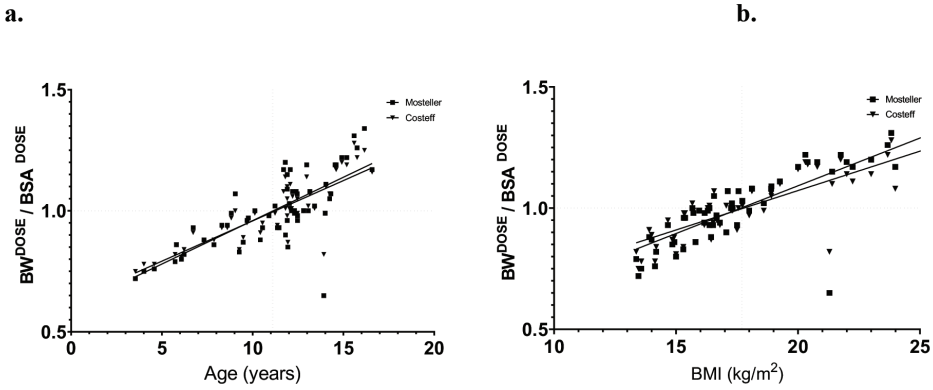

Figure 2. a) The ratio between the actual dose given in body surface area (calculated according to Mosteller's and Costeff's formulas) and the dose calculated per body weight versus age (years) is shown. The equations for the slopes are as following: Costeff's formula: Y = 0.03331*X + 0.6254; Mosteller's formula: Y = 0.03587*X + 0.6009. b) Body surface area/body weight -based dose ratio versus body mass index (kg/m^2). The equations for the slopes are as following: Costeff's formula: Y = 0.03250*X + 0.4222; Mosteller's formula: Y = 0.03939*X + 0.3043. Squares indicate doses calculated based on Mosteller's formula, triangles indicate doses calculated based on Costeff's formula

BW: body weight, BSA: body surface area, BMI: body mass index

Table 2. The comparison of groups that were relatively over- and underdosed

Characteristics	All patients, (n = 60)	Height gain at goal, (n = 36)	Height gain not at goal, (n = 24)	p
Relatively over-dosed group	32/60 (53%)	14/32 (44%)	18/32 (56%)	**0.006**[a]
Relatively under-dosed group	28/60 (47%)	22/28 (79%)	6/28 (21%)	
Prepubertal subgroup, (n = 35)				
Relatively over-dosed group	11/35 (31%)	3/11 (27%)	8/11 (73%)	**0.01**[b]
Relatively under-dosed group	24/35 (69%)	18/24 (75%)	6/24 (25%)	
Pubertal subgroup, (n = 25)				
Relatively over-dosed group	21/25 (84%)	11/21 (52%)	10/21 (48%)	0.125[b]
Relatively under-dosed group	4/25 (16%)	4/4 (100%)	0/4 (0%)	

Number (n) of patients with percentages (%) are presented. Dose ratio was calculated as following: Dose given according to body weight [body weight (BW); mg]/dose calculated according to body surface area (BSA); mg. Over-dosed group indicates a dose ratio of BW-to-BSA greater than 1; under-dosed group indicates a dose ratio of less than or equal to 1. BSA was calculated according to Mosteller's formula.
[a]: Pearson chi-square test, [b]: Fisher's exact test

been established (6,28,29,30,31). Both strategies were assumed to be equally effective in treatment, with significant differences only observed at the extremes of weight and in very young patients (32). In contrast to the general belief of equal efficacy, Hughes et al. (7) demonstrated that even slight increases in BW-based doses could correspond to higher values when converted to mg/m^2/week. The results of the present study indicate that the difference between BW- and BSA-based dosing increased proportionally as patients' age and BMI values increased. The actual and estimated doses were equal at the age of approximately 11 years with a BMI level of 18 kg/m^2. Thus, older patients with higher BMIs would be given higher doses if BW-based methods were chosen over BSA-based calculations.

Due to the variations in the pharmacokinetics of medications resulting from changes in growth and maturity, dosage recommendations for children are often subdivided into age categories of 2-6 years, 6-12 years, 12-18 years, and 18-21 years (33). Likewise, the Pediatric Pharmacy Advocacy Group (33,34) also recommends BW-based dosing for children weighing less than 40 kg. Even though these strategies have not been generalized for patients who are receiving rhGH treatment, different dosing strategies have also been explored among girls with Turner syndrome (8). Similar results were seen in the homogenous group of patients with IGHD with similar characteristics in the present study, also suggesting that different efficacy and safety profiles may result for different age groups.

Differences were also found between the estimations based on BW and BSA when patients were stratified by response to treatment in terms of height gained. Among patients in the HAG or NHAG groups, the percentages of patients who would be have been given relatively higher or lower doses of rhGH differed significantly. Fifty six percent of all patients classified as NHAG, most of whom were pubertal, received higher doses when using BW-based in comparison to BSA-based calculations. Furthermore, almost three-quarters of pre-pubertal patients (73%) classified as NHAG were given relatively higher doses using BW-based calculation. This could suggest that this method based on BW would be preferable in both prepubertal and pubertal groups with inadequate height gain, since BSA-based estimates would result in the administration of relatively lower doses to those with poor growth responses. However, these associations should be interpreted carefully. Although the differences were not significant, patients in the NHAG group had relatively shorter target heights and older bone ages, with statistically significant lower height SD scores at start compared to patients in the HAG group, all of which may be indicative of poor growth response (35). Moreover, it is also impossible to predict whether higher doses would have resulted in better growth responses.

Futhermore, dosing based on BW resulted in lower but adequate doses for those exhibiting the expected growth response (HAG group). In other words, if dosing based on BSA had been chosen, the majority of patients with HAG (79%) would have received unnecessarily high doses of rhGH, whereas patients in the NHAG group (56%) would have been dosed relatively inadequately low. For patients with the expected height gain in the first year (HAG), subgroup analysis showed that the relative dose difference in favor of BW-based calculations was mostly attributable to the prepubertal group. Among the prepubertal HAG patients, 75% would have been dosed relatively higher if the BSA-based method had been chosen. Similarly, Hughes et al. (7) suggested that BSA-based dosing would result in overtreatment for most children, including those with Turner syndrome and GHD, but not excluding those with obesity. Similar to our findings, both Schrier et al. (8) indicated that younger children would receive more rhGH doses based on BSA in comparison to BW-based doses (11).

These results are important because of the two main drawbacks associated with relative overtreatment with rhGH. Firstly, excessive rhGH may result in potential adverse effects. IGF-1 and IGFBP-3 levels were monitored and were in the reference ranges for our cohort who actually received rhGH doses based on BW while if relatively higher doses had been given using BSA-based methods, there may have been a need for more frequent dose adjustments or clinic visits. Secondly, prescribing higher GH doses to good responders would also result in unnecessary expenditure. Schrier et al. (8) also demonstrated that, despite comparable efficacy, the predicted financial savings for rhGH doses based on BSA and BW would be significantly different. Similarly, the ratio of BSA-to-BW extended to 1.53, indicating that if dosing based on BSA rather than BW had been adopted, the costs would have been 53% higher, despite equal efficacy. Considering that GH treatment is expensive, with an average annual cost of up to 7,088 Euros for a 30-kg child with GH deficit, for children younger than 11 years with BMI levels less than 18, the BSA-based dosage would not be cost-effective in comparison to the BW-based calculations (36).

Study Limitations

Our study was retrospective and we were not able to prescribe both dosings randomly to a larger cohort due to ethical and logistical barriers. Furthermore, due to small group size, we were not able to draw robust conclusions concerning the pubertal group. Another weakness was that the study included two different centers but both

centers have been following identical strategies regarding follow-up and rhGH dosing. The greatest strength of our study was that none of the patients had been taking any additional medications, and the safety of treatment was strictly controlled by routinely measured IGF-1/IGFBP-3 values. Thus, the two dosing strategies were hypothetically compared in a homogenous cohort with IGHD who were not overweight and did not have any co-morbidities.

Conclusion

BW- and BSA-based strategies were compared in an homogenous cohort of patients with IGHD receiving rhGH. GH doses based on BW compared to BSA-based dosing may result in the administration of higher doses to children older than 11 years of age with BMI greater than 18 kg/m^2 and lower doses to children younger than 11 years of age with BMI less than 18 kg/m^2. Dosing based on BW may be preferable in both prepubertal and pubertal children who do not show adequate growth responses. In prepubertal children, relatively lower doses calculated based on BW rather than BSA provide similar efficacy at lower costs.

Ethics

Ethics Committee Approval: This study was approved by the Ethical Committee of Dokuz Eylül University Faculty of Medicine (approval number: 2022/42-14, date: 28.12.2022) and performed according to the principles of the Declaration of Helsinki.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Ayhan Abacı conceived the idea for this work. Özge Besci and Ayhan Abaci designed the study. Özge Besci, Sezen Ersoy, Reyhan Deveci Sevim, Kübra Yüksek Acinikli, Gözde Akın Kağızmanlı, Ahmet Anık, and Tolga Ünüvar have collected and interpreted the data. Özge Besci wrote the first draft. Korcan Demir, Ece Böber and Ayhan Abacı edited and revised the manuscript critically. All authors read and approved the final manuscript.

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Endocrinological Approach to Adolescents with Gender Dysphoria: Experience of a Pediatric Endocrinology Department in a Tertiary Center in Turkey

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What is already known on this topic?

In Turkey, the number of transgender adolescents seeking hormonal treatment has tended to increase in recent years. However, there are generally very few centers, which are providing gender-affirming care for transgender adolescents.

What this study adds?

This study conducted in a tertiary pediatric endocrinology department in Turkey contributes to the literature regarding medical interventions for adolescents with gender dysphoria. A multidisciplinary approach in the follow-up of these individuals may improve their general health care and also support choosing their gender identity.

Abstract

Objective: A significant rise in the number of trans adolescents seeking medical interventions has been reported in recent years. The aim of this study was to report the clinical features, treatment, and follow-up of adolescents with gender dysphoria (GD) with our increased experience.

Methods: Twenty-six male-to-female (MTF) and twenty-seven female-to-male (FTM) adolescents who were referred to the GD-outpatient clinic between 2016 and 2022 were reviewed. The clinical and laboratory findings of thirty transgender adolescents (15 FTM /15 MTF) who received medical intervention were evaluated retrospectively.

Results: Most individuals (60.4%) were admitted between 2020 and 2022, and the remaining (39.6%) were admitted between 2016 and 2019. At the time of referral, median age was 16.3 years [interquartile range (IQR) 1.53; range 13.2-19.4] in 26 MTF, and 16.4 years (IQR 1.74; range 11.7-21.6) in 27 FTM adolescents. The median age at pubertal blockage with gonadotropin-releasing hormone analog and androgen receptor blocker was 16.4 years (IQR 1.4; range 11.7-17.8) in 22 adolescents (9 MTF, 13 FTM), and 17.4 years (IQR 1.4; range 15.5-19.4) in 6 MTF individuals, respectively. Cross-sex hormone therapy was commenced in 21 adolescents (12 MTF, 9 FTM) at the median age of 17.7 years (IQR 0.61; range 16-19.5). Fifteen individuals (8 MTF, 7 FTM) have been transferred to the adult endocrinology department in transition clinics.

Conclusion: All treatments were generally well tolerated and effective, including bicalutamide, and no significant side effects were observed. Transition clinics played an important role in the better management of gender reassignment processes.

Keywords: Transgender, gender reassignment, gonadotropin-releasing hormone treatment, bicalutamide, cross-sex hormone, transition, multidisciplinary follow-up

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Introduction

Gender dysphoria (GD) is characterized by incongruence between the experienced gender and the natal sex, which also affects various aspects of daily life. Previously defined as "gender identity disorder" by "Diagnostic and Statistical Manual of Mental Disorders: DSM-5", the term has been updated in DSM-5 with distinct diagnostic criteria for children and also adolescents with GD (1).

Following psychiatric evaluation, medical treatment consists of three phases in adolescents with severe and persistent GD (2). The first phase is the suppression of puberty with gonadotropin-releasing hormone agonist analogs (GnRHa) in those who have reached at least pubertal stage 2. In the second phase, cross-sex steroid hormones (CSH) are added to GnRHa treatment to induce sex characteristics consistent with the appropriate gender (3,4). Most adolescents have sufficient mental capacity by 16 years of age to give informed consent to gender-affirming hormone treatment (3). The final phase is genital/gonadal surgery procedures after the age of 18 years, when the individuals reach legal adulthood, and also maintaining the use of CSH (2,3). Legal procedures are in line with the recent guidelines, and surgical interventions are made in adulthood with the approval of the related committees in Turkey.

The start of the gender reassignment process for transgender adolescents in adulthood leads to problems in the social roles of these individuals due to their undesirable secondary sex characteristics. It has been observed that when puberty is suppressed at an early age before the full development of secondary sex characteristics, there is greater patient satisfaction in the postoperative period (3).

However, the knowledge and experience regarding hormonal therapies for adolescents with GD are not widespread in many pediatric endocrinology departments (4,5,6,7). During this challenging process, a multidisciplinary team is effective while accessing the necessary reports, and providing the correct guidance while making the process more suitable for these individuals (2).

This is the first study to be carried out in adolescents with GD in a tertiary pediatric endocrinology clinic in Turkey. The aim was to raise awareness about these individuals by presenting the clinical features and follow-up during hormonal therapy and by emphasizing the benefits of support from a multidisciplinary team.

Methods

Subjects

The medical records of all adolescents diagnosed with GD following at least six months of psychiatric follow-up and who were referred to our GD outpatient clinic between the years 2016 and 2022 were reviewed retrospectively.

Medical intervention was commenced in individuals who were diagnosed with GD based on DSM-5 diagnostic criteria (1) by a mental health professional (MHP). Medical treatment was administered in adolescents, in whom informed consent was taken from both themselves and their legal guardians, and follow-up of these individuals was performed at an interval of 3-6 months. In addition, all transgender adolescents and their parents were informed about fertility preservation.

Pubertal suppression therapy consisted of intramuscular (IM) injections of the GnRHa (triptorelin acetate, 3.75 mg every 4 weeks, or leuprolide acetate 3.75 mg every 4 weeks-11.25 mg every 12 weeks). The selection of GnRHa preparation was dependent on availability in the market. Oral vitamin D (1000-2000 U/day) and calcium (Ca) (500-1000 mg/day) were commenced during pubertal suppression.

In older male-to-female (MTF) adolescents, in whom insurance coverage of GnRHa therapy could not be provided, the potent androgen receptor blocker bicalutamide was started at a dose of 25 mg/day and gradually increased up to 50 mg/day. Bicalutamide was combined with transdermal estradiol (E2) in the initial phase of this treatment. Cyproterone acetate was also commenced in adolescents, who chose not to receive bicalutamide.

During GnRHa therapy, CSH was added in incremental doses to induce novel puberty in both female-to-male (FTM) and MTF adolescents who were evaluated by a MHP (with expertise in gender identity) for emotional readiness to cope with the treatment. Female and male puberty was induced by administering transdermal/oral E2 and IM injections of testosterone (T) -esters (a mixture of T -propionate, -phenylpropionate, -isocaproate, and decanoate) respectively. The management of hormonal therapy was planned according to recent guidelines (2).

Adolescents who reached the legal age (18 years) were transferred to the adult endocrinology department through transition clinics. Legal procedures for surgical operations were also managed in those who received at least one year of CSH therapy by a multi-disciplinary team. This multi-disciplinary team consisted of at least one specialist from the departments of adult and pediatric psychiatry, adult and pediatric endocrinology, gynecology, urology, plastic surgery, and forensic medicine.

Physical Examination

All physical examinations were done by the same examiner at each visit (EKÖ). Heights and weights of all individuals

and their parents were measured using a wall-mounted calibrated Harpenden Stadiometer (Holtain Ltd, Crymych, United Kingdom) sensitive to 0.1 cm and an electronic scale sensitive to 0.1 kg. Body mass index (BMI) was calculated as weight (kg)/height (m^2). All measurements were expressed as standard deviation scores according to age and birth-registered sex in accordance with the national standards (8). Pubertal development was assessed using the Tanner-Marshall scale (9). Since most of these adolescents are generally very sensitive about genital examination, it was only performed in those who gave consent.

Laboratory Investigations

Complete blood count, glucose and lipid profile, and serum concentrations of Ca, phosphorus (P), alkaline phosphatase, 25-OH vitamin D, aspartate aminotransferase, and alanine aminotransferase were performed at the third and sixth months of treatment and every six months thereafter.

Luteinizing hormone (LH), and follicle-stimulating hormone were analyzed by electro-chemiluminescence immunoassay (Cobas, Roche Diagnostics, Mannheim, Germany). Prolactin (PRL), T, and E2, were measured by immune-chemiluminescence assay (Immulite 2000 system, Siemens AG, Berlin and Munich, Germany).

Imaging

Bone mineral density (BMD) of the spine (L1-L4) was measured using dual-energy X-ray absorptiometry (Hologic QDR 4500A Fan Beam X-ray Bone Densitometer, Hologic, Bedford, MA, USA). BMD results are presented in z-scores, which were calculated according to age and birth-registered sex appropriate for this device.

Ethical Approval

Medical ethical approval was granted by the Local Medical Ethics Committee of İstanbul University with the file number 2021/559, date: 12.04.2021. Informed consent revealing the risks and benefits of medical treatments has been obtained from individuals and their legal guardians.

Statistical Analysis

Statistical analysis was performed using IBM Statistical Package for the Social Sciences Statistic Base, version 22.0 (Chicago, IL., USA). The prevalence analysis stating frequency is indicated both as number (n) and percentage (%). Values are stated as median [interquartile range (IQR); range]. The distribution of variables was evaluated using the Kolmogorov-Smirnov test. In addition, the Mann-Whitney U test was used for the analysis of quantitative data. The significance of a p value is considered to be ≤0.05.

Results

Twenty-one individuals (39.6%) were admitted between 2016 and 2019, and the remaining thirty-two (60.4%) were admitted between 2020 and 2022. The distribution of transgender adolescents according to presentation year is illustrated in Figure 1.

At the time of referral, the median age was 16.3 years (IQR 1.53; range 13.2-19.43) in 26 MTF and, 16.4 years (IQR 1.74; range 11.74-21.64) in 27 FTM adolescents.

The majority of individuals (n = 38, 71.7%) were uncomfortable with their natal sex from early ages (< 10 years) and their discomfort had increased especially during puberty. In the remaining individuals (n = 15, 28.3%), this disturbance had started at pubertal ages (> 10 years). While forty-three (81.3%) of the individuals were referred by a MHP, the remaining ten individuals (18.7%) were brought by legal guardians regarding suspicions of hormonal disorders. Five FTM adolescents have been taking hormonal medications without a prescription. Before presenting to a MHP, five (4 FTM, 1 MTF, 9.4%) had attempted suicide.

At the time of referral, the pubertal stage of four adolescents (3 MTF, 1 FTM) had not reached Tanner 4. Except for an 11.7-year-old FTM who was in stage 4 puberty, all FTM were postmenarchal and in stage 5 puberty. No signs of a difference/disorder of sex development were detected, and all subjects had appropriate sex characteristics of the sex assigned at birth. Seven FTM adolescents were diagnosed with polycystic ovary syndrome (PCOS), and PCOS treatment was refused by these adolescents. A MTF individual had metabolic syndrome and metformin was started due to impaired glucose tolerance.

Out of 53 adolescents, 23 were not suitable for hormonal therapy, and 2 adolescents began their treatments in adult endocrinology. Twenty-eight individuals (13 FTM, 15 MTF)

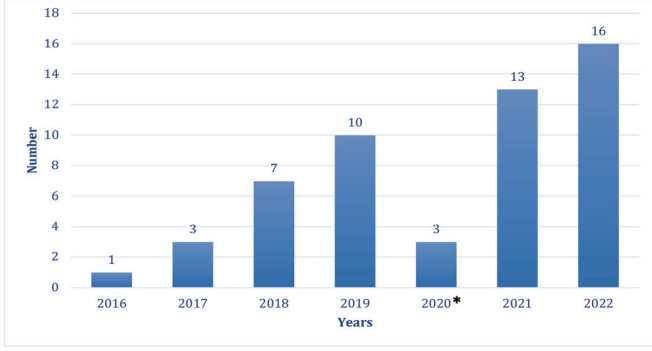

Figure 1. The distribution of transgender youth according to presentation year to outpatient clinics

**During the COVID-19 pandemic*

started hormonal therapy. Out of these, 21 (9 FTM, 12 MTF) continued with cross-sex hormone therapy and transition to adult units was conducted for 15 individuals (8 MTF, 7 FTM). A flowchart of adolescents' inclusion in the study is illustrated in Figure 2 and the details of the hormonal therapy of individuals are shown in Supplemental Table 1.

The median L1-L4 spine BMD z-score was lower in those with low BMI (Figure 3) ($p = 0.0006$, $R^2 = 0.4$). This correlation was stronger in MTF subjects ($p = 0.0135$, $R^2 = 0.5$). The basal median L1-L4 spine BMD z-score of MTF and FTM adolescents was -1.1 (IQR 3.4) and 0.4 (IQR 1.9), respectively, no statistical difference was detected ($p = 0.254$).

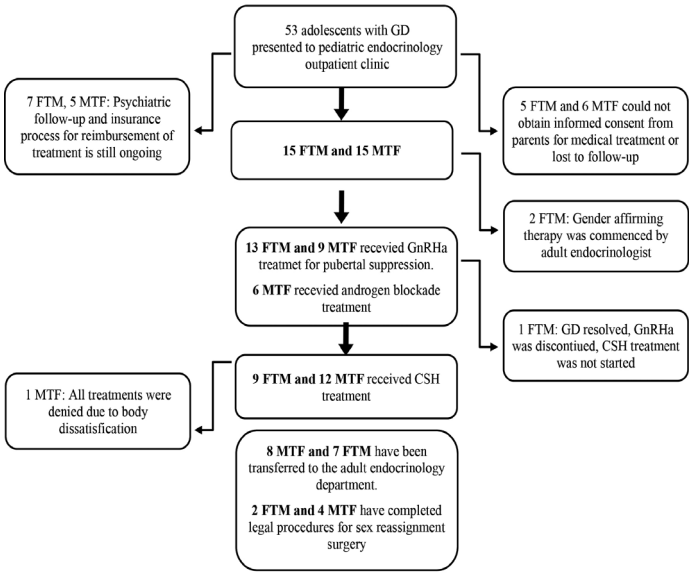

Figure 2. A flowchart of adolescents with gender dysphoria receiving medical treatment

GD: gender dysphoria, FTM: female-to-male, MTF: male-to-female, GnRHa: gonadotropin-releasing hormone analog, CSH: cross-sex hormone, GnRHa: gonadotropin-releasing hormone analog

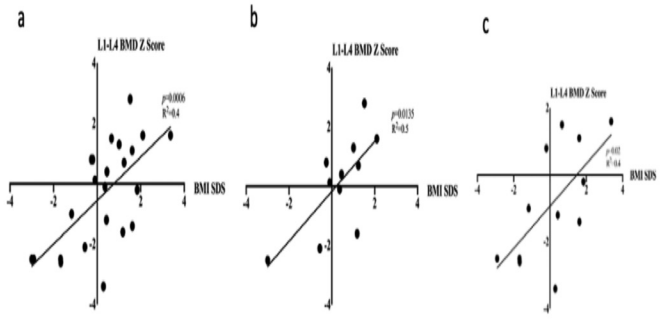

Figure 3. The relationship between basal BMI SDS and L1-L4 BMD z scores in individuals receiving medical interventions. The median L1-L4 spine BMD z-score was lower in those with low BMI SDS in all groups (a). While this correlation was strong in MTF individuals (b), it was weak in FTM individuals (c)

BMI: body mass index, SDS: standard deviation score, BMD: bone mineral density, MTF: male-to-female, FTM: female-to-male

During medical interventions, complete blood counts and biochemical parameters (liver and renal function) were within normal ranges in all subjects. The other details of clinical and laboratory findings and comparison of parameters are shown in Table 1.

Pubertal Blockage with GnRHa

Twenty-two individuals (9 MTF, 13 FTM) commenced GnRHa treatment [3.75 mg/4 weeks (n = 7), 11.25 mg/12 weeks (n = 15)]. The median age at the start of treatment was 16.4 years (IQR 1.4; range 11.7-17.8) and the median duration from presentation to pediatric endocrinology clinic to treatment (provided that at least 6 months followed by psychiatry) was 0.2 years (IQR, 0.2; range, 0-2.5).

The median duration of GnRHa monotherapy in nine MTF and six FTM individuals was 0.9 years (IQR 0.4; range 0.04-1.12) and 0.6 years (IQR 0.5; range 0-1.9) respectively. In an FTM adolescent (S4), GnRHa treatment was continued as this had been started two years earlier in another center.

The data of clinical characteristics of adolescents receiving medical intervention (including age at the first presentation, age at the start of hormone treatment, type of hormone treatment, anthropometry at presentation and last examination, and history of surgery) are shown in Table 1.

Three months after starting GnRHa treatment, menstrual suppression was achieved and persisted throughout the treatment process in twelve FTM. The median LH concentration was 2.0 mIU/mL (IQR 3.1). Vaginal bleeding occurred in two FTM adolescents (S4, S9), in one at the start of the GnRHa treatment, and in the other two years after starting GnRHa treatment, at the time of CSH treatment. Menstrual cessation was obtained with oral norethisterone (10 mg/day).

In a FTM adolescent (S5) whose GD started in the pubertal period, GD resolved after one year of GnRHa monotherapy and she did not continue with CSH treatment.

Pubertal suppression was sustained in 3 MTF (S1, S2, S9) receiving 3.75 mg of GnRHa every 4 weeks (mean LH 0.83 mIU/mL), however, spontaneous erections persisted in 3 MTF (S3, S4, S6). The mean level of LH was 12.2 mIU/mL in one of them receiving 3.75 mg of GnRHa every 4 weeks, and in the remaining individuals receiving 11.25 mg of GnRHa every 12 weeks, it was 5.2 mIU/mL and 7.25 mIU/mL respectively. The dose of GnRHa was increased to 7.5

Table 1. The comparison of clinical and laboratory findings of adolescents receiving medical interventions at the onset of treatments

	GnRHa			CSH				
	MTF median (IQR)	FTM median (IQR)	p	MTF median (IQR)			FTM median (IQR)	p**
				After GnRHa	p*	Combined androgen receptor blocker		
n	9	13		6		6	9	
Age (yrs)	16.7 (1.2)	16.7 (1.0)	**0.797**	17.3 (1.1)	**0.931**	17.5 (1.3)	17.8 (0.4)	**0.159**
Height (cm)	176.1 (3.0)	160.8 (5.0)	**< 0.001**	177.9 (2.0)	**0.329**	169.4 (3.3)	164.9 (7.4)	**0.019**
Height SDS*	0.6 (0.9)	-0.2 (1.5)	**0.331**	0.3 (0.5)	**0.329**	0.4 (1.3)	-0.4 (1.4)	**0.587**
BMI (kg/m^2)	24.8 (5.8)	23.1 (4.5)	**0.290**	20.1 (0.7)	**1.000**	19.8 (5.9)	24.5 (6.9)	**0.243**
BMI SDS*	0.7 (1.5)	0.7 (1.5)	**0.845**	-1.2 (0.7)	**1.000**	-0.9 (2.1)	1.1 (2.9)	**0.247**
Systolic BP (mmHg)	110 (20)	107.5 (11)	**0.081**	105 (10)	**0.913**	105 (15)	110 (14)	**0.199**
Diastolic BP (mmHg)	80 (10)	69 (8.8)	**0.023**	67.5 (6.3)	**0.737**	62.5 (8.8)	70 (7.5)	**0.300**
LH (mIU/mL)	4.5 (1.7)	6.7 (4.1)	**0.420**	5.0 (4.7)	**0.937**	4.5 (2.8)	2.3 (3.0)	**0.214**
FSH (mIU/mL)	4.1 (2.9)	4.7 (3.3)	**0.357**	NA	**NA**	4.3 (3.5)	NA	**NA**
E2 (pg/mL)	25.9 (18.0)	68.9 (58.6)	**0.008**	18.5 (10.5)	**0.167**	27.3 (28)	9.3 (1.0)	**0.881**
T (ng/mL)	5.3 (1.8)	0.3 (0.3)	**< 0.001**	0.3 (0.7)	**0.082**	6.3 (3.2)	0.4 (0.1)	**1.000**
PRL (ng/mL)	8.4 (10.8)	15.2 (4.1)	**0.078**	16.1 (3.8)	**0.381**	12.3 (4.0)	12.7 (5.1)	**1.000**
Glucose (mg/dL)	86.5 (5.1)	86 (10.5)	**0.955**	90 (5.0)	**0.714**	93.5 (7.8)	89 (4.0)	**0.369**
Insulin (µg/mL)	13.3 (16.6)	9.0 (4.4)	**0.137**	9.7(3.2)	**0.786**	7.1 (2.3)	12.6 (3.2)	**0.151**
HbA1c (%)	5.4 (0.4)	5.0 (0.2)	**0.052**	5.6 (0)	**0.203**	5.1 (.0.5)	5.2 (0.1)	**0.230**
Total cholesterol (mg/dL)	183 (54.9)	157.7 (47.5)	**0.484**	167.8 (45.3)	**0.610**	166.3 (14.1)	158 (46)	**0.576**
Triglycerides (mg/dL)	115 (26.8)	63 (13.9)	**0.005**	87.5 (59.4)	**1.000**	74.6 (31.4)	57 (14.1)	**0.396**
HDL (mg/dL)	40 (1.9)	55 (4.7)	**< 0.001**	59.1 (19.3)	**0.831**	55.5 (16.1)	52.3 (16.1)	**0.364**
LDL (mg/dL)	130 (48.5)	91.5 (50.3)	**0.381**	95.3 (20.8)	**0.476**	90 (17.3)	94.5 (42.5)	**0.868**
25-hydroxyvitamine (ng/mL)	26.8 (10.6)	15.7 (6.9)	**0.009**	16.7 (3.4)	**0.329**	15.9 (2.3)	21.9 (17.9)	**0.787**
L1-L4 BMD z-score	-1.0 (3.3)	0.4 (1.9)	**0.412**	-0.91 (3.1)	**1.000**	-1.2 (2.3)	-0.9 (1.0)	**0.940**

#SDS was calculated according to birth-registered sex.
*Comparison of parameters between group GnRH analog and group androgen receptor blocker.
**Comparison of parameters between MTF and FTM Groups receiving CSH.
GnRHa: gonadotropin-releasing hormone analog, CSH: cross-sex hormone, IQR: interquartile range, FTM: female-to-male, MTF: male-to-female, BMI: body mass index, SDS: standard deviation score, BP: blood pressure, LH: luteinizing hormone, FSH: follicle-stimulating hormone, E2: estradiol, T: testosterone, PRL: prolactin, HDL: high-density lipoprotein, LDL: low density lipoprotein, BMD: bone mineral density, NA: not available, yrs: years

mg/month in three individuals. After increasing the dose to 7.5 mg/month, the mean levels of LH were 1.3 mIU/mL, 3.2 mIU/mL, and 0.6 mIU/mL respectively.

Cross-sex Hormone Therapy (Combined with GnRHa or Androgen Receptor Blockers)

Sixteen adolescents (7 MTF, 9 FTM) received CSH treatment in addition to GnRHa treatment. The median age at the start of the induction of CSH was 17.7 years (IQR 0.6; range 16-19.5). In 5 MTF adolescents (S10-14) the median age at the start of combined treatment (bicalutamide and E2) was 17.4 years (IQR 1.4; range 16.2-19.5). At presentation, a 17.3 years old MTF adolescent (S15) was receiving cyproterone acetate (12.5 mg/day) and sublingual E2 (2 mg divided into 3 doses) for 3 weeks; this treatment was continued.

Although the median serum E2 concentration was higher [54.4 (IQR 26.5) pg/mL] in bicalutamide with CSH group when compared to the GnRHa with CSH group [32.5 (IQR 18.8) pg/mL], there was no statistical difference between the groups (p = 0.429). The details of clinical and laboratory findings of MTF individuals receiving E2 combined with androgen receptor blockers or GnRHa are given in Table 2.

In the first six months of transdermal E2 treatment, in one MTF (S5), a transient elevation in PRL concentration (up to 63.3 ng/mL) was detected. In the first year of treatment, the serum PRL concentrations returned to normal ranges without any intervention. In a FTM (S10) who received combined bicalutamide and E2 treatment, a mild elevation in PRL concentrations (up to 31.8 ng/mL) was detected.

Table 2. The clinical and laboratory findings of transwomen during the treatment of cross-sex hormone following GnRH analog or combined androgen receptor blockers

Subjects	The age of treatment onset (yrs)	Duration of CSH treatment (yrs)	At initial time				Last evaluation			
			Breast development (Tanner stage)	Serum E2 level (pg/mL)	Serum T level (ng/mL)	Serum PRL level (ng/mL)	Breast development (Tanner stage)	Serum E2 level (pg/mL)	Serum T level (ng/mL)	Serum PRL level (ng/mL)
GnRHa + CSH										
1	17.8	0.9	1	39.1	7.4	-	2	17.6	0.4	-
2	17.7	0.7	1	25.9	5	16.1	2	-	-	-
3	17.8	0.3	1	16	5.2	-	-	39.7	0.22	-
4	16.6	1.7	1	34.8	9.6	11.2	3	110	0.24	19.8
5	16	1.9	1	12	-	5.7	#	33	0.4	27
6	18.2	0.8	1	-	5.5	-	2	36.7	0.26	11.3
Androgen receptor blocker + CSH										
10	19.5	0.5	1		8.8	10.8	3	54	12.9	24
11	16.3	1.0	1	26	7.5	9.6	3	34	6.4	9
12	17.9	0.7	1	28.6	7.2	13.9	2	69.1	9.5	16.1
13	16.2	0.4	1	20.5	3.9	13.9	2	54.7	4.7	20
14	17.7	0.7	1	13.1	5.3	8.8	3	18.6	5.7	7.9
15*	17.3	0.4	1	74.9	0.2	30.9	4	139	0.03	27

#Breast augmentation.
*The individual using cyproterone acetate 12.5 mg/day and sublingual E2 divided into 3 doses.
GnRHa: gonadotropin-releasing hormone analog, CSH: cross-sex hormone, E2: estradiol, T: testosterone, PRL: prolactin

At the start of T therapy, hemoglobin (HGB) levels were normal in all FTM, but when the dose of T was increased to the full dose, HGB levels increased to 15.5 g/dL in two FTM adolescents (S1, S2). On further follow-up, no subsequent increase in HGB levels was detected, thus no additional treatment was needed. These adolescents were also cigarette smokers.

Surgery

Two individuals (S1, S2) underwent mastectomy surgery at another center on their own and their families' initiative without our direction. Five MTF individuals (S1-S5) had voice and facial feminization surgery and one of them received breast augmentation without our initiative at another centre. Due to body dissatisfaction related to cosmetic appearance after the surgery, a MTF adolescent (S5) developed depression and refused to go outside. Therefore all treatments were discontinued in this adolescent and psychiatric follow-up was recommended. Fifteen individuals (8 MTF, 7 FTM) have been transferred to the adult endocrinology department.

Discussion

This is the first comprehensive study conducted in a tertiary pediatric endocrinology clinic in Turkey that evaluated clinical follow-up of transgender adolescents during medical interventions. It was possible to facilitate the transgender adolescents' treatment management and transitions to the adult clinic after 18 years of age within the multidisciplinary team. Legal requirements for the gender reassignment process were also provided by this team.

Although presentation numbers of transgender adolescents decreased during the lockdown because of the COVID-19 pandemic, it is remarkable that half of the presentations occurred in the last year (Figure 1). In parallel with the increasing experience of our multidisciplinary team, the number of transgender individuals who presented to our center has increased gradually over the years. Similarly, a significant rise in the number of trans people seeking treatment for GD has also been reported in the literature (10).

In the present study, 53 % of all adolescents (n = 28) were able to start medical intervention in our department. The legal guardians of 11 adolescents with GD (21 % of the total) did not give consent, so they were not able to start any treatment and they were lost to follow-up. However, there is a genuine concern that improper drug use may spread among individuals whose access to standard treatment is somehow blocked. In our cohort, hormonal medication use

without a prescription was observed in five MTF adolescents. Inappropriate hormone use among trans adolescents may be problematic, which could deteriorate their general health and lead to physical and mental problems (10).

We believe that an increase in the number of gender health care clinics and multidisciplinary committees for transgender adolescents in our country may provide improved access to available and monitored treatment, as well as preventing negative outcomes and drop-out.

We observed anecdotal improvements in the quality of life of individuals following appropriate hormone therapy, although we did not conduct a survey specifically into this subject. Furthermore, we did not observe drop-out in adolescents receiving hormonal treatment except for one MTF individual. Despite close follow-up, this individual attempted suicide during CSH treatment, due to persistent body dissatisfaction, which led to severe depression. She refused all medical interventions because of the assumption that medical interventions did not work in her body. Beginning GD medical interventions in an older adolescent period may result in persistent physical disturbance and depression, based on relatively slow physical changes. It is also reported that major depression and suicide attempts are more frequent among trans individuals when compared to the general population (11).

Pubertal suppression with GnRHa at standard dose was relatively inadequate in MTF adolescents. Therefore, we had to increase the dose. It was observed that in older adolescents, 3-monthly injections of 11.25 mg may not be adequate. To cope with body dissatisfaction and rapid physical changes, a relatively rapid dose increase of CSH may be more suitable in older individuals (older than 16 of age). Neyman et al. (12) reported that in their cohort, 84.6% of the adolescents aged between 12 and 18.4 years on bicalutamide treatment had breast development ranging from Tanner stage 2-5. In our cohort, 5 MTF individuals aged between 16.2 and 19.5 years received bicalutamide. The breast development stage was 2 at the third month of treatment in two individuals and it was 3 in the remainder, whereas breast development reached Tanner stage 3 at most, in those who received GnRHa and CSH treatment. Indeed, the median serum E2 concentration was higher in the bicalutamide group, although there was no statistical difference between the groups. This result may be due to the limited number of individuals. Another noteworthy finding regarding treatment was that in an MTF adolescent receiving cyproterone acetate combined with sublingual E2 (divided into 3 doses), serum E2 level was quickly increased and breast development was quite fast as it progressed from Tanner stage 1 to stage 3 in one month. Thus, potent androgen receptor blockers, such as bicalutamide, may be effective and promote rapid feminization in older MTF adolescents who want to experience rapid physical changes. Additionally, although it is limited to the observation of only one case in our cohort, divided doses and a sublingual route of E2 seems more effective for obtaining a feminizing effect.

It has been reported that prepubertal-onset GD may be transient in the majority of adolescents by late pubertal periods (13). However, we observed that prepubertal-onset GD was persistent and it was exacerbated in the pubertal period. This difference may be related to the fact that most of the children with prepubertal-onset GD are not referred to pediatric endocrinology departments. Indeed, the majority of our cohort consisted of adolescents, who were in the late pubertal period. Besides, we observed that in our cohort GD was resolved only in one FTM individual, who notably had pubertal-onset GD, and decided not to continue with CSH after GnRHa. Therefore, pubertal suppression with GnRHa in the first step is suitable, even in adolescents who present late to the endocrinology department, and whom the recent guideline is also applicable for (2). Briefly, the varying range of attitudes, ranging from impatience to indecisiveness, were observed among these individuals in our cohort. Therefore, we highlighted the importance of close psychiatric follow-up, which is crucial at each step taken toward the transition to cope with the effects of these challenging processes.

During CSH induction, increased serum E2 levels may result in a transient elevation in PRL concentrations. It has been reported that this elevation may be observed often in the first few months of E2 treatment (14). We observed significant PRL elevation in only one MTF adolescent, but this was not persistent and returned to normal ranges. Additionally, in two FTM individuals, a transient T-induced increase in hematocrit concentration was remarkable in the first months, but again this decreased on follow-up. Indeed, during all medical interventions, we did not observe a situation requiring interruption of the treatment.

The negative effect on peak bone mass of pubertal suppression among MTF adolescents has been previously reported (15,16). The lower physical activity among transgender girls might be responsible for a decrease in muscle mass, which is mandatory to reach the peak bone mass in young adulthood (16). At presentation, the median BMD z-scores of the spine of MTF adolescents were below the population mean, albeit with sufficient vitamin D levels. Moreover, following CSH administration, no significant improvement in the median BMD z-scores was observed, again with sufficient vitamin D and Ca replacement. This result was consistent with previous reports in MTF populations (15,16). In MTF, the median L1-L4 BMD z-score was normal at the

start of treatment despite insufficient vitamin D levels. Yet, it decreased during pubertal suppression. This decrease in the z-score was especially evident in those with a lower BMI. A relatively high-dose CSH therapy may be appropriate in this period, which is important to achieve peak bone mass and density. However, due to the relatively small number of transgender adolescents evaluated, the changes in bone health could not be reliably evaluated.

Gender reassignment surgery procedures were discussed in multi disciplinary councils after the transition to adults' department after 18 years of age according to international guidelines. Some individuals in our cohort who were under the age of 18, underwent minor plastic surgical interventions (without our direction) and they generally reported satisfaction afterwards. Nevertheless, the importance of psychiatric follow-up was stressed in these cases as well.

Study Limitations

Conducting surveys is necessary to measure the quality of life. In this retrospective study, difficulties were encountered in accessing data for certain cases.

Additionally, during the COVID-19 period, face-to-face meetings with individuals could not be conducted. Furthermore, evaluating responses to all treatments requires larger population studies.

Conclusion

The follow-up and hormonal therapy of transgender adolescents is clinically challenging. Until recently, there were very few centers in our country providing gender-affirming care for transgender adolescents. However, with the increase in the number of healthcare centers for transgender adolescents, we believe that parental awareness about GD, and accordingly trans adolescents' access to treatment will increase. Potent drugs or rapid dose increases of CSH may be a solution for older adolescents who expect rapid physical change. GnRHa treatment is also reasonable as the first step and provides support during the diagnostic process in young adolescents. Psychiatric evaluation is mandatory during treatment at each step. Thus, we are guiding these individuals medically and also supporting them on the journey to increase their quality of life and thus overall satisfaction with their lives. Moreover, we believe that multidisciplinary teams and multidisciplinary transition clinics, in which endocrinologists (pediatric/adult) and psychiatrists (pediatric/adult) play important roles, will contribute to better management of gender reassignment processes according to the physical and mental conditions of transgender adolescents.

Ethics

Ethics Committee Approval: Medical ethical approval was granted by the Local Medical Ethics Committee of İstanbul University with the file number 2021/559, date: 12.04.2021.

Informed Consent: Informed consent revealing the risks and benefits of medical treatments has been obtained from individuals and their legal guardians.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Medical Practices: Esin Karakılıç Özturan, Ayşe Pınar Öztürk, Firdevs Baş, Ayşe Burcu Erdoğdu, Seven Kaptan, Aslı Derya Kardelen Al, Şükran Poyrazoğlu, Melek Yıldız, Neşe Direk, Şahika Yüksel, Feyza Darendeliler, Concept: Esin Karakılıç Özturan, Feyza Darendeliler, Design Esin Karakılıç Özturan, Feyza Darendeliler, Data Collection or Processing: Esin Karakılıç Özturan, Ayşe Pınar Öztürk, Firdevs Baş, Ayşe Burcu Erdoğdu, Seven Kaptan, Aslı Derya Kardelen Al, Şükran Poyrazoğlu, Melek Yıldız, Neşe Direk, Şahika Yüksel, Feyza Darendeliler, Analysis or Interpretation: Esin Karakılıç Özturan, Firdevs Baş, Feyza Darendeliler, Literature Search: Esin Karakılıç Özturan, Firdevs Baş, Şahika Yüksel, Feyza Darendeliler, Writing: Esin Karakılıç Özturan, Firdevs Baş, Şahika Yüksel, Feyza Darendeliler.

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Click for Supplementary Table 1. The data of clinical characteristics of adolescents receiving medical intervention

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An Endocrinological Perspective on 22q11.2 Deletion Syndrome: A Single-center Experience

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What is already known on this topic?

22q11.2 deletion syndrome is typically known for its triad of features, cardiac anomalies, immunodeficiency, and hypoparathyroidism. While it has been established that there is a typical facial appearance in this syndrome, many previously published articles and reviews have focused on philtrum and mouth anomalies.

What this study adds?

Regarding typical facial features, drooping and/or swelling of the lateral eyelids (hooded eyelids), a long and prominent philtrum, and a thin upper lip are the reported findings that deserve special attention. There was no significant difference between permanent and transient hypoparathyroidism cases in terms of parathyroid hormone levels to predict permanent or temporary forms. Vitamin D deficiency is a risk factor for hypocalcemia and may be a cause of transient hypoparathyroidism in 22q11.2 deletion syndrome.

Abstract

Objective: 22q11.2 deletion syndrome (22q11.2 DS) is the most common chromosomal microdeletion disorder. Associated problems in 22q11.2 DS may include cardiac abnormalities, immune dysfunction, facial dysmorphism, with endocrine, genitourinary and gastrointestinal problems, and developmental delay. The aim of this study was to evaluate and present all endocrinological findings of patients with 22q11.2 DS from a single center.

Methods: All participants had confirmed 22q11.2 DS by fluorescence in situ hybridization with hypoparathyroidism. Data were retrieved by retrospective review of patient records.

Results: A total of 17 patients were reviewed. On physical examination, all patients had similar dysmorphic features. The median age at diagnosis was 45 days (1 day-13 years). Most cases (64.7%, 11/17) were diagnosed with hypoparathyroidism incidentally after routine tests. At the time of diagnosis, mean calcium was 7.04 ± 0.80 mg/dL, phosphorus was 6.2 ± 1.1 mg/dL, and median parathyroid hormone (PTH) was 11.5 (3.7-47.6) ng/L. Transient hypoparathyroidism was detected in five cases (29.4%). There was no significant difference between patients with permanent or transient hypoparathyroidism regarding gender, age at diagnosis, calcium, phosphorus, and PTH levels. However, vitamin D levels were significantly lower in the transient group ($p = 0.036$). During follow-up, short stature, obesity, and type 2 diabetes mellitus were absent. Thyroid autoantibodies were detected in two patients with normal thyroid function tests. Despite there being no pathological short stature, final stature was shorter than the general population (mean height standard deviation score: -0.94 ± 0.83).

Conclusion: Hypocalcemia may be detected during acute illness in some cases where hypocalcemia appears at later ages. There was no significant difference between permanent and transient hypoparathyroidism cases in terms of PTH level. Recognition of the more specific facial findings is important to trigger investigation of genetic variants, additional anomalies, and for follow-up.

Keywords: 22q11.2 deletion syndrome, DiGeorge syndrome, hypoparathyroidism, hooded eyelids, immunodeficiency, Tetralogy of Fallot, vitamin D deficiency

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Introduction

The 22q11.2 deletion syndrome (22q11.2 DS) is the most common chromosomal microdeletion disorder. This syndrome may involve cardiac abnormalities, immune dysfunction, facial dysmorphism, with endocrine, genitourinary and gastrointestinal problems, developmental delay, and neuropsychiatric disease. Chromosome 22q11.2 deletion may be diagnosed as DiGeorge syndrome, velo-cardio-facial syndrome, conotruncal anomaly face syndrome, autosomal dominant Opitz G/BBB syndrome, or Cayler cardio-facial syndrome, depending on the phenotype (1). Dysmorphic facial features are characteristic of 22q11.2 DS. Example of facial abnormalities include hooded eyelids, pseudoptosis of the upper eyelids, narrow palpebral fissures, telecanthus, hypertelorism, tubular nose, bulbous nasal tip, anteverted nostrils, low-set and posteriorly rotated ears, ear helix abnormalities, microtia, long/short philtrum, malar flattening, retrognathia, and chronic open mouth posture (2,3). Commonly associated endocrine disorders include hypocalcemia and hyperphosphatemia, due to parathyroid gland hypoplasia, growth retardation, obesity, skeletal anomalies, and thyroid dysfunction (4,5,6,7). It has been reported that short stature may occur due to cardiovascular abnormalities, recurrent infections due to immune deficiency, and nutritional problems might also become apparent (4). It is known that thyroid dysfunction may occur due to autoimmunity and thyroid hypoplasia (4). While certain features, such as immunodeficiency, cardiovascular anomalies, and hypoparathyroidism in the case of 22q11.2 DS have been commonly reported, few papers that present all the endocrinological abnormalities in 22q11.2 DS have been published (4,5,7,8,9).

The aim of this study was to retrospectively evaluate and present all endocrinological findings of patients with 22q11.2 DS from a single center.

Methods

Patients

All patients with 22q11.2 DS and endocrinological manifestations followed in Bursa Uludağ University Hospital, Clinic of Pediatric Endocrinology were included in the study. The study included only those patients with fluorescence in situ hybridization (FISH)-confirmed 22q11.2 DS who also had hypoparathyroidism. Patients without endocrinological findings (hypoparathyroidism, hypothyroidism, and/or short stature absent), genetically unconfirmed diagnosis and/or those over 18 years old were excluded. Age at diagnosis, clinical presentation, gender, and birth characteristics (week of gestation, birth height, birth weight) were retrospectively evaluated. Detailed laboratory analyses, including blood levels of calcium (Ca), phosphorus (P), alkaline phosphatase (ALP), parathyroid hormone (PTH), 25 hydroxyvitamin D [25(OH)D], albumin, free T4 (fT4), free T3 (fT3), thyroid-stimulating hormone (TSH), thyroid autoantibodies [anti-thyroid peroxidase antibody (anti-TPO), anti-thyroglobulin antibody (anti-TG) and TSH receptor antibody] were also evaluated. In addition, insulin-like growth factor-1 values were investigated in patients with height standard deviation (SD) score (SDS) <-2 during follow-up. Final height SDS in 5 patients and additional skeletal anomalies were evaluated. Cardiac malformation, immunodeficiency, and autoimmunity were also noted. Height and weight SDS values were calculated according to the reference values of Turkish children (10).

Laboratory Analysis

TSH, fT4, fT3, and 25(OH)D values were analyzed with a Chemiluminescent Microparticle Immuno Assay method using the Abbott Architect Plus i2000 Immunoassay Analyzer device (Abbott, Abbott Park, Illinois, USA). Ca, P, PTH, and ALP values were measured by a spectrophotometric method using the Abbott Architect c-16000 Clinical Chemistry Analyzer (Abbott, Abbott Park, Illinois, USA).

Genetic Analysis

Deletion of the chromosome 22q11.2 region was investigated using FISH, using Vysis DiGeorge Region LSI N25 Spectrum Orange/LSI ARSA Spectrum Green Probes (Abbott, Abbott Park, Illinois, U.S.A).

Ethics

A consent form was filled out by all parents and participants. Written informed consent was obtained from the families of two patients who allowed the publication of clinical facial photographs. The study was approved by the Uludağ University Local Ethical Committee (approval number: 2021-19/22, date: 22.12.2021).

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences for Windows, version 23.0 (IBM Inc., Armonk, NY, USA). To assess the significance of the differences between the groups, the normality of variables was tested with the Kolmogorov-Smirnov test. The Mann-Whitney U and chi-square tests were also used. Results were reported as median (interquartile range) or mean $\pm$ SD. A two-sided p value of <0.05 was considered statistically significant.

Results

A total of 17 patients were included. The F/M (8/9) ratio was 0.88:1. The mean week of gestation was 38 weeks, birth weight was 3161±626 grams (0.22±1.15 SDS), and birth length was 48.5±3.7 cm (0.02±1.27 SDS). On physical examination, all cases had similar facial findings (Figure 1). The most striking facial findings of the patients were droopy and/or swollen lateral eyelid (hooded eyelids), telecanthus, bulbous nasal tip, anteverted nostrils, long and prominent philtrum, and thin upper lip.

All cases had hypocalcemia (n = 17), and the age at diagnosis was variable, ranging from 1 day to 13-years-old but with a median of 45 days. Eight cases were diagnosed in the neonatal period (8/17). Most cases (64.7%, 11/17) were incidentally diagnosed with hypoparathyroidism after routine tests. Hypocalcemia was diagnosed due to convulsions in two cases, tetany in two cases, and antenatal genetic diagnosis in two cases. The following serum levels were found at diagnosis: mean Ca 7.04 ± 0.80 mg/dL, P 6.2 ± 1.1 mg/dL, median PTH 11.5 (3.7-47.6) ng/L, ALP 158.5 (65-426) U/L and 25(OH)D 18.2 (5-36.1) µg/L. Ca treatment and calcitriol were started immediately after the diagnosis, and vitamin D supplementation was given to cases with vitamin D deficiency. Transient hypoparathyroidism was detected in five cases (29.4%) (Table 1). Three of these were diagnosed in the neonatal period during routine monitoring (Patients 1, 3, and 10) and all three had vitamin D deficiency. Maternal 25(OH)D level was only examined in the mother of patient 3, which was low (10.3 µg/L). Ca treatment was stopped on the 10th, 35^{th,} and 90th days. Hypocalcemia did not recur during follow-up. In the other cases (Patients 11 and 16) diagnosed at 14 months and 3.5 years, hypocalcemia was detected during major cardiac surgery. Ca treatment was discontinued at the 4th month and on the 20th day of treatment, respectively. No hypocalcemia was observed during follow-up.

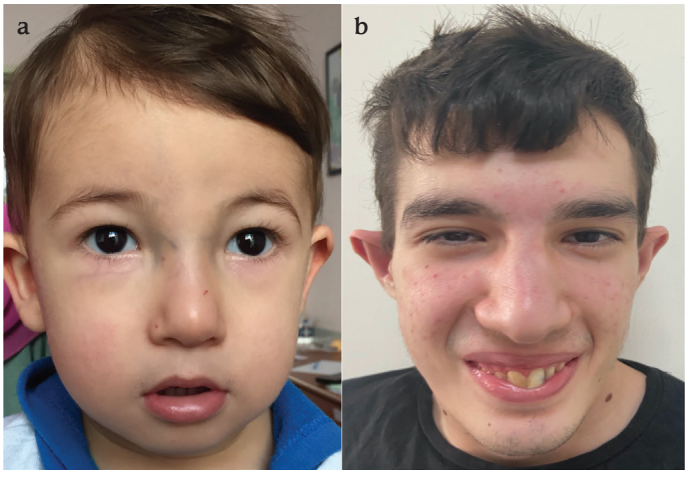

Figure 1. Typical facial appearance for 22q11.2 DS in different age groups a: Childhood (patient number: 15), b: Adulthood (patient number: 9)

22q11.2 DS: 22q11.2 deletion syndrome

The median age at diagnosis in the 12 patients with permanent hypoparathyroidism was 7.2 months (1 day to 13 years) (Table 2). In these patients mean Ca level was 7.1 ± 0.8 mg/dL, P 6.14 ± 0.98 mg/dL, median PTH 9.7 (3.7-47.6) ng/L, ALP 158.5 (87-426) U/L and mean 25(OH) D level were 19.9 ± 9.5 µg/L at diagnosis. All cases received calcitriol monotherapy, and no symptomatic hypocalcemia was detected during follow-up. No significant difference between permanent and transient hypoparathyroidism cases was found in terms of gender, age at diagnosis, Ca, P, or PTH (p = 0.523, p = 0.425, p = 0.689, p = 0.716 and p = 0.182, respectively). However, 25(OH)D levels were significantly lower in the transient hypoparathyroidism group, although this was only measured in 3/5 patients (p = 0.036).

Other endocrinological findings were evaluated. Short stature (mean height SDS: -0.94 ± 0.83), obesity, and type 2 diabetes mellitus were not found during follow-up, and the mean height SDS of the five patients who had reached their final height was -0.94 ± 0.94. Thyroid autoantibodies (anti-TPO and/or anti-TG) were detected in two cases with normal thyroid function tests. Two cases were diagnosed with primary hypothyroidism in the neonatal period, one by neonatal screening, the other in the intensive care unit.

While scoliosis was detected in three of the cases, no other skeletal anomaly was found. A Brown tumor was detected in the left parietal bone of the calvarium in a patient with scoliosis.

Some cardiac defect was observed in 13 (76%) cases (Tables 1 and 2). No significant difference was detected in height SDS between patients with and without cardiac anomaly (p = 0.400). While severe combined immunodeficiency was detected in one patient, B cell and/or T cell immunodeficiencies were reported in ten cases (58.8%). There was no significant difference in height SDS between patients with and without these immunodeficiencies (p = 0.983). Other rare additional findings are shown in Tables 1 and 2.

Discussion

22q11.2 DS is the most common microdeletion disease with conotruncal anomalies. Cardinal findings are cardiac defect,

Table 1. Diagnostic features of transient hypoparathyroidism cases

Patient number	Gender	Age at diagnosis with hypoparathyroidism (age)	Gestational age (week)	Birth weight SDS	Birth height SDS	Diagnosis	Calcium at diagnosis (mg/dL)	Phosphorus at diagnosis (mg/dL)	ALP at diagnosis (U/L)	PTH at diagnosis (ng/L)	25(OH) D at diagnosis (µg/L)	Cardiac anomaly	Rare additional findings
1	M	2 days	39	1.98	-1.1	Incidentally	7.5	4.6	169	19.5	6	Terralogy of Fallot, ASD, right-sided aortic arch	No
3	F	2 days	38	0.37	-0.55	Incidentally	6.5	7.4	202	12.4	8	Truncus arteriosus, VSD, ASD	No
10	F	1 day	36^{+4}	1.95	0.33	Incidentally	5.9	7.6	150	20.3	5	VSD, Aortic coarctation, interrupted aortic arch, ASD	No
11	M	14 months	38	N/A	N/A	During cardiac surgery	7	5.9	65	38.8	N/A	Terralogy of Fallot	Unilateral ureteropelvic stenosis, bifid uvula
16	M	3.5 years	N/A	N/A	N/A	During cardiac surgery	7.7	N/A	N/A	N/A	N/A	Terralogy of Fallot, ASD	Strabismus and unilateral undescending testes

M: male, F: female, N/A: not applicable/available, PTH: parathyroid hormone, ALP: alkaline phosphatase, SDS: standard deviation score, ASD: atrial septal defect, VSD: ventricular septal defect, 25(OH)D: 25 hydroxyvitamin D

immunodeficiency, and hypoparathyroidism (1,11). The physical examination findings of the cases are also important in the diagnosis, and typical facial findings have been highlighted (2,3). In the present study, droopy and/or swollen lateral eyelid (hooded eyelids), long and prominent philtrum, and thin upper lip were the most striking and helpful signs for rapid diagnosis.

Due to the abnormal parathyroid function, hypocalcemia has been reported to be the most common endocrine abnormality in 22q11.2 DS (12). Therefore, only cases with hypoparathyroidism were included in this study. In many studies, it has been reported that hypoparathyroidism is most common in the neonatal period (90-99%) and may be temporary due to the recovery of parathyroid function over time (5,7). In the present study, transient hypoparathyroidism was found in 29.4% of cases, 60% of which were diagnosed in the neonatal period. The lower incidence of neonatal transient hypoparathyroidism in this population may be due to not all facial findings being fully recognized, that there were no cardiac defects in some of the cases, and that there was no follow-up after the newborn period when the infant became normocalcemic. The detection of hypocalcemia after cardiac surgery in two of the patients, at 14 months and 3.5 years of age, with no previous symptoms shows that hypocalcemia may be evident during hypermetabolic states (7). It should be kept in mind that hypocalcemia may develop during acute illness in cases where hypocalcemia is not detected until older. In addition, cases with transient hypocalcemia should be carefully monitored for hypocalcemia in similar situations. A significant difference was found between the transient and permanent groups in 25(OH)D levels. Therefore, vitamin D deficiency was found to be a risk factor for hypocalcemia in 22q11.2 DS, especially the transient group. It seems reasonable that transient hypoparathyroidism would result from vitamin D deficiency in 22q11.2 DS, most probably due to the limited secretion capacity of the parathyroid gland. Therefore, it is important to avoid vitamin D deficiency in patients with 22q11.2 DS. It was noted that transient hypoparathyroidism due to vitamin D deficiency in 22q11.2 DS was previously reported in only one case in the neonatal period, secondary to maternal hypovitaminosis D

Table 2. Diagnostic features of persistent hypoparathyroidism cases

Patient number	Gender	Age at diagnosis with hypoparathyroidism (age)	Gestational age (week)	Birth weight SDS	Birth height SDS	Diagnosis	Calcium at diagnosis (mg/dL)	Phosphorus at diagnosis (mg/dL)	Magnesium at diagnosis (mg/dL)	ALP at diagnosis (U/L)	PTH at diagnosis (ng/L)	25(OH) D at diagnosis (µg/L)	Cardiac anomaly	Rare additional findings
2	M	45 days	38	-1.5	-0.8	Seizure	6	4.9	1.8	181	6.5	N/A	Tetralogy of Fallot, pulmonary artery hypoplasia	Unilateral renal agenesis and inguinal hernia
4	F	10.5 years	38	-0.86	N/A	Incidentally	6.9	5.2	N/A	158	31	N/A	Truncus arteriosus, VSD	No
5	M	8 years	39	-0.11	N/A	Incidentally	8.9	5.6	N/A	157	9.1	18.27	No	No
6	M	12.5 years	39	N/A	N/A	Incidentally	7	5.9	N/A	229	36.4	26.4	No	Inguinal hernia
7	M	13 months	38^{+5}	-0.43	N/A	Tingling in hands	7.8	7.6	2	140	7.4	22.2	No	No
8	F	30 days	N/A	N/A	N/A	Seizure	6.7	7.8	N/A	134	6.8	21.1	Tetralogy of Fallot, ASD	No
9	M	13.5 years	N/A	N/A	N/A	Incidentally	5.8	6.4	N/A	128	10.6	24.7	Dilatation of aortic root	Unilateral vanishing testes
12	F	1 day	34^{+1}	-1.02	-1.38	Incidentally	6.6	7.3	3.1	426	4.1	8.9	Truncus arteriosus	No
13	F	11.5 years	N/A	N/A	N/A	Tingling in hands	7.1	6.2	N/A	159	23.8	26.8	No	No
14	F	1 day	39^{+1}	0.77	2.51	Incidentally	6.8	5.3	1.9	195	47.6	7.9	Pulmonary stenosis, VSD, ASD	No
15	M	2 days	40	0.24	0.28	Antenatal	7.8	6.3	2.6	177	3.7	7	Truncus arteriosus, VSD, right-sided aortic arch	No
17	F	1 day	36^{+6}	1.09	0.87	Antenatal	7.8	5.2	3.8	87	10.3	36.1	Truncus arteriosus, VSD, pulmonary atresia	Cleft palate

M: male, F: female, N/A, not applicable/available, PTH: parathyroid hormone, ALP: alkaline phosphatase, SDS: standard deviation score, ASD: atrial septal defect, VSD: ventricular septal defect, 25(OH)D: 25 hydroxyvitamin D

(13). Although it has been reported in a few studies that PTH levels may be undetectable in patients with permanent hypoparathyroidism (5), no significant difference was found in PTH levels between permanent and transient hypoparathyroidism cases in this study. PTH levels did not help predict transient or permanent hypoparathyroidism.

Growth retardation, thyroid dysfunction, and obesity are endocrinological problems seen frequently in 22q11.2 DS (4,5,7,8,9). Choi et al. (5) reported intrauterine growth retardation in 26.2% of their cases. In the present study, both birth weight and height were found to be in the normal reference range. It has been reported that the cause of growth retardation may be related to cardiac anomalies, recurrent infections, and nutritional problems, due to velopharyngeal anomalies (4,14). Levy-Shraga et al. (4) reported an association between heart defect and short stature, but not with recurrent infection and palatal defects. In the present study, no significant difference was detected in height SDS in patients with cardiac anomaly or T cell and/or B cell immunodeficiencies It is thought that this may be because cardiac surgery was quickly as early as possible, when necessary, in our cohort and there was no history of frequent infections in the cases with T cell and/or B cell immunodeficiencies. Goldberg et al. (14), suggested that short stature, seen in 30% of children, may be constitutional short stature since it is seen in only 10% of adults. These authors found no relationship between cardiac defect and short stature; growth hormone deficiency (GHD) was found in 4% of the cases. GHD was reported by Weinzimer et al. (11) in four cases, with growth hormone therapy improving the patient's final height. At the Children's Hospital of Philadelphia, short stature was reported in 10-40% of patients. GHD was found in some children below the 5th percentile for height (15). GHD was not detected in any patient in our cohort. This may be due to the larger number of patients in the earlier studies. Shprintzen et al. (16) described short stature in 39% of their patients. In another study, the height of patients who reached their final adult height was below -1 SDS (4). Habel et al. (17) and Tarquinio et al. (18) also reported that the cases were shorter than the general population. Similarly, in our study, the mean height was reported as -0.94 ± 0.83 SDS on follow-up, and the mean final height of five patients was -0.94 ± 0.94. Although no pathological short stature was seen in our cohort, the final stature of those reaching final height was indeed around -1 SD shorter than the general population.

Autoimmune (Graves and Hashimoto) and non-autoimmune (thyroid hypoplasia) thyroid diseases have been reported in 22q11.2 DS cases, affecting between 0.7-7% of patients (4,5,7,8,9). In the present study, thyroid autoantibodies were detected in two cases (11.7%) with normal thyroid function, and two cases (11.7%) were diagnosed with primary hypothyroidism in the neonatal period and started levothyroxine treatment. This rate is likely higher than the literature, which may suggests that thyroid diseases can be seen more frequently in 22q11.2 DS cases if hypoparathyroidism is present. But in our study, the number of the patient is very limited and there are many factors can affect thyroid metabolism. It has been reported that skeletal system anomalies, such as cervical spinal region anomalies, scoliosis, syndactyly, and patellar dysfunction are affect between 17-47% in 22q11.2 DS (19). In the present study, the rate of scoliosis was found to be similar to that in the literature. Previous reports have suggested that urogenital anomalies may affect approximately 30% of patients with 22q11.2 DS (20). There were four patients (23.5%) with urogenital anomalies in our study cohort. Thus, assessment of the skeletal system and investigation for urogenital anomalies should be performed in patients with 22q11.2 DS. Although the incidence of palate anomalies has been reported to be 69-100%, we found only two cases (11.8%) (19). Only patients with 22q11.2 DS and associated hypoparathyroidism were included in the present study, so the results may have been affected. Furthermore, given the low number of patients, our results may not accurately reflect the overall incidence of this anomaly.

Study Limitations

It was not possible to access data for some cases due to technical issues. In addition, long-term follow-up of some cases was not performed in our hospital. Although studies into hypoparathyroidism were numerous, the frequency of other endocrine findings is not clear because of the paucity of data.

Conclusion

This article describes various endocrine manifestations in patients with 22q11.2 DS. These findings suggest that careful endocrine evaluation is necessary for patients with this microdeletion syndrome, particularly those with hypoparathyroidism or thyroid dysfunction. It is known that many cases are diagnosed in the neonatal period, but some

may be missed due to transient hypocalcemia and loss of follow-up. These cases may present with hypocalcemia in acute hypermetabolic situations later in life. Therefore, patients with hypocalcemia in the neonatal period should be carefully monitored for 22q11.2 DS. Vitamin D deficiency was found to be a risk factor for hypocalcemia in 22q11.2 DS, especially in the transient group. It is important to carefully investigate cases presenting to pediatric endocrinology with hypoparathyroidism, especially in terms of cardiac and urogenital anomalies. Recognition of the more specific facial findings, particularly the hooded eyelids along with the better known dysmorphic features, may improve earlier diagnosis and trigger genetic diagnosis, screening for additional anomaly, and routine follow-up. The endocrinological findings and immunodeficiency types and prevalences in patients with 22q11.2 DS should be evaluated in larger series.

Ethics

Ethics Committee Approval: The study was approved by the Uludağ University Local Ethical Committee (approval number: 2021-19/22, date: 22.12.2021).

Informed Consent: Written informed consent was obtained from the families of two patients who allowed the publication of clinical facial photographs.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Yasemin Denkboy Öngen, Erdal Eren, Concept: Yasemin Denkboy Öngen, Erdal Eren, Design: Yasemin Denkboy Öngen, Erdal Eren, Data Collection or Processing: Yasemin Denkboy Öngen, Erdal Eren, Analysis or Interpretation: Yasemin Denkboy Öngen, Şebnem Özemri Sağ, Şehime Gülsün Temel, Erdal Eren, Literature Search: Yasemin Denkboy Öngen, Erdal Eren, Writing: Yasemin Denkboy Öngen, Erdal Eren.

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Psychometric Properties of the Turkish Validity and Reliability of the Parent Diabetes Distress Scale

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What is already known on this topic?

Families of adolescents with type 1 diabetes mellitus (T1DM) experience many difficulties in different areas related to diabetes.

What this study adds?

The adaptation and validation of the Turkish version of the Parent Diabetes Distress Scale (PDDS) is reliable and valid. The Turkish PDDS may be used to assess parental distress when their adolescent children are diagnosed or living with T1DM.

Abstract

Objective: The aim of this study was to evaluate the validity and reliability of the Turkish translation of the Parent Diabetes Distress Scale (PDDS).

Methods: The PDDS is a 5-point Likert-type scale with 20 items. After obtaining permission from the scale developers, the study commenced. First, a systematic adaptation of the scale into the Turkish language was performed including translation, expert panel review, back translation, and pilot study. Test-retest was applied to 35 participants. After these procedures, data collection was undertaken using the adapted PDDS and a demographic data collection form. The collected data were analyzed for reliability, including stability of the scale with test-retest and internal consistency of the scale (Cronbach's α), and validity including construct validity of the scale with confirmatory factor analysis (CFA).

Results: The parents of 210 teenagers, aged >11 and <18 years, who had been diagnosed with type 1 diabetes mellitus for at least one year were included. Of these parents, 71.9% (n=151) were mothers and 53.3% (n=112) of the children were girls. The Cronbach's α value was 0.906. The results of the CFA were $\chi^2/df=4.406$, $p<0.001$, comparative fit test 0.704, and goodness of fit tests 0.749. The mean total PDDS score was 2.2 ± 0.7. These results indicate that scores of 1.6 points or less was evaluated as "little or no distress" 1.7-2.4 as "moderate distress," and >2.4 points as "high distress". This showed that the majority of the parents in the study experienced moderate or severe diabetes-related distress.

Conclusion: The Turkish version of the PDDS fulfilled the validity and reliability tests at an acceptable level.

Keywords: Type 1 diabetes, adolescent, scale, reliability, validity, parent stress

Introduction

Type 1 diabetes mellitus (T1DM) is a common chronic disease caused by pancreatic β-cell damage in children and adolescents (1,2). The 10th edition of the *International Diabetes Federation Atlas* estimated that 1,211,900 children and adolescents under 20 years of age have T1DM worldwide (3). The incidence of childhood-onset T1DM is

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reported to be increasing in many countries. Although the rate of increase varies geographically, the overall global annual increase is estimated to be around 3% (4).

T1DM burdens the whole family of affected children in many ways and is thus a cause for clinical concern (5). Although it is generally accepted that childhood diabetes affects all family members, few studies have focused specifically on parents (5,6,7). Furthermore, relatively few studies have examined the relationships between parental stress and family demographic factors. The young age of the sick adolescent, long-term illness, low socioeconomic level, and being a single parent cause higher levels of stress in parents (5,8,9). It has been reported that in adolescents with poor glycemic control and diabetes self-management, family conflict and parental distress tend to be at a higher level (8). Increased parental emotional distress has been associated with more parental depressive symptoms, lower quality of life, and more family stress (9,10,11).

A number of different scales related to the stress caused by type 1 and type 2 diabetes have been published (12,13). However, some of these scales only measure the distress caused by diabetes (14). There are few scales evaluating diabetes-related stress in parents of children with T1DM (12,14,15). The scale developed by Katz et al. (12), on the other hand, measures the familial effect of T1DM in children. Adolescence is a particularly risky period for families in terms of diabetes management because during adolescence family conflicts tend to be increased (16). Adolescents may experience more difficulties in self-management of their chronic diabetes (5). Therefore, in 2016, Hessler et al. (17) developed the Parent Diabetes Distress Scale (PDDS), a 5-point Likert-type scale with 20 items. This scale measures the effect of adolescent T1DM on the family and has been widely used. To the best of our knowledge, however, there is no Turkish version of the PDDS. Therefore, the aim of this study was to test the factor structure, reliability and validity using the PDDS translated into Turkish.

Methods

Design and Setting

This study used a methodological design. The survey was conducted between October and December 2021.

Participants

The sample of the study consisted of the parents of adolescents over the age of 11 and under the age of 18 who attended Sivas Cumhuriyet University Faculty of Medicine, Pediatric Endocrinology outpatient clinic and had been diagnosed with T1DM for at least one year. As the scale contained 20 items and it is recommended that a sample of 5-10 times the number of items should be reached in cross-cultural scale adaptation (18), the required sample size was calculated as at least 200 participants (20 items x 10). The targeted sample was achieved by administering the research questionnaire to 210 people. It was administered by one of the researchers using the face-to-face interview method. The participants were informed about the study beforehand, and their consent was obtained. Either the mother or father was included in the study. Other relatives were excluded. Parents who were illiterate were also excluded.

Process

The participants answer the items in the PDDS with responses ranging from *Not at all* to *A great deal*, according to how they felt about the scale items in the last month. There are no negatively scored items in the scale. To score the scale, the participants' responses to the items are summed and divided by the number of items in the scale. The scores that can be obtained from the scale range from 0 to 5. High scores are associated with increased stress levels. Hessler et al. (17) defined four sub-dimensions of the scale (personal distress, teen management distress, parent/teen relationship distress, and healthcare team distress). In the original study of Hessler et al. (17), the Cronbach's α value for the whole scale was 0.94.

In this study, the Turkish version of the PDDS and a data form created by the researchers were administered to the participants. The data form gathered information about the sociodemographic characteristics of the parents, the demographic characteristics of the teenagers, and the characteristics of their diabetes. Permission to use the PDDS was obtained by mail (info@behavioraldiabetes.org) from Dr. Polonsky, one of the original authors. The adaptation stages of the scale were conducted in line with World Health Organization intercultural adaptation guidelines, as well as recommendations in the literature review (Figure 1). The scale was translated into Turkish by two translators. Then the authors committee decided on a common translation. The translated scale was sent to experts and assessed and the content validity index (CVI) was calculated. The Turkish scale was back-translated into English, and after translation was completed, a second confirmation was received from Dr. Polonsky by e-mail. The Turkish version of the scale was administered to 10 people as a pilot study. Then 30 people were tested-retested on the final version of the scale. Correlation analysis between the test-retest was examined. Afterwards, the data collection form and PDDS were administered to 210 participants for the main study. Figure 1 shows the intercultural adaptation stages applied.

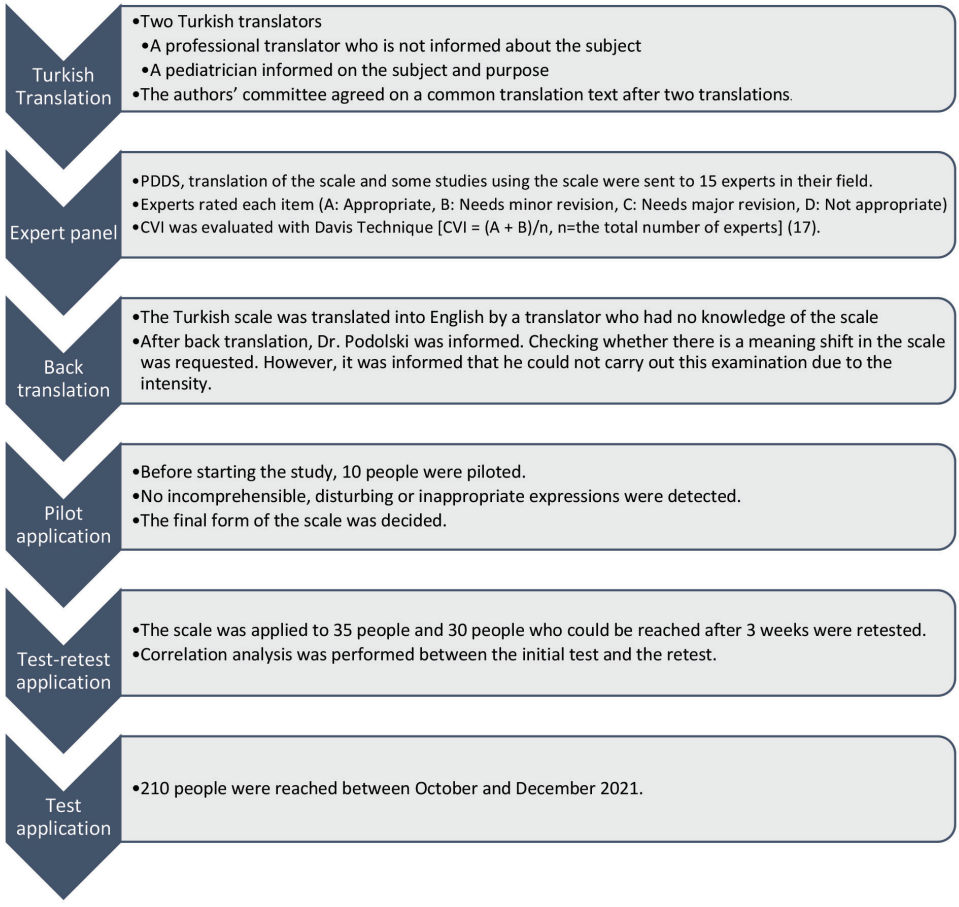

Figure 1. Intercultural adaptation stages applied in the research

CVI: content validity index, PDDS: Parent Diabetes Distress Scale

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) for Windows, version 25 and IBM SPSS Amos 20 were used for statistical analysis (IBM Inc., Armonk, NY, USA). Normality analysis was performed with the Shapiro-Wilk test for numerical values. Descriptive statistical analyses were calculated for sociodemographic data and items of the scale. The Student's t-test was used to compare normally distributed numerical data between two categorical variables. The factors affecting the PDDS score were evaluated with a multiple linear regression model analysis (enter method). The presence of multicollinearity among the independent variables was tested with the variance inflation factor (VIF) value. Within the scope of the reliability analysis of the scale, the stability of the scale was determined by test-retest, and the internal consistency of the scale was evaluated with Cronbach's α. Content validity was evaluated with the Davis technique to test the validity of the scale (19). Construct validity was tested with confirmatory factor analysis (CFA). The suitability of the scale for factor analysis was evaluated with the sphericity method of Kaiser-Meyer-Olkin (KMO) and Bartlett's test. CFA results were reported with total variance values and factor loads, χ^2/df, comparative fit test (CFI), goodness-of-fit test (GFI), and root mean square error (RMSEA) values of approximate approximation. A p value of less than 0.05 was considered to indicate statistical significance, with a 95% confidence interval.

Results

Descriptive Characteristics of the Participants

The parents of 210 teenagers were included in the study. The mean age of their children was 14.0 ± 2.1 years and the mean duration of diabetes diagnosis was 56.7 ± 37.8 months. Eighty (38.1%) of the parents had completed primary school. The demographic characteristics of the participants are shown in Table 1. The children of 84 (40.0%) of the participants experienced severe hypoglycemia (< 50 mg/

dL) every month. The median monthly frequency of those who had hypoglycemia was 2 (minimum: 1 - maximum: 6; interquartile range: 2.0).

Diabetes-Related Stress of Parents and Affecting Factors

The PDDS mean score of the parents was 2.2 ± 0.7. The scores were divided into three categories: ≤1.6 points, 1.7-2.4, and >2.4". The interpretation of these three categories was: ≤1.6 points evaluated as "little or no distress," 1.7-2.4 points as "moderate distress," and >2.4 points as "high distress." The cut-off value was not calculated in this study. It is recommended that further studies using this scale determine their own cut-off value. The effect of variables on PDDS scores is shown in Table 2.

Diabetes-related stress levels were found to be higher in mothers compared with fathers and in those living in rural areas compared with those living in the city center ($p < 0.05$). Diabetes-related stress levels were also found to be higher in families whose children used an insulin pen, those who had been hospitalized in the last year, those who had difficulty complying with the diabetes regimen, and those who had experienced severe hypoglycemia in their children ($p < 0.05$). In the multiple linear regression model created, the diabetes-related stress score was confirmed as higher in mothers compared with fathers, those living in rural areas compared with those living in the city center,

Table 1. Parent and teen characteristics and diabetes mellitus-related features

Demographic characteristics	n = 210
Parent (% mother)	151 (71.9%)
Domicile situation n (%)	
City	138 (65.7%)
Rural	72 (34.3%)
Education level of parent*	
Primary school (5 years)	81 (38.6%)
Secondary school (3 years)	55 (26.2%)
High school (3 years)	42 (20.0%)
University and above	32 (15.2%)
Income level	
Minimum wage and below	86 (41.0%)
Above minimum wage	124 (59.0%)
Family structure	
Nuclear	158 (75.2%)
Extended	41 (19.5%)
Separated	11 (5.2%)
Number of children	2.1 (0.9)
Teen age	14.0 (2.1)
Teen gender (% female)	112 (53.3%)
Months since diagnosis	56.7 (37.8)
HbA1c (percent)	8.6 (1.9)
Frequency of self-monitoring blood glucose per day	6.8 (2.0)
Insulin delivery method	
Pen	193 (91.9%)
Pump	17 (8.1%)
Additional chronic disease n (%)	62 (29.5%)
Hospitalization in the last year n (%)	114 (54.3%)
Intensive care hospitalization in the last year n (%)	44 (21.0%)
Diabetic diet compliance	
Difficult	98 (46.7%)
No difficulty	112 (53.3%)

*Educational status of the parent who answered the questionnaire was shown and illiterate people were not included in the study.
HbA1c: glycated hemoglobin

Table 2. Effect of variables on PDDS score

	Mean score of PDDS	p
Gender of teenager		
Female	2.2 (0.7)	0.941
Male	2.2 (0.7)	
Parent		
Mother	2.3 (0.7)	**< 0.001**
Father	1.9 (0.7)	
Parent education		
Lower than high school education	2.2 (0.7)	0.856
High school and above education	2.2 (0.8)	
Domicile situation		
City	2.0 (0.6)	**< 0.001**
Rural	2.5 (0.8)	
Method of Insulin delivery		
Pen	2.2 (0.7)	**0.001**
Pump	1.6 (0.4)	
Hospitalization in the last year		
Yes	2.4 (0.7)	**< 0.001**
No	1.9 (0.6)	
Intensive care hospitalization in the last year		
Yes	2.6 (0.6)	**0.046**
No	2.3 (0.7)	
Regime compliance		
Easy	2.0 (0.7)	**0.006**
Not easy	2.3 (0.7)	
Presence of additional chronic disease		
Yes	2.4 (0.8)	**0.011**
No	2.1 (0.7)	
Presence of severe hypoglycemia (< 50 mg/dL) every month		
Yes	2.3 (0.7)	**0.005**
No	2.1 (0.7)	

PDDS: Parent Diabetes Distress Scale

those who were hospitalized in the last year compared with those who were not hospitalized, and the presence of severe hypoglycemia (< 50 mg/dL) compared to those not have ($p < 0.05$) (Table 3). Adjusted R^2 was calculated as 0.327. It shows that this established model explains 32.7% of the diabetes-related stress on parents. The VIF values of the independent variables of the model ranged from 1.088 to 1.318. Since the VIF value is below ≤4, there is no multi collinearity problem (20).

Validity and Reliability Analyses of the PDDS

Content validity analysis was evaluated with the Davis technique to test the validity of the scale. With this technique a CVI > 0.80 will indicate content validity (18). The CVI for all items of the Turkish version of the PDDS was above 0.80 (Table 4).

The Cronbach's α value calculated within the scope of the internal consistency analysis of the scale was 0.906. The item-total correlation coefficients ranged from 0.208 to

Table 3. Examination of the effects of variables on parent diabetes distress using multiple regression analysis model

	β	%95 CI	p
Female teen gender (compared to male)	0.087	-0.270 – 0.095	0.345
Female parent gender (compared to fathers)	0.213	0.011 – 0.415	**0.039**
Parent's lower than high school education level (compared to above)	-0.070	-0.012 – 0.153	0.094
Number of children	0.194	0.097 – 0.291	**< 0.001**
Living in the city center (compared to rural)	-0.440	-0.628 – -0.251	**< 0.001**
Using insulin pen (compared to pump)	0.335	-0.675 – 0.005	0.053
Months since diagnosis	0.002	0.000 – 0.004	0.074
Hospitalization in the last year (compared to not)	0.294	0.097 – 0.492	**0.004**
Easy regime compliance (compared to harder)	0.145	-0.323 – 0.034	0.111
Presence of additional chronic disease (compared to not)	0.309	0.107 – 0.512	**0.003**
HbA1c	-0.014	-0.061 – 0.032	0.543
Presence of severe hypoglycemia (< 50 mg/dL) every month (compared to not)	0.209	0.012 – 0.407	**0.038**

n = 210, adjusted $R^2 = 0.327$.
CI: confidence interval, HbA1c: glycated hemoglobin

Table 4. The results of CVI on PDDS using the Davis technique

	Appropriate	Needs minor revision	Needs major revision	Not appropriate	CVI
Item 1	11	2	2	0	0.86
Item 2	14	0	1	0	0.93
Item 3	13	1	1	0	0.93
Item 4	15	0	0	0	1.00
Item 5	9	4	2	0	0.86
Item 6	10	3	2	0	0.86
Item 7	8	5	2	0	0.86
Item 8	15	0	0	0	1.00
Item 9	14	1	0	0	1.00
Item 10	15	0	0	0	1.00
Item 11	12	1	2	0	0.86
Item 12	15	0	0	0	1.00
Item 13	11	2	2	0	0.86
Item 14	7	6	2	0	0.86
Item 15	14	1	0	0	1.00
Item 16	13	1	1	0	0.93
Item 17	15	0	0	0	1.00
Item 18	12	1	2	0	0.86
Item 19	7	6	1	1	0.86
Item 20	15	0	0	0	1.00

CVI: content validity index, PDDS: Parent Diabetes Distress Scale

Table 5. Test-retest and Cronbach's α analyzes of the PDDS

	Test retest reliability (Pearson's r)	Total correlation	Cronbach's α if item deleted
Item 1	0.743**	0.355	0.906
Item 2	0.854**	0.503	0.902
Item 3	0.797**	0.595	0.900
Item 4	0.809**	0.599	0.900
Item 5	0.912**	0.677	0.898
Item 6	0.912**	0.599	0.900
Item 7	0.953**	0.700	0.897
Item 8	0.832**	0.464	0.903
Item 9	0.874**	0.649	0.898
Item 10	0.660**	0.542	0.902
Item 11	0.535*	0.208	0.909
Item 12	0.815**	0.495	0.903
Item 13	0.820**	0.646	0.898
Item 14	0.798**	0.643	0.899
Item 15	0.729**	0.477	0.903
Item 16	0.836**	0.410	0.905
Item 17	0.861**	0.609	0.900
Item 18	0.830**	0.588	0.900
Item 19	0.909**	0.469	0.903
Item 20	0.892**	0.552	0.901
Total	0.942**	Cronbach's α of total scale: 0.906	

* < 0.05, ** < 0.001.
PDDS: Parent Diabetes Distress Scale

0.700. There was a single value below 0.30, but since there was no change in the Cronbach α value when this item was removed, it was decided to retain the item in the scale. The reliability of the scale was also measured using the test-retest method. The test-retest correlation coefficients of the items of the scale ranged from 0.535 to 0.953. The test-retest correlation coefficient of the total score was calculated as 0.942 (Table 5). The KMO coefficient, which was calculated within the scope of the construct validity of the scale, was 0.842. Bartlett's sphericity test results were $\chi^2 = 2003.303$, $p < 0.001$. In the CFA performed on the sub-dimensions defined in the scale, the χ^2/df value was calculated as 4.406 ($p < 0.001$), GFI 0.749, CFI 0.704 and RMSEA 0.128. Figure 2 shows the CFA model of the PDDS.

Discussion

Families who have teenagers with T1DM experience many difficulties, which may include their own personal distress, difficult relationships with their teenagers, and teenagers' problems with managing their diabetes. Identifying and supporting families' diabetes-related distress plays a key role in disease management. Adolescence is already a challenging period for young people, in which many changes occur physically and psychologically. It is more difficult for teenagers to struggle with a chronic illness, such as T1DM, during this period. A happy and psychologically

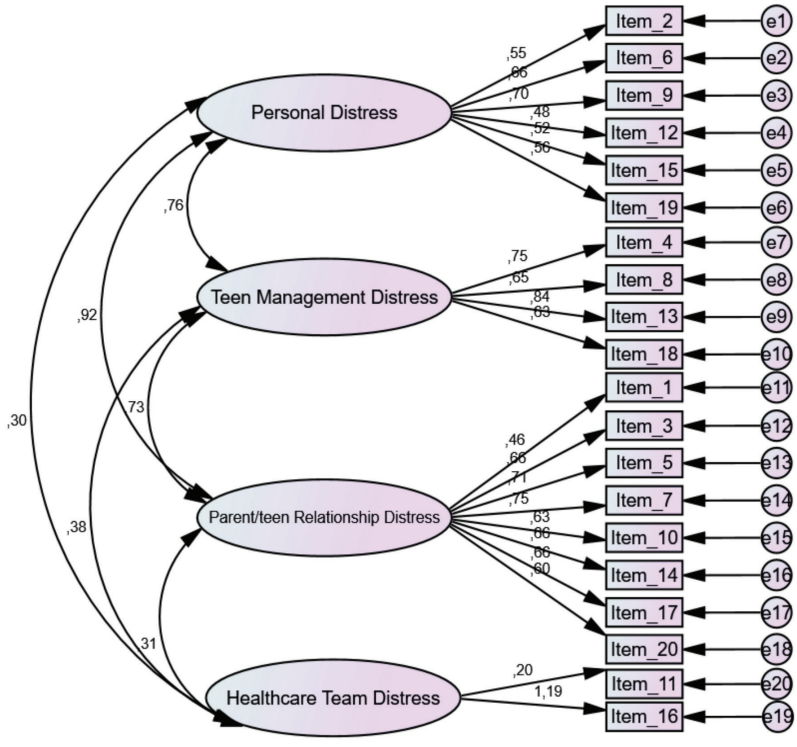

Figure 2. Confirmatory factor analysis model of the PDDS

PDDS: Parent Diabetes Distress Scale

strong parent can better support their child in this process. The current study describes the adaptation of the PDDS for use in Turkey and the subsequent validity testing in order to provide a tool to assess the stress experienced by parents of adolescents with T1DM.

Content validity analysis of the scale was conducted. The CVI value of all items of the PDDS scale were higher than 0.80, which was interpreted as the Turkish version of the PDDS having content validity (20). The Cronbach's α, item-total correlation, and test-retest correlation analyses were performed within the scope of reliability analyses of the scale. The Cronbach's α value calculated for the Turkish version of the PDDS was 0.906 which was comparable with 0.940 reported by Hessler et al. (17) following the original development of the scale. In the literature, a value of 0.60 and above is acceptable for the Cronbach's α value (21) although some researchers suggest that values above 0.70 are reliable (22). Item-total correlation analysis, one of the reliability analyses, explains the relationship between the scores obtained from the test items and the total score of the test. If this value is 0.30 and above, it may be interpreted as the discrimination rate of the items is high (18). Only one of the 20 items of the Turkish version of the PDDS scale had a value below 0.30, but when this item was removed it did not change the Cronbach's α value so it was decided to retain the item in the scale. The test-retest method is the reliability calculation applied to determine the stability of a scale. In this assessment, the time between testing and retesting is important. Deciding the delay should be done according to the feature measured. Generally, it is stated that a period of 2-3 or 4-6 weeks is sufficient (23). In the present study, the test was administered again to 30 of the original 35 participants three weeks after first completion. The test-retest reliability for the present study was 0.942 and there was a strong and significant positive correlation between the two sessions.

In the present study, KMO and Bartlett tests were applied to determine the adequacy of the sample and whether the data were suitable for factor analysis. The present study found a KMO value of 0.842 while Bartlett's sphericity test results were significant at $\chi^2 = 2003.303$ and $p < 0.001$. A KMO value greater than 0.60 and a significant Bartlett test indicate that the data are suitable for factor analysis (18). In the CFA performed on the sub-dimensions defined in the scale, χ^2/df, GFI, CFI, and RMSEA values were used to evaluate the fit index. A χ^2/df, value of ≤3 is a very good indicator of model fit. GFI and CFI values of 0.90 and above indicate adequate good fit, while values between 0.80 and 0.90 indicate that the structure is suitable for a good fit. Regarding the RMSEA value, while some researchers accept < 0.06 as being good, others accept 0.07 as the threshold value (24,25,26). In our study, the CFA, performed in accordance with the study of Hessler et al. (17), showed that the fit indexes of the sub-dimensions were low. For this reason, it was concluded that the scale did not show any sub-dimensions in the analyses conducted in our sample and it should therefore be evaluated using the total score.

The results of the present study in more than 200 parents of Turkish adolescents with T1DM found that diabetes-related parental stress was common, which is similar to previous studies (8,11) and the findings of the original scale. The PDDS scale mean score of the parents was 2.2 ± 0.7 which falls towards the upper end of the moderate distress category. This suggests that most of the study participants experienced moderate or severe DDS. Furthermore, earlier studies reported that mothers experience more stress than fathers when caring for a child with diabetes (27,28,29). This finding was replicated with the Turkish version of the PDDS. Common stressors include food management, family conflict related to diabetes, injecting insulin, and monitoring blood sugar (30).

There may be differences between the sexes in terms of adaptation to chronic disease due to hormonal and metabolic changes during adolescence. While it is more difficult for boys to adapt to diabetes during childhood, it is more difficult for girls to adapt during adolescence. This situation also makes a difference in the impact of the disease on their families. Different results have been found in the literature on this issue (31,32). In the study of Hessler et al. (17), it was reported that parents of sons experienced more diabetes-related stress. In another study, it was found that families of adolescent girls experienced more stress (33). The present study found no significant patient gender effect and the scale score.

In the study of Hessler et al. (17), it was reported that parents of sons experienced more diabetes-related stress. Hessler et al. (17) reported no correlation between the duration of diabetes diagnosis and stress and this was also found in the present study. Many studies have reported a relationship between degree of glycemic control and diabetes-related stress (34,35,36). In the present study, there was no relationship between stress level and glycated hemoglobin (HbA1c) level, but a positive correlation was found with increasing frequency of severe hypoglycemia. Hessler et al. (17) found a positive correlation between both frequency of hypoglycemia and HbA1c level and stress. In a study among Lithuanian youth, parents of young people with good diabetes control had lower stress levels (33).

Parents of children with T1DM experience fear and stress during insulin injection and glucose-testing procedures.

Previous studies reported that 13.6% of mothers experienced needle phobia and related stress following the diagnosis of diabetes (37,38). In the present study, higher levels of stress were reported by parents of adolescents who used an insulin pen for injections compared with those who used pumps. This was similar to the original report of Hessler et al. (17) but in a later study by Polonsky et al. (38) research, the opposite was the case.

Studies have shown that the most frequent conflicts between families and young people were around adolescents' blood sugar control and adherence to their diets (39,40). In the present study, parents of adolescents who had difficulties in adapting to their diabetes regimen had increased stress. Frey et al. (41) found that low income level was associated with difficulties in coping with the disease and also with more stress in mothers. Mothers had higher levels of diabetes-related stress than fathers in the present study, in line with earlier reports.

Approximately 20-30% of parents of children with T1DM experience clinically significant depressive symptoms and anxiety (42). These symptoms are mostly related to parents' involvement in their child's diabetes management tasks (43). In addition, parents' fear of hypoglycemia and adolescents' poor glycemic control also increased parents' stress (5,44). In the present study, stress increased in the families of adolescents who had severe hypoglycemia in the month preceding completion of the Turkish version of the PDDS, or if their children had been hospitalized in the previous year, or had an additional chronic disease. These findings are again in line with previously published reports.

Study Limitations

The study data were collected from a single center in the central part of the country. Statistical power was limited. Therefore, the generalization of results by country are likely to be affected. However, the present study also has several strengths. First of all, to the best of our knowledge, this is the first study conducted in Turkey in which this scale was adapted and used. Therefore, this study indicates that the Turkish version of the PDDS may be used in both clinical and research settings, and the validity and reliability of the Turkish version are at a sufficient level. In addition, cross-cultural comparative studies can be carried out using this scale.

Conclusion

Diabetes-related stress is a common and important problem for parents of adolescents with T1DM. This stress is associated with sociodemographic characteristics of families and adolescents' diabetes management. This study examined the psychometric properties of the Turkish version of the PDDS and the results showed that the Turkish version of the scale was a valid and reliable measurement tool.

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Ethics

Ethics Committee Approval: Ethics approval was granted by the Sivas Cumhuriyet University Non-Interventional Clinical Research Ethics Committee (approval number: 2021-01/52, date: 13.01.2021).

Informed Consent: The participants were informed about the study beforehand, and their consent was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Seher Karahan, Ezgi Ağadayı, Concept: Seher Karahan, Seda Aybüke Sarı, Nurullah Çelik, Ayça Kömürlüoğlu Tan, Esra Döğer, Design: Seher Karahan, Ezgi Ağadayı, Seda Aybüke Sarı, Nurullah Çelik, Ayça Kömürlüoğlu Tan, Esra Döğer, Data Collection or Processing: Seher Karahan, Ezgi Ağadayı, Seda Aybüke Sarı, Nurullah Çelik, Ayça Kömürlüoğlu Tan, Esra Döğer, Analysis or Interpretation: Ezgi Ağadayı, Literature Search: Seher Karahan, Ezgi Ağadayı, Writing: Seher Karahan, Seda Aybüke Sarı.

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CASE REPORT

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Congenital Hyperinsulinism and Maple Syrup Urine Disease: A Challenging Combination

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What is already known on this topic?

Glucose homeostasis is maintained through multiple pathway interactions. Congenital hyperinsulinism is the most common cause of persistent hypoglycemia in infancy. Maple syrup urine disease is a rare cause of neonatal hypoglycemia caused by impairment of branched-chain alpha-keto acid dehydrogenase complex.

What this study adds?

This patient is perhaps the only known case of the co-occurrence of congenital hyperinsulinism with maple syrup urine disease. This case report describes the challenging clinical management of both conditions in combination.

Abstract

Congenital hyperinsulinism (CHI) is the most common cause of persistent hypoglycemia in infancy. CHI is a challenging disease to diagnose and manage. Moreover, complicating the course of the disease with another metabolic disease, in this case maple syrup urine disease (MSUD), adds more challenges to the already complex management. We report a term neonate who developed symptomatic, non-ketotic hypoglycemia with a blood glucose (BG) level of 1.9 mmol/L at 21-hours of life. A critical sample at that time showed high serum insulin and C-peptide levels confirming the diagnosis of CHI. Tandem mass spectrometry done at the same time was suggestive of MSUD which was confirmed by high performance liquid chromatography. The diagnosis of both conditions was subsequently confirmed by molecular genetic testing. His hypoglycemia was managed with high glucose infusion with medical therapy for CHI and branched chain amino acids (BCAA) restricted medical formula. At the age of four months, a near-total pancreatectomy was done, due to the failure of conventional therapy. Throughout his complicated course, he required meticulous monitoring of his BG and modified plasma amino acid profile aiming to maintain the BG at ≥3.9 mmol/L and levels of the three BCAAs at the disease therapeutic targets for his age. The patient is currently 29 months old and has normal growth and development. This patient is perhaps the only known case of the co-occurrence of CHI with MSUD. Both hypoglycemia and leucine encephalopathy can result in death or permanent neurological damage. The management of CHI and MSUD in combination is very challenging.

Keywords: Hypoglycemia, congenital hyperinsulinemia, maple syrup urine disease, *ABCC8* mutation, *BCKDHA* mutation

Introduction

Neonatal hypoglycemia is a medical emergency with multiple possible underlying causes. Congenital hyperinsulinism (CHI) is the most common cause of persistent hypoglycemia in infancy. CHI comprises a group of heterogenic genetic disorders with the common finding of recurrent episodes of hyperinsulinemic hypoglycemia due to inappropriate secretion of insulin by the pancreatic β-cells (1). The most common causes of CHI are mutations in *ABCC8* (OMIM #600509) and *KCNJ11* (OMIM #600937). Recessive mutations in these genes cause diffuse hyperinsulinism that is a severe, drug-resistant form,

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which may require resection of the pancreas. In this case, 18F-fluoro-L-DOPA positron emission tomography (PET) scan is not indicated. Dominant mutations in these genes have been associated with diazoxide-responsive disease (1,2).

Maple syrup urine disease (MSUD) is a rare cause of neonatal hypoglycemia. It is an autosomal recessive disease affecting the metabolism of branched chain amino acids (BCAA). The genetic defects result in impairment of branched-chain alpha-keto acid dehydrogenase complex. This complex is the rate-limiting step in breaking down the BCAA; leucine, isoleucine, and valine.

The combination of those two diseases makes the management very challenging, as there are multiple interactions between insulin and BCAA. Leucine is the most potent insulin stimulus among BCAA. It increases insulin secretion indirectly by inhibition of K_{ATP} channel activity via its intermediate metabolite; α-ketoisocaproate. Moreover, it regulates insulin release by acting on glutamate dehydrogenase. Additional mechanisms include triggering calcium oscillations in pancreatic β-cells and stimulation of the mTOR signaling pathway. The mTOR pathway mediates nutrient sensing and regulates protein synthesis in the pancreas. Inhibition of mTOR by rapamycin impairs glucose-induced insulin secretion in the pancreatic β-cells which might explain the risk for new-onset diabetes in organ transplanted patients (3,4). In contrast, insulin has a pronounced leucine lowering effect, hence it is used in the treatment of MSUD catabolic status (5).

In this case report, we present a child with CHI and MSUD who underwent near-total pancreatectomy. The main objective is to describe the management challenges in pre- and post-pancreatectomy status.

Case Report

A currently 29-month-old boy was born to a double first-cousin parent with no significant family history. His mother was 30 years old, G4P3 with no significant medical comorbidity. The pregnancy was uneventful. He was born at a peripheral hospital at 37 weeks of gestation via lower segment caesarian section (LSCS) as his mother had previous LSCS. His APGAR scores were 9 and 10 at 5 and 10 minutes, respectively. His birth weight was 3.46 kg (46th percentile), head circumference 35 cm (35th percentile), and length 51 cm (61st percentile). Breastfeeding was started immediately following delivery. At 21 hours of life, the baby was noted to be dusky, not breathing and he was resuscitated with bag-mask ventilation and transferred to the neonatal intensive care unit. He was found to be hypoglycemic, with a blood glucose (BG) of 1.9 mmol/L. He received four boluses of 10% dextrose and a critical blood sample, taken during hypoglycemia, showed high insulin, 58.7 mIU/L (2.6-24.9 mIU/L) and C-peptide of >1160 ng/mL (0.5-2 ng/mL). The urine was negative for ketones. The baby required nasal cannula oxygen 2 L/min for the first two days of life and then gradually weaned to room air.

On examination, he had no dysmorphic features; his anterior fontanelle was level and there was no organomegaly. He was noted to have absent sucking and rooting reflexes with normal Moro's reflex. No abnormal body odor was noted and there were no abnormal movements. Feeding was started with 30 mL expressed breast milk plus glucose powder via nasogastric tube. The BG level was very labile between the days two and nine of life, needed a glucose infusion rate (GIR) of 15.6 mg/kg/minute to maintain his BG at a safe level. On day ten of life, he was started on octreotide 14 mcg/kg/day divided every 4 hours (as diazoxide was not available). Three days later, diazoxide (15 mg/kg/day) in three divided doses was added with hydrochlorothiazide 3.5 mg twice a day. On day 12 of life, the tandem mass spectrometry which was done as part of the critical sample at 21hours of life was reported. It showed a high serum (leucine and isoleucine) and valine suggesting the diagnosis of MSUD. Following this result, he was kept nil per mouth on a high glucose infusion and shifted to our hospital for further management. MSUD diagnosis was further confirmed by plasma amino acid analysis by HPLC with the presence of allo-isoleucine at 101 µmol/L, and elevated levels of the three BCAA: Leucine 724 µmol/L (45.0-160.0 µmol/L), isoleucine 240 µmol/L (28.0-95.0 µmol/L), and valine 390 µmol/L (60.0-294.0 µmol/L). The patient was started on oral feeding formula consisting of MSUD Anamix, Similac 1, and maxijul with adjustment of BCAA supply based on regular profiling of plasma BCAA levels; MSUD profile was performed three times weekly, on average. Along with enteral feeding, he continued to require intravenous dextrose infusion giving a GIR of 12.5 mg/kg/min to maintain his glucose. Medical therapy started initially was continued with adjustment of doses reaching the maximum dose of diazoxide (20 mg/kg/day). The dose of octreotide was built up reaching a maximum of 35 mcg/kg/day. Despite that, he kept requiring high GIR and had recurrent episodes of hypoglycemia associated with attempts of weaning the dextrose infusion requiring subcutaneous injections of glucagon. Diazoxide was stopped on day 20 of life due to a lack of clinically significant response.

Brain magnetic resonance imaging was performed at 24 days of life and showed an abnormal white matter signal with areas of restricted diffusion in the brainstem, cerebral peduncles, and cortical spinal tract features consisting with leucine encephalopathy.

CHI was genetically confirmed through the finding of a known pathogenic homozygous variant *c.3748C>T, p.Arg1250*, in the *ABCC8* gene. A homozygous variant in the *BCKDHA a (c.1087, p.Arg363Trp)* was identified supporting the diagnosis of MSUD that was confirmed biochemically.

His management course was complicated with central venous line (CVL) related issues, including central line-related infections, cardiac arrest due to CVL migration-related pericardial effusion, and the need for multiple CVL insertions. As the patient continued to need high GIR with the maximum dose of octreotide to maintain his BG, he was transferred to another local center for near-total pancreatectomy at the age of 4 months. Pre-operatively, PET-scan was not done as the patient had been genetically confirmed to have diffuse disease. This was subsequently also confirmed on histopathology which showed diffuse hyperplasia of beta islet cells. Post-operatively, he had a stormy course; with central line-related septicemia, venous thrombosis, and difficulty establishing enteral feeding. In the immediate postoperative days, he had persistent hyperglycemia requiring IV insulin infusion initially, and then shifted to insulin infusion pump at a basal rate only.

At the age of 5 months, he was discharged home on an insulin pump and BCAA-restricted formula via nasogastric tube. Initially, he was only requiring basal rate insulin, later he required bolus insulin for feeds on follow-up.

Three months after the discharge, the patient presented with frequent episodes of hypoglycemia, hence insulin was stopped. Subsequently, he continued to have recurrent episodes of hypoglycemia so he was restarted on diazoxide with hydrochlorothiazide for which he showed a good response. Parents stopped the medication on their own after one month for a claim of episodes of hypotension in the peripheral hospital. The child continued to have hypoglycemic episodes on a daily basis, especially at night. Thus, uncooked cornstarch was added to bedtime formula once daily. With that, he continued to have recurrent hypoglycemia so a gastrostomy tube was inserted and continuous night feeding via feeding pump was started. Afterward, the glycemic control was much better.

The child was admitted twice at the age of six and eight months respectively with hyperglycemia associated with significant ketosis mainly due to feeding intolerance. These episodes need to be identified as either diabetic ketoacidosis or metabolic decompensation related to his MSUD. With further investigations doing a blood gas the child was not acidotic hence the ketosis was most likely related to metabolic decompensations.

As his *BCKDHA* variant was novel the patient was given a trial of thiamin for two months with no marked reduction in leucine as would be expected in patients with thiamin-responsive MSUD.

Upon the last visit at 29 months, the patient was not on any medication but was still on continuous overnight feeding through a gastrostomy tube. Overall, his BG readings were within normal. His assessment revealed that his development was appropriate for his age with a normal neurological examination. His growth parameters were all acceptable for his age; with a height of 87 cm (15th percentile), weight: 11.8 kg (12th percentile), and head circumference of 48 cm (21st percentile).

Discussion

Non-ketotic hypoglycemia is a rare neonatal presentation, associated with disorders of fructose or galactose metabolism, hyperinsulinism, fatty acid oxidation, and growth hormone deficiency. MSUD, which is an aminoacidopathy, is usually marked by early encephalopathy, sweet odor, and ketosis. In a country where there is no newborn screening for MSUD, the diagnosis of MSUD with non-ketotic hypoglycemia presentation may be missed. As reported by Haymond et al. (6), lethal hypoglycemia in MSUD is rare and it's due to a defect in gluconeogenesis. Given the high insulin level in the critical sampling, and the absence of ketone and sweet-smell, the initial hypoglycemia in the presented patient was likely mainly secondary to CHI.

As previously reported, the homozygous variant in the *ABCC8* gene in the presented patient is associated with a diffuse and severe CHI that is diazoxide unresponsive and required near-total pancreatectomy (7). Postoperatively, the hypoglycemia was less severe, as shown in Figure 1, which may explain the better response to diazoxide as reported before (8).

In the pre-pancreatectomy period, the leucine level was fluctuating and difficult to predict compared to the post-procedure period. This is thought to be partially explained by concordant hyperinsulinemia. The relatively mild MSUD course so far, in which leucine maximally reached 500 umol/L under stress conditions (surgery), was thought to be a beneficial effect of hyperinsulinemia. However, in the post-procedure period, leucine levels remain reasonably controlled. This was supported by Wilcoxon signed-ranking test which does not show a statistically significant difference in the leucine level during the pre- and post-operative period (shown in Figure 1). We hypothesize that the mild course of MSUD is most likely related to the genetic variant effect rather than CHI concurrence.

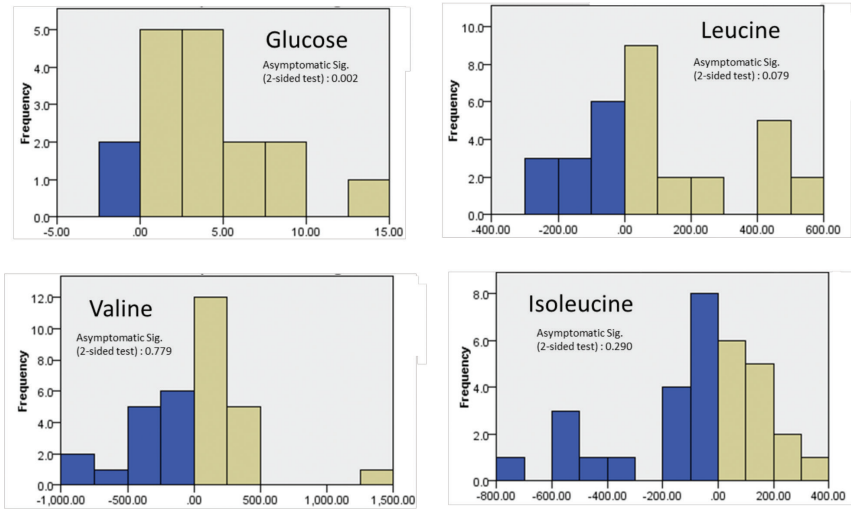

Figure 1. Wilcoxon signed-ranking test related samples for glucose, leucine, valine and isoleucine level. The test conducted on the serum level that was taken four months before the pancreatectomy and four months after the procedure. It showed statistically significant difference on glucose level before and after the procedure. On the other hand, there was no statistically significant difference on the BCAA level. This might indicate indirectly that hyperinsulinemia has no major rule in BCAA status in pre and post pancreatectomy

BCAA: branched chain amino acids

As this patient showed no response to conventional medical therapy, the use of sirolimus, an mTOR inhibitor was discussed. However, given currently limited data of its success and the complexity of our case, near-total pancreatectomy was the preferable option (9). The discussion of an early liver transplant was raised but due to uncertainty of the MSUD status post pancreatectomy, this option was delayed.

The patient course in the post pancreatectomy period has followed the expected route in which 40-60% of patients developed persistent hypoglycemia (8). He had an initial period of persistent hyperglycemia followed by persistent hypoglycemia that is now responding to overnight feeding.

The outcome for this patient remains unpredictable. The post-pancreatectomy glucose level has become significantly high which might indicate an early sign of diabetes. There are still multiple challenging aspects in his management, like the long-term risk of developing diabetes and its interaction with MSUD, the need for a liver transplant for his MSUD control, and the overall quality of life. We intend to continue reporting the progress of this patient in the future, given the possibly unique nature of his conditions.

Acknowledgments

We would like to acknowledge our dietitian, neonatal intensive care unit team at sultan Qaboos University hospital and the pediatric surgery, metabolic and gastroenterology teams at the Royal Hospital for their incredible role in the management of our patient.

Ethics

Informed Consent: Informed consent for publishing this case was obtained from the patient's parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Azza Al Shidhani, Abdulhamid Al Hinai, Khalid Al Thihli, Hilal Al Mandhari, Saif Al Yaarubi, Irfan Ullah, Nadia Al-Hashmi, Fathiya Al Murshedi, Concept: Azza Al Shidhani, Data Collection or Processing: Azza Al Shidhani, Abdulhamid Al Hinai, Analysis or Interpretation: Azza Al Shidhani, Abdulhamid Al Hinai, Khalid Al Thihli, Hilal Al Mandhari, Saif Al Yaarubi, Irfan Ullah, Nadia Al-Hashmi, Fathiya Al Murshedi, Literature Search: Azza Al Shidhani, Abdulhamid Al Hinai, Writing: Azza Al Shidhani, Abdulhamid Al Hinai.

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CASE REPORT

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Vasculitis-like Palpable Purpuric Rash Induced by Decapeptyl in a Pediatric Patient Diagnosed Central Precocious Puberty

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What is already known on this topic?

Drug-induced vasculitis is an inflammation of blood vessels caused by pharmaceutical agents and is the most common form of vasculitis. Pathogenesis is not fully undersdtood but many different drugs may cause similar clinical features, suggesting a common mechanism. Skin manifestations are most common, followed by renal involvement or lung involvement usually manifesting as interstitial pneumonia or acute respiratory distress syndrome. There are several systemic adverse reactions reported due to gonadotropin-releasing hormone analogs, including vasculitis-like rashes.

What this study adds?

This is one of the few reported pediatric cases of central precocious puberty (CPP) associated with vasculitis-like rash due to tripotelin (Decapeptyl Depot®) injection. This case will raise awareness of this association so that if pediatric patients with CPP and who develop side-effects such as cutaneous vasculitis, vasculitis-like rashes or other systemic adverse reactions, the management may continue with an alternative preparation.

Abstract

Central precocious puberty (CPP) is defined as the appearance of secondary sexual signs in girls younger than eight years of age or the onset of menarche before the age of 10 years. Gonadotropin-releasing hormone analogs (GnRHa) are the most effective therapy in CPP. Drug-induced hypersensitivity vasculitis is an inflammation of blood vessels, which may be due to the use of a number of pharmacologic agents. This case report describes drug-induced vasculitis in a girl being treated with Decapeptyl. A 7.25 year-old girl was admitted to Pediatric Endocrinology outpatients with premature breast development. She was diagnosed with CPP on the basis of physical examination and laboratory findings and tripoteline acetate (Decapeptyl®) treatment was initiated. She experienced multiple widespread skin rashes and mild abdominal pain with high temperature eight hours after the second dose of Decapeptyl. She was admitted to hospital with the diagnosis of drug-induced vasculitis and a single dose of intravenous methyl-prednisolone (1 mg/kg) and oral cetirizine was given. Her blood and urine analysis revealed no other organ involvement, other than skin. On the third day, the purpuric lesions began to resolve and had completely disappeared by the sixth day. Her treatment for CPP was switched to Depot Leuprolide acetate and she continued her treatment for two years uneventfully. To the best of our knowledge, this is the first report of a child with CPP experiencing drug-induced vasculitis due to tripotelin injection. Effective treatment may be continued by switching to an alternative gonadotropin releasing hormone analog.

Keywords: Drug-induced, central precocious puberty, vasculitis, vasculitis-like rash

Presented in: This case report was presented as a poster at the 63rd Turkish National Pediatric Congress in November 2019.

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Introduction

Central precocious puberty (CPP) is defined as the appearance of secondary sexual signs in girls younger than eight years of age or the onset of menarche before the age of 10 years. CPP may lead to premature epiphysial fusion with compromise in final adult stature and thus usually requires medical intervention (1).

The incidence of CPP is estimated as 1 in 5000 to 10,000 children and is more common in girls. The idiopathic form of CPP is due to the early maturation of the hypothalamic-pituitary-gonadal axis in the absence of other pathological causes.

Gonadotropin-releasing hormone analogs (GnRHa), which are able to desensitize the pituitary gland to endogenous GnRH are the most effective therapy in CPP. In the early 1980s, several different formulations of GnRHa were developed with different durations of action and routes of administration. However, during the past decade, there has been an increase in the number of extended-release formulations of GnRHa. GnRHa have a good safety profile. The most commonly reported adverse events are injection-site reactions which are typically mild and self-limited (1).

Drug-induced hypersensitivity vasculitis is an inflammation of blood vessels that has been linked to the use of several pharmacologic agents. Decapeptyl is a GnRHa which is widely used in *in vitro* fertilization processes, sex hormone-dependent malignancies and CPP. Recent studies have demonstrated the immune-modulatory effects of GnRHa and sex steroids; estrogens have an activating role in some autoimmune disorders by enhancing humoral responses while testosterone enhances suppressor T cells (2,3). GnRHas also play a role in sex-differences evident in several autoimmune diseases, probably because of their immunostimulatory effect and may also modulate the development of autoimmune disorders or worsening of preexisting diseases. However, this association between GnRH and autoimmunity remains hypothetical due to the lack of conclusive evidence. There are, however, a few case reports of the exacerbation of systemic lupus erythematosus-related thrombocytopenia and lupus nephritis after GnRHa administration (4,5), two cases of GnRHa induced vasculitis and one case of polymyositis in a patient treated with leuprolide acetate (6). All of these reports were in adult patients. We present a case of vasculitis induced by GnRHa treatment for CPP in a seven year old girl, which, to the best of our knowledge is the first published report of pediatric GnRHa-induced vasculitis.

Case Report

A girl, aged 7.4 years, was admitted to the University of Kyrenia, Dr. Suat Günsel Hospital, Pediatric Endocrinology outpatient clinic with a six-month history of premature breast development. She did not take any medication nor have any significant medical history. Her family history revealed only Hashimoto thyroiditis in her father. On physical examination, she had a weight of 24 kg (-0.01) and height of 126 cm (0.52) with normal psychomotor development. Breast development was Tanner stage 2 with no pubic or axillary hair. The rest of the physical examination was unremarkable. Bone age according to Greulich and Pyle atlas was 9 years. Pelvic ultrasound revealed a uterine long axis of 27.8 mm, anteroposterior diameter of 15 mm, transverse diameter of 6.1 mm consistent with prepubertal measurements, while right and left ovarian volumes were 1.3 cm^3 and 1.6 cm^3 which were highfor chronological age. Baseline luteinizing hormone (LH) was 0.37 mIU/mL, follicle stimulating hormone was 3.45 mIU/mL, estradiol < 10 pg/mL and prolactin 6.32 pg/mL. LH releasing hormone stimulation test results, assessed by chemiluminescence microparticle immunoassay, are given in Table 1. Her cranial magnetic resonance imaging scan was normal. On the basis of these findings she was diagnosed with CPP and tripoteline acetate (Decapeptyl®) treatment was initiated at 3.75 mg/25 days. She did not experienced any side effects with the first dose of tripoteline acetate.

After 25 days, she was given the second dose and she experienced multiple rashes on her body with mild abdominal pain starting eight hours after drug administration. Lesions gradually spread towards the upper leg and gluteal region and she was admitted to the emergency unit. Her temperature was 39 °C and other vital signs were normal. Physical examination revealed a few maculopapular rashes on her arms and body (Figure 1)

Table 1. LHRH stimulation test results

Time (min)	LH (mIU/mL)	FSH (mIU/mL)	Estradiol (pg/mL)
0	0.34	4.33	16
30	5.85	11.94	
60	5.61	13.29	
90	4.56	14.31	17

LHRH: luteinizing hormone releasing hormone, FSH: follicle stimulating hormone

and non-blanching purpuric lesions on her legs and gluteal region (Figure 2), conjunctival hyperemia (Figure 3) and abdominal tenderness with palpation. Blood tests showed normal biochemical parameters and C-reactive protein (CRP) was 0.92 mg/dL. She had mild microcytic anemia with hemoglobin level 11.1 mg/dL, mean cell volume 58.2 fL, white cell count 8,300/mm^3 and platelet count 498,000/mm^3. Urine analysis and abdominal ultrasound were normal. The patient was hospitalized in order to continue observation. Her temperature fell with a single dose of paracetamol and did not rise again thereafter. A single dose of intravenous methyl-prednisolone (1 mg/kg) and oral cetirizine were given in the emergency department on admission. On the next day, there were no new purpuric lesions and her abdominal pain had resolved. Her stool was checked for occult blood and was negative in three samples. On the second day of hospitalization she was discharged from the hospital because her family wanted to continue the treatment at home. Diagnostic skin biopsy was recommended but was refused. On the third day, all the purpuric lesions began to resolve and there was no evident lesions by the sixth day. This adverse reaction, apparently due to the tripoteline acetate medication, was reported to the national health authorities and the manufacturer. Informed consent was obtained from her parents. For medical exam and publication.

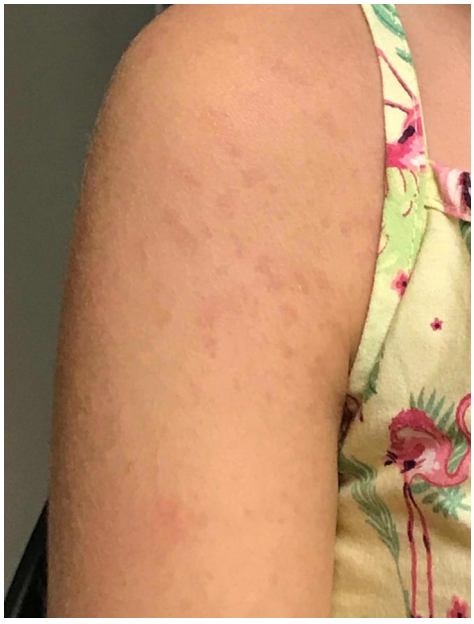

Figure 1. Maculopapular rashes on arms

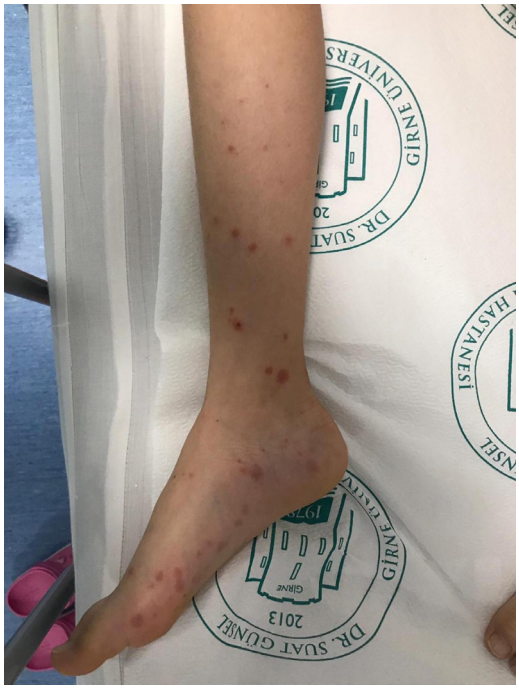

Figure 2. Non-blanching purpuric lesions on her legs

Discussion

Drug-induced clinical syndromes have been recognized for many years and range from self-limiting to life threatening. Some may trigger autoimmune events or may be confused with autoimmune disease. Drug-induced vasculitis is an inflammation of blood vessels caused by pharmaceutical agents and is the most common form of vasculitis. Inflammation may be short term (acute) or long-term (chronic) with variable non-dermatological organ involvement (7). The systemic form of drug-induced vasculitis is rarely seen in patients on long term therapy, whereas cutaneous vasculitis is more common (8,9).

Drug-induced vasculitis usually affects skin and, rarely, the kidneys and lungs (10,11). Pathogenesis is not clear but

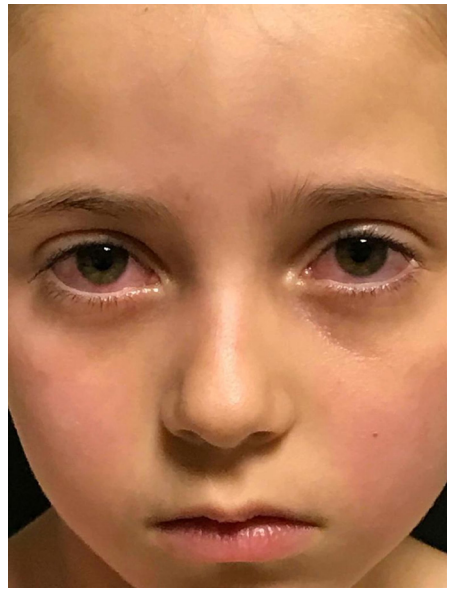

Figure 3. Conjunctival hyperemia

many different drugs may cause similar clinical features, suggesting a common mechanism.

A type of anti-neutrophil cytoplasm antibodies (ANCA) associated vasculitis is described in 2000's related to long term use of anti-thyroid medications (12). Since then many other drugs such as antibiotics, anti-tumor necrosis factor alpha agents or psychoactive agents have been associated with ANCA-associated vasculitis (7). Tests for ANCA and tissue biopsies are recommended for diagnosis and differential diagnosis of drug-induced vasculitis (13,14).

Usually clinical manifestations of drug-induced vasculitis are similar to primary vasculitic syndromes. Skin manifestations are most common, followed by renal involvement with varying symptoms, such as hematuria, proteinuria, or elevated serum creatinine (15). Some patients may suffer only lung involvement with interstitial pneumonia or acute respiratory distress syndrome (16,17).

There are no specific laboratory tests for diagnosis of drug-induced vasculitis, but some laboratory markers may help to distinguish drug induced vasculitis from idiopathic autoimmune diseases. These biomarkers include ANCA, anti-double-stranded DNA antibodies or antiphospholipid antibodies. Some laboratory findings may indicate organ involvement. Anemia is common but acute-phase reactants such as the erythrocyte sedimentation rate or CRP which are usually elevated in autoimmunity, are not sensitive or specific for drug-induced vasculitis (13,18). The presented case presented with anemia and slightly elevated CRP level.

Treatment options for drug-induced vasculitis depends on the patients' individualized maintenance. Clinical course and specific organ involvement in each case of drug-induced vasculitis will determine optimum treatment and there is no standard approach but the first step is the discontinuation of the likely causative medication, as was recommended in this case. Corticosteroids, cyclophosphamide, azathioprine and mycophenolate mofetil are among the available treatment options (19).

There are a few case reports describing vasculitis or vasculitis-like rashes following treatment with GnRHa. The first was reported in 1993 in a 23-year old woman after the first course of Decapeptyl used for *in vitro* fertilization (20). The patient experienced purpuric papular rashes, similarly to those seen in this pediatric case, and lesions resolved in a few days with oral antihistamine and topical corticosteroid cream (20). The second case was a 67-year old man who experienced fever, rash and arthritis after the second dose of leuprolide (Lucrin®) for prostate cancer and was subsequently treated with steroids (6). Another interesting case, reported in 2010, was a 26-year old woman with a history of previous autoimmune and neuromuscular disease, experiencing polymyositis and vasculitis five days after GnRH analogue (Decapeptyl) administration (21).

Kirkgoz et al. (22) recently reported nine pediatric cases experienced systemic hypersensitivity reactions to GnRHa during the treatment of CPP. One of the cases in this report had similarities to the currently presented case with palpable purpuric rash on her legs, which was assumed to be Henoch-Schönlein purpura by her pediatrician. This case also resolved without treatment in one week and the patient was successfully switched to Leuprolide acetate.

Conclusion

In summary, we described a pediatric case of CPP experiencing vasculitis-like rashes due to tripoteline acetate (Decapeptyl Depot®) injection. Due to the mild clinical course, absence of extracutaneous organ involvement and rapid recovery, no further tests or biopsies were performed. However, this represents a limitation of this report as we could not clarify the diagnosis with histopathological confirmation. Her treatment for CPP was switched to Depot Leuprolide acetate (Lucrin Depot® 11.25/3 months) and she continued her treatment successfully for two years. This makes us think that apart from the active ingredient of the drug, solvents may also cause such side effects. It should be kept in mind that in pediatric CPP, patients who develop side effects such as cutaneous vasculitis may be successfully treated by changing the GnRHa preparation.

Ethics

Informed Consent: Informed consent was obtained from her parents.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Nilüfer Galip, Nermin Ankay, Rüveyde Bundak, Concept: Nilüfer Galip, Rüveyde Bundak, Design: Nilüfer Galip, Rüveyde Bundak, Data Collection or Processing: Nermin Ankay, Literature Search: Nilüfer Galip, Writing: Nilüfer Galip, Rüveyde Bundak.

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CASE REPORT

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Syndrome of Congenital Insulin Resistance Caused by a Novel *INSR* Gene Mutation

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What is already known on this topic?

Leprechaunism (Donohue syndrome) is the most severe form of insulin resistance caused by autosomal recessive mutations in the *INSR* gene and is characterized by extreme insulin resistance leading to hyperinsulinemia, impaired glucose homeostasis and prenatal and postnatal growth retardation. Moreover, dysmorphic features, hypertrichosis, acanthosis nigricans, macrogenitosomia, and polycystic ovaries and breast enlargement in females are also present. Common complications include hypertrophy of internal organs. Patients with leprechaunism usually die in the first year of life.

What this study adds?

The study describes a novel mutation in the *INSR* gene in a patient with Donohue syndrome. This case highlights the importance of genetic testing of the *INSR* gene, which is crucial for genetic counseling as well as for improving the prognosis of patients with severe insulin resistance syndromes; the prognosis may be dependent on the type and location of the *INSR* gene mutation. Moreover, until now, there have been no reports of fatty liver disease in these patients with proven loss of function of the *INSR*, which raises the question of whether it plays a role in prognosis. The study also highlights the challenges faced by clinicians in the management of this complex, rare condition.

Abstract

Mutations in the *INSR* gene result in rare inherited syndromes causing insulin resistance, such as leprechaunism (Donohue syndrome), Rabson-Mendenhall syndrome and insulin resistance type A. Leprechaunism is an autosomal recessive disorder associated with extreme insulin resistance that leads to hyperinsulinemia, impaired glucose homeostasis, fasting hypoglycemia and postprandial hyperglycemia. Impaired insulin action causes prenatal and postnatal growth retardation. Lipoatrophy, dysmorphic facies, hypertrichosis, macrogenitosomia and hypertrophy of internal organs are also present. A male infant with congenital insulin resistance was born at term after a normal pregnancy with a weight of 1905 g (< 3 c), a length of 48 cm (< 3 c), and an Apgar score of 10. Intrauterine growth retardation, transient hypoglycemia, pneumonia, urinary tract infection and heart defects [patent foramen ovale (PFO); patent ductus arteriosus (PDA)] were diagnosed after birth. At 5 weeks of age, he was admitted to the regional hospital with severe fever, diarrhea and dehydration. Hyperglycemia was observed (672 mg/dL), and insulin was administered. He was referred to a hospital at 7 weeks of age for suspected neonatal diabetes and hypertrophic cardiomyopathy. The physical examination revealed a loud systolic heart murmur, tachycardia, tachypnea, dysmorphic facies, hypertrichosis, acanthosis nigricans, hypotonia, swollen nipples and enlarged testicles. Glycemic fluctuations (50-250 mg/dL) were observed. The serum insulin concentration was high (maximum 1200 IU/mL) at normoglycemia. Ultrasound of the heart confirmed progressive hypertrophic cardiomyopathy. Leprechaunism was confirmed by genetic analysis of *INSR*, in which a novel c.320C > G; p. Thr107Arg homozygous missense mutation was found in exon 2.

Keywords: Insulin receptor, insulin resistance, leprechaunism

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Introduction

Insulin participates in a variety of biochemical processes, such as the metabolism of carbohydrates, lipids and proteins. It also influences cell proliferation, differentiation and apoptosis. Insulin fulfills the role of a growth factor and a regulator of gene expression and contributes to protein biosynthesis (1).

Insulin acts through a specific INSR, which is encoded by a gene (Gene ID: 3643, OMIM: *147670) located on the short arm of chromosome 19 (19p13.3) composed of 22 exons (2). The mature INSR is a heterotetramer, consisting of two dimers of two subunits (α and β, respectively). The α subunit is an extracellular ligand binding domain, whereas the intracellular β subunit functions as the catalytic domain of the receptor (3,4,5,6). Mutations in the *INSR* gene result in rare inherited syndromes of insulin resistance, such as leprechaunism (Donohue syndrome; OMIM: #246200), Rabson-Mendenhall syndrome (RMS; OMIM # 262190), insulin resistance type A (OMIM ID: 610549) and lipoatrophic diabetes mellitus (7,8,9,10,11).

Donohue syndrome is the most severe form of insulin resistance. It is a rare autosomal recessive disorder associated with extreme insulin resistance leading to hyperinsulinemia, impaired glucose homeostasis, fasting hypoglycemia and postprandial hyperglycemia. Impaired insulin action causes prenatal and postnatal growth retardation.

Dysmorphic features, such as prominent eyes, thick lips, a flattened nasal bridge with upturned nostrils, low-set posteriorly rotated ears and thick skin with a lack of subcutaneous fat are present. Hypertrophy of internal organs, including cardiomegaly, hepatosplenomegaly, and hypertrophy of the ovaries is also a common complication. Moreover, hypertrichosis, acanthosis nigricans, macrogenitosomia, and polycystic ovaries and breast enlargement in females are also characteristic of children with Donohue syndrome, and in some, mental retardation has been observed. Patients with leprechaunism usually die in the first year of life (10,12,13,14,15).

Patients with RMS develop constant hyperglycemia, diabetic ketoacidosis and other complications of diabetes mellitus, which usually leads to death in the first or second decade of life (16,17,18,19,20,21).

Case Report

A male infant, who was the offspring of consanguineous, young, healthy parents from the Roma population, was admitted to the department of pediatric endocrinology at the age of seven weeks with suspected neonatal diabetes and hypertrophic cardiomyopathy. He was born at term after a normal pregnancy (Apgar 10). He was small for his gestational age, with a birth weight of 1905 g [standard deviation score (SDS) -3.73] and length of 48 cm (SDS -1.2). Intrauterine growth retardation, transient hypoglycemia, neonatal pneumonia, urinary tract infection and heart defects including patent foramen ovale (PFO) and patent ductus arteriosus were diagnosed just after birth. He was released from the neonatology unit twelve days after birth. At the age of five weeks, he was admitted to the regional hospital severely unwell with fever, diarrhea and dehydration. Hyperglycemia (672 mg/dL) was observed so insulin (i.v.) was administered for several days. Fluctuations in the blood glucose levels were observed afterwards (40-410 mg/dL) but unexpectedly normalized spontaneously thereafter.

The child was referred to the department of pediatric endocrinology at the age of seven weeks with suspected neonatal diabetes. His body weight was 3000 g (SDS -2.5), and physical examination revealed a loud systolic heart murmur, tachycardia, tachypnea, and inspiratory-expiratory dyspnea. He had multiple phenotypic anomalies, including low-set large ears, coarse facial features, a flattened nasal bridge, thickened lips, generalized hypertrichosis, acanthosis nigricans in the skin folds and over the knees, decreased subcutaneous fat, hypotonia, prominent nipples and enlarged testicles (4 mL). Glycemic fluctuations (50-250 mg/dL) were initially observed. Postprandial hyperglycemia normalized spontaneously. Subsequently, at the age of eight weeks, recurrent episodes of hypoglycemia occurred, and the patient required intravenous infusions of 10% glucose. The serum insulin concentration was elevated (maximum 1200 IU/mL) concurrent with normoglycemia (Table 1). Glycated hemoglobin A1c was at the lower normal limit (4.1%; normal 4.0-6.2%), which indicated the predomination of hypoglycemic episodes over postprandial hyperglycemic episodes. Serum liver enzymes and lipids concentration fluctuated around the upper range of normal

Table 1. The glucose and insulin values for the patient serum

Age (weeks of life)	Seventh	Seventh	Eighth	Eighth	Eighth
	Fasting	Postprandial	Fasting	Postprandial	Postprandial
Glucose (mmol/L)	2.9 (NR < 5.6)	13.9 (NR < 140)	2.7 (NR < 100)	10.1 (NR < 140)	4.7 (NR < 140)
Insulin (mIU/L)	300 (NR < 12)	-	-	453 (NR < 75)	1200 (NR < 75)

NR: normal range

limits. Moreover, serum insulin-like growth factor 1 (IGF-1) and IGF binding protein 3 (IGFBP3) were decreased at <25 ng/mL (normal 55-327) and <0.5 ug/mL (normal 0.7-3.6), respectively. Inherited metabolic disorders (amino acid and acylcarnitine profiles) were excluded based on the mass spectrometric results. Echocardiography confirmed hypertrophic cardiomyopathy with obstruction of the left ventricular outflow. A congenital heart defect (PFO) was still present and a beta-adrenoreceptor antagonist (metoprolol) was administered. Subsequent echocardiography revealed the progression of hypertrophic cardiomyopathy. A radiogram of the chest showed cardiomegaly and inflammatory changes in the lungs. Pneumonia was treated with antibiotics. The infant was stable during the subsequent four weeks following his admission to the ward. Diuretics were administered due to fluid retention. During the fifth week of hospitalization, fever occurred, and the patient's general state deteriorated. Physical examination revealed tachycardia, a loud systolic heart murmur, intense dyspnea, wheezing, hepatomegaly and peripheral edema. Echocardiography revealed the progression of ventricular septum hypertrophy and pulmonary valve vegetation. Sepsis, endocarditis and progression of hypertrophic cardiomyopathy with severe left ventricular constriction were diagnosed. The patient was managed with antibiotic therapy and intravenous gammaglobulins after the implantation of a central venous Broviac catheter, but he went into acute postoperative respiratory failure. The infant was admitted to the intensive care unit where sudden circulatory arrest occurred. Despite immediate resuscitation, the child died in the thirteenth week of postnatal life. The postmortem examination demonstrated major hypertrophy of both heart ventricles, features of left and right heart insufficiency, hepatomegaly, fatty liver and digestive tract hemorrhage. The syndrome of congenital insulin resistance was diagnosed based on the clinical picture, laboratory tests and molecular analysis results.

Genetic Analysis of the *INSR* Gene

A blood sample was collected and frozen at -20 °C until analysis. Genetic analysis of the *INSR* gene was performed according to all the relevant national regulations and institutional policies in accordance with the tenets of the Helsinki Declaration and was approved by the Local Ethics Committee of the Poznan University of Medical Sciences (no. 146/10).

Molecular studies of the *INSR* gene were performed using PCR and direct sequencing. The parents of the patient were not available for genetic testing. Genomic DNA was isolated from peripheral blood leucocytes using the QIAamp® DNA Blood Mini Kit (QIAGEN). Twenty-two sets of primers were used to amplify all exons of the *INSR* gene. All the oligonucleotides used in this study came from the Institute of Biochemistry and Biophysics, Polish Academy of Science, Warsaw, Poland, and their sequences are available on request. Polymerase chain reactions (PCR) were performed in a 10-20-µL mixture using HotStarTaq® DNA Polymerase (QIAGEN) with the following parameters: denaturation at 95 °C for 15 min, followed by 40 cycles of 95 °C for 60 s, 60 °C or 58 °C for 30 s, and 72 °C for 45-90 s (depending on the primer pair used). A final amplification at 72 °C for 7 min completed the PCR program. The PCR products were separated by electrophoresis on a 1.2%-1.5% agarose gel in the presence of ethidium bromide (Merck), purified from the gel using a QIAquick® Gel Extraction Kit (QIAGEN) and directly sequenced with the same primer pair used for PCR. All sequencing reactions were performed using the BigDye Terminator v 3.1 cycle sequencing kit (Applied Biosystems) on an ABI Prism 3130XL Genetic Analyzer (Applied Biosystems). Finally, the sequences were analyzed using VectorNTI 9.0 Software (Invitrogen) with the reference sequence NC_000019.08 (accession date: 03.03.2008).

Results

The serum glucose and insulin values for the patient are presented in Table 1.

Results of the Genetic Analysis of the *INSR* Gene

The sequence analysis revealed the presence of a novel, homozygous missense mutation, c.320C>G, in exon 2 that changed the polar threonine at position 107 into a basic arginine (p. Thr107Arg) (Figure 1). This variant was not found in control samples in the current version of the

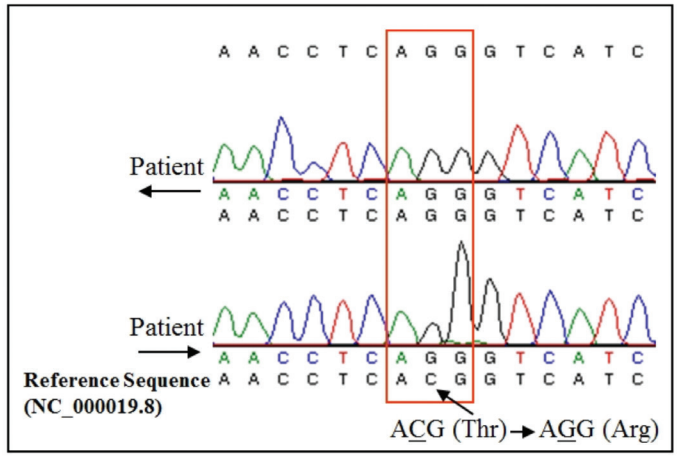

Figure 1. The sequence analysis of the *INSR* gene

ESP6500 dataset deposited in the NHLBI Exome Variant Server, which comprises a set of 2203 African-American and 4300 European-American unrelated individuals with 6503 samples (13,006 chromosomes) in total (accession date: 25.01.2013), or in the ExAC and 1000G databases.

Three predictive tools were used to estimate the overall effect of the p. Thr107Arg mutation in the presented patient.

Analysis using SIFT (Sorting Intolerant From Tolerant, http://sift.jcvi.org/), which predicts the possible impact of a substitution based on sequences of similar peptides, assigned a score of 0 to the p. Thr107Arg mutation. This indicates that the mutation has a damaging effect on the INSR protein (the SIFT scale designates scores of less than 0.05 as deleterious, while scores of 0.05 or greater are predicted to be tolerated). Similarly, analysis using Mutation Taster (http://www.mutationtaster.org/) predicted the p. Thr107Arg mutation to be "disease causing" (score: 1.94; probability ($p = 0.99$). Finally, PolyPhen-2 (Polymorphism Phenotyping v2) software, which predicts the effects of a substitution based on the structure and function of a human protein using straightforward physical and comparative considerations (http://genetics.bwh.harvard.edu/pph2/), predicted this mutation to be "probably damaging" based on scores of 1.000 (HumDiv, sensitivity: 0.00; specificity: 1.00) and 0.999 (HumVar, sensitivity: 0.09; specificity: 0.99).

Discussion

We present an infant boy with clinical features and laboratory data typical of leprechaunism whose diagnosis was confirmed by genetic analysis of the *INSR* gene, in which a novel mutation was found. Donohue syndrome (leprechaunism) was first identified in 1948 by W. L. Donohue (22). The incidence of leprechaunism has been estimated to be at least 1 in 4 million live births (7). The molecular background of insulin resistance in leprechaunism has been associated with recessive mutations of the *INSR* gene. Due to such mutations, aberrant receptors can no longer serve their function. Mutations in the *INSR* gene are extremely rare, which is why most cases of inherited severe insulin resistance result from consanguineous parents (12,14,23), as was the case for the infant described here.

Molecular studies carried out in the presented case led to the identification of the novel homozygous missense mutation c. C320 > G in exon 2 in the *INSR* gene. Only one single nucleotide polymorphism (SNP) C/T at position 320 in the *INSR* gene (rs 140762552, c.320C > T; p. Thr107Met) has been deposited in the SNP database (dbSNP, http://www.ncbi.nlm.nih.gov/snp) to date, but the variant described was of unknown significance. The impact of the mutation c.C320 > G identified in the presented case on receptor function is unknown; thus, the clinical diagnosis of leprechaunism was confirmed by *in silico* analysis. This novel mutation was predicted to disrupt a single-stranded right-handed beta-helix in the L-domain of the α subunit of INSR. This domain contains a cysteine-rich region composed of eight disulfide bonds. The three L-domains located in the α subunit of INSR surround a central space that is large enough to accommodate a hormone. Although the protein fragment comprising residues 1-462 does not bind insulin on its own, this central site exhibits many of the features crucial for insulin binding and ligand specificity (24). Therefore, the p. Thr107Arg mutation may disrupt the ability of INSR to bind its ligand.

Longo et al. (23) investigated several patients with inherited insulin resistance syndromes and different survival times that ranged from a few weeks to several years. They distinguished two phenotypes in their patients, leprechaunism and RMS, and tried to establish the genotype-phenotype correlation. They identified new mutations in the *INSR* gene and analyzed the correlation between these mutations and the survival rate. Mutations that completely or markedly impaired insulin binding to the receptor resulted in the most severe phenotype with early death (leprechaunism), while mutations resulting in residual insulin-binding activity were associated with a longer lifespan (RMS).

The patient presented herein who had severe insulin resistance, had a phenotype consistent with the descriptions of patients with leprechaunism reported in the literature (14,22,25). He demonstrated all of the relevant disorders of carbohydrate metabolism: fasting hypoglycemia, postprandial hyperglycemia, hyperinsulinemia and severe insulin resistance. A combination of decreased hepatic glucose output in the fasting state and decreased hepatic glycogen synthesis during feeding due to a postreceptor defect in insulin action leads to fasting hypoglycemia and postprandial hyperglycemia (26).

Another consequence of insulin resistance is defects in fatty acid metabolism, which are responsible for the pathogenesis of fatty liver disease (26). The postmortem examination of our patient showed the presence of fatty liver. To date, there have been no reports of fatty liver disease in patients with proven loss of function of the *INSR*. Donohue and Uchida presented various results of liver histological examinations, including the absence of abnormalities, nonspecific lesions consisting of focal degeneration or necrosis, and focal increases in glycogen and iron deposits (14).

Despite extreme insulin resistance, patients with primary defects at the level of the INSR (generalized insulin resistance) do not manifest metabolic dyslipidemia. Despite higher plasma free fatty acids and glucose levels and massively increased plasma insulin levels, liver fat measurements were normal in the patients with INSR mutations. Theoretically, this could be a result of either reduced hepatic lipogenesis or increased oxidation or excretion of liver triglycerides (27,28). We hypothesize that the presented patient may have had rudimentary INSR activity and therefore developed fatty liver disease.

Intrauterine growth retardation and postnatal failure to thrive are part of the clinical picture of congenital insulin resistance. Psiachou et al. (29) suggested that the primary defect in leprechaunism is in the *INSR* gene and that a secondary defect is probably responsible for the impaired response to endogenous growth hormone and thus growth retardation. According to Kadowaki et al. (30), the *INSR* may regulate the function of IGF-I receptors. They suggested that the defect in the *INSR* gene impaired the functioning of receptors for other growth factors. Our patient had serum levels of IGF-1 and IGFBP-3 below the normal limits. IGF-1 plays an important role in prenatal and postnatal growth. Thus, both the defects in insulin action and the impaired synthesis of IGF-1 and IGFBP-3 resulted in growth retardation in this child. Nakae et al. (31) proved that treatment with reecombinant human insulin-like growth factor 1 (rhIGF-1) normalized glucose metabolism and was effective in preventing postnatal growth retardation in a patient with leprechaunism. Other authors, however, reported that administration of recombinant human growth hormone (rhGH) and rhIGF-1 had little or no influence on glucose homeostasis and none on growth stimulation. They hypothesized that this could be due to a postreceptor defect in IGF-1 signaling caused by the absence of insulin function (13).

There is evidence that insulin at high concentrations acts as a growth factor through IGF-I receptors. IGF-I receptors are present in the ovaries, kidneys and heart. This fact could explain the enlargement of these organs reported in patients with leprechaunism (30).

Recurrent bacterial infections presented an additional problem in the presented patient. Published data show that infections occur with increased frequency in patients with leprechaunism. It is proposed that a congenital leptin deficiency due to defective insulin activity in the adipose tissue negatively influences T-cell function (23,32).

There is no effective therapy for severe forms of inherited insulin resistance. The life expectancy of children with leprechaunism is poor, and early death is certain. Most patients die during the first year of life (14,22,25). Some patients with milder forms of this disease have lived longer, which may be related to partial activity of INSR (33).

Conclusion

In summary, we present a novel homozygous mutation in the *INSR* gene in a patient with severe clinical manifestations of insulin resistance, which confirmed the diagnosis of Donohue syndrome (leprechaunism). This patient also had fatty liver and, to date, there have been no other reports of fatty liver disease in patients with proven loss of function of the INSR. The fatal outcome in this child demonstrated the high risk in subjects with the most severe form of the disease if the mutation occurs in the ligand-binding domain of the receptor. This rare disorder represents a challenge for future clinical trials that may improve the prognosis of such patients.

Ethics

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices - Concept - Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: Aleksandra Rojek, Beata Wikiera, Anna Noczynska, Marek Niedziela.

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CASE REPORT

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Novel Homozygous Nonsense Mutation in the *LRP5* Gene in Two Siblings with Osteoporosis-pseudoglioma Syndrome

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What is already known on this topic?

Osteoporosis-pseudoglioma syndrome (OPPG) is a rare autosomal recessive disorder characterized by severe juvenile osteoporosis, increased bone fragility and congenital blindness, due to mutations in the *LRP5* genes.

What this study adds?

A novel homozygous nonsense mutation present in two siblings with OPPG but with differing phenotype is described which will add to the spectrum of *LRP5* mutations leading to OPPG.

Abstract

Osteoporosis-pseudoglioma syndrome (OPPG) is a rare autosomal recessive disorder characterized by severe osteoporosis and eye abnormalities that lead to vision loss. In this study, clinical findings and genetic study of two siblings with OPPG are presented. Whole exome sequencing of DNA enriched for exonic regions was performed with SureSelect 38Mbp all exon kit v. 7.0. The two siblings presented with different clinical manifestations of OPPG. The younger female sibling had blindness and severe osteoporosis with multiple fractures, while her older brother was also blind but with less severe osteoporosis and no fractures. On analysis, a novel homozygous nonsense mutation (c.351G>A) in exon 2 of *LRP5* (NM_002335) was found, predicted to change a tryptophan at 117 to a stop codon (p.Trp117Ter). Thus, a variable phenotype was associated with an identical variant in these two siblings. The novel mutation reported herein expands the spectrum of the underlying genetic pathology of OPPG.

Keywords: Osteoporosis-pseudoglioma syndrome, *LRP5* gene, nonsense mutation

Introduction

Osteoporosis-pseudoglioma syndrome (OPPG) is a rare autosomal recessive disease characterized by severe osteoporosis, increased bone fragility, defects in the fetal ocular fibrovascular system, convulsions and intellectual disability. Osteoporosis causes vertebral compression, kyphosis, short stature, bowing of long bones, and vertebral and recurrent long bone fractures that may lead to skeletal abnormalities and physical disabilities (1). Ocular complications, usually presenting at birth or in early infancy, are due to vitreoretinal degeneration and manifest as phthisis bulbi, microphthalmia, retinal detachment, and/or exudative retinopathy, leading to congenital or juvenile blindness (2).

The WNT signaling pathway plays an important role in the regulation of skeletal homeostasis, osteoblast differentiation, and bone formation. A characteristic feature of WNT signaling is dose-dependency, which results in different phenotypic disorders. In the case of WNT activation, binding of WNT ligands to the seven-pass transmembrane Frizzled (Fzd) receptor and its co-receptor, low-density lipoprotein receptor-related protein-5 or -6 (LRP5/6), it triggers a series

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of events. This includes the dimerization of the two receptors on the cell surface and subsequent structural changes in these receptors.

In vertebrates, there are indeed 19 different WNT ligands and 10 Fzd receptors. However, out of these receptors, only two - LRP5 and LRP6 - are directly involved in WNT signaling. LRP5/6 play an important role in the initiation of WNT signal transduction and inhibit their function in WNT/Fzd signaling. The N-terminus of the Fzd receptor is indeed the primary region responsible for binding to WNT ligands. LRP5 and LRP6, as co-receptors of WNT ligands, play a crucial role in canonical WNT signaling. They are key components of the Wnt receptor complex and are essential for transmitting the WNT signal into the cell (3).

Homozygous or compound heterozygous inactivating mutations in the gene encoding low-density lipoprotein receptor-related protein 5 (*LRP5*) causes OPPG, while gain of function mutations in *LRP5* resuls in a high bone mass phenotype (hyperostosis and osterosclerosis) secondary to increased WNT signaling (4,5). Here, we report a novel mutation in the human *LRP5* gene in two Iranian siblings with OPPG.

Case Reports

Case 1

The proposita was a 12-year-old girl as the second child of a consanguineous marriage of Iranian descent. She was born at term with weight, head circumference and length of 3100 g (25% percentile), 35 cm (50% percentile) and 52 cm (75-90% percentile), respectively. At presentation the patient had multiple fractures in the wrist, femur, and tibia without any obvious trauma (Figure 1). She had bilateral microphthalmia, corneal opacities and pseudoglioma and was congenitally blind, which was diagnosed at two months old. Her first recognized long bone fracture was in the femur at the age of two years during occupational therapy. At the age of three years, her wrist broke, but did not have any further fractures until she was seven. After the age of seven years, she had a fracture in her femurs, tibia/fibula or wrists every year. There was no chest deformity. The patient had an autism spectrum disorder and she could neither talk nor communicate with others and was not teachable. Serum calcium, phosphorus, magnesium, alkaline phosphatase, parathyroid hormone, thyroid function tests, lipid profile, liver transaminases and uric acid were in all within the normal range.

Bone turnover markers were not investigated as these were unfortuanetly not available. The bone mineral density (BMD) was investigated with an absorptiometry (DEXA) method using a Hologic Discovery W S/N 83407 (Hologic Inc., Marlboro, MA, USA). BMD measurement showed severe osteoporosis with the absolute value of 0.369 g/cm^2 in the lumbar region (L1-L4) (Z score -3.1) and 0.309 g/cm^2 in the femur (Z score -4.4).

After the diagnosis of OPPG, pamidronate was started with infusions of 1 mg/kg daily for three consecutive days every three months from the age of three years and continued up to 11 years old. In addition, she received 1000 units of vitamin D and 500 mg of oral calcium daily during this period.

During treatment with pamidronate, she had multiple tibial fractures without any obvious trauma, while at the age of 11 years hip fractures occurred. In general, there was no improvement in her physical activity during treatment with pamidronate and she suffered from bone pain and recurrent bone fractures despite pamidronate. BMD at 10 years old showed the absolute value of 0.532 g/cm^2 in the lumbar region and 0.372 g/cm^2 in the femur, while BMD Z score according to height was -1.1. Therefore, no significant increase in BMD was observed despite continuous pamidronate therapy. We declare that the patient's mother has given her informed

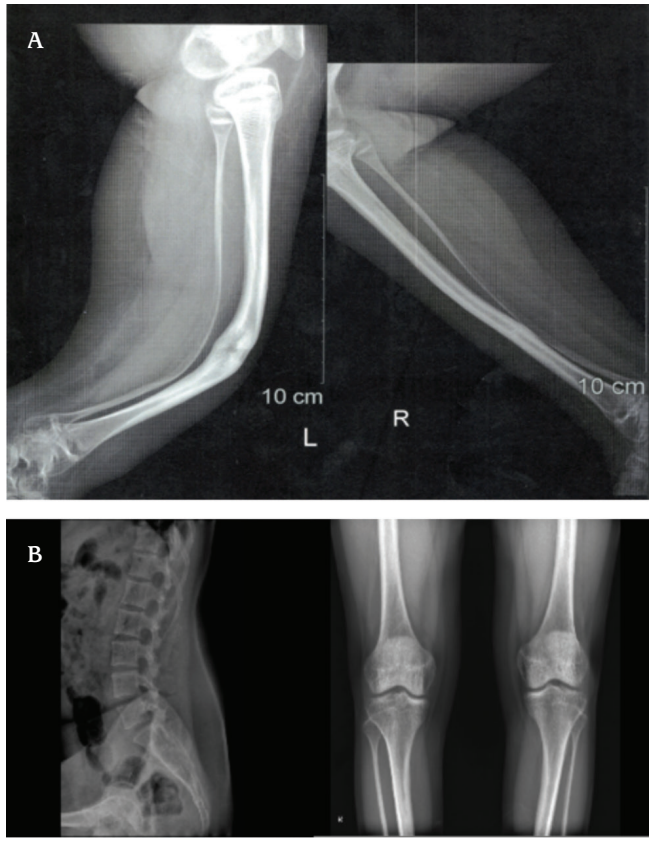

Figure 1. A) X-ray radiography of the patient's tibia and fibula, B) X-ray radiography of the patient's lumbar spine and knees

written consent to the publication of her children's file in accordance with the Helsinki Declaration.

Case 2

The patient, brother of the proposita, was 18 years old at the time of first evaluation and a student at law school. He was born preterm at 33 weeks by normal vaginal delivery, with birth weight, height, and head circumference of 2000 g (<3%), 46 cm (10%), and 33 cm (10%), respectively. His weight and height at presentation were 52 kg [-2 standard deviation (SD)] and 152 cm (-4 SD). In early infancy, ophthalmological evaluation revealed bilateral microphthalmia with corneal opacities, persistent hyperplasia of the primary vitreous and lesions in the anterior and posterior chambers. His cardiovascular and neurological examinations were normal. Of note, he had no history of long bone fractures. Furthermore, the patient did not have any long bone or chest deformities and there was no vertebral compression on his lumbar X-ray (Figure 1B). He did not complain of back or limb pain.

All laboratory data, including biochemical and hormonal tests, including serum calcium, phosphorus, magnesium, alkaline phosphatase, parathyroid hormone, thyroid function tests, lipid profile, liver transaminases and uric acid were normal. At first examination when he was 18 years old, BMD by DEXA showed severe osteoporosis with absolute values of 0.635 g/cm^2 in the lumbar (Z score -4.1) and 0.439 gr/cm^2 in the femur (Z score of -3.6). BMD at 22 years old after three years of treatment with alendronate (70 mg per week, starting at age 19 years) showed absolute value of 0.516 g/cm^2 (Z score -3.0) in the lumbar and 0.615 g/cm^2 (Z score -2.8) in the femur. Thus there there was a relative increase BMD in the lumbar area. The patient received 1000 mg calcium and vitamin D3 1000 IU (25 mcg) daily, in addition to the oral alendronate. The measured height of the mother was 167 cm and the father's height was 174 cm.

Genetic Studies

DNA was extracted from peripheral blood leukocytes using a commercial kit (High Pure PCR Template Preparation, Roche). Whole exome sequencing on DNA enriched for exonic regions was performed with SureSelect 38Mbp All exon kit v. 7.0 (Agilent Technologies, Santa Clara, CA, USA), and the samples were prepared according to manufacturer protocols. Prepared DNA samples were sequenced on HiSeq2000 (Illumina Inc., San Diego, CA, USA) using 75 x 2 bp paired-end sequencing with a mean coverage of 80-120x. The preliminary whole exome data analysis was performed through BWA and GATK software (6,7) to generate a BAM and a VCF file, respectively. Annotations of the VCF files were carried out using the wANNOVAR software, and the data was manually analyzed for the presence of candidate pathogenic variants.

In these patients, a novel, homozygous, nonsense mutation (c.351G>A) in exon 2 of *LRP5* (NM_002335) was found (Figure 2, 3), This was predicted to change tryptophan 117

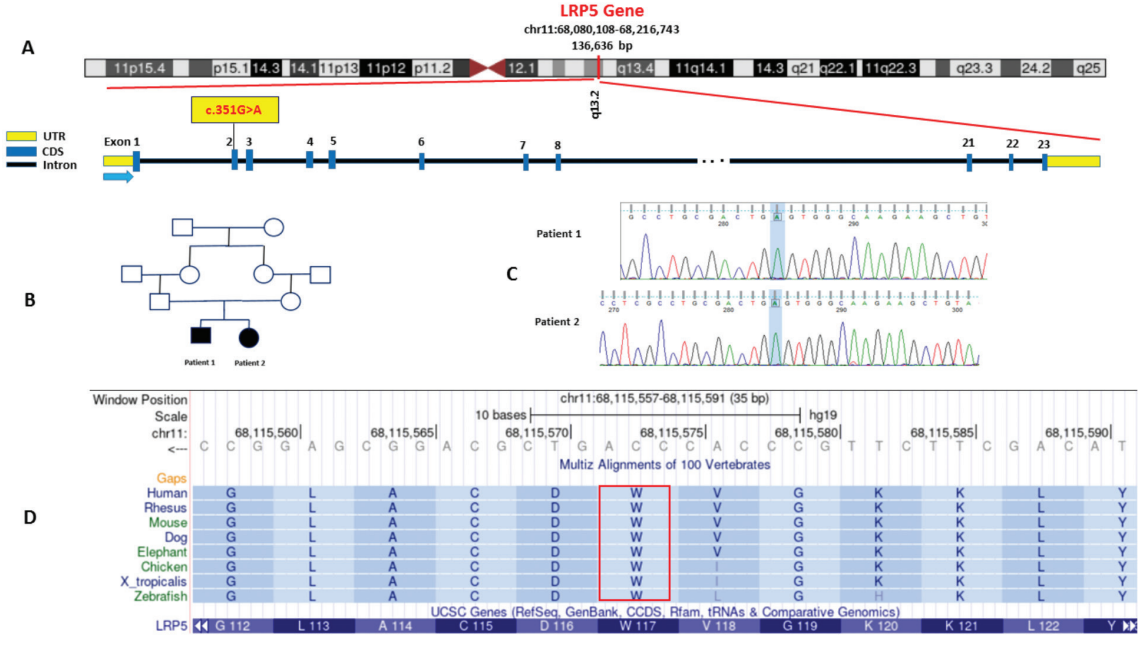

Figure 2. A) *LRP5* gene structure and the mutation. B) Family pedigree. C) Electropherograms from Sanger confirmation in family members showing *LRP5* (c.351G>A, p.Trp117*), homozygous mutant. D) The highly conserved state of the variant amino acid across evolution of species

to a stop codon (p. Trp117Ter). The identified mutation in *LRP5* was validated using Sanger sequencing. Segregation analysis revealed that the mother was heterozygous at the mutation position (Figure 2) but the father was not available for testing (Figure 2). Detailed computational predictive analysis of p. Trp117Ter mutation indicated a disease-causing alteration using PolyPhen-2, SIFT, and Mutation Taster. The local NGS database (8), currently consisting of 1406 whole exome sequencing data from healthy controls as well as public databases including the 1000 Genomes Project, the Genome Aggregation Database (gnome AD), and the Genome Aggregation Consortium (ExAC), were also interrogated and no samples exhibited the identified mutation in *LRP5* found in these siblings, indicating a novel, previously unreported variant.

Discussion

In the present study, the OPPG phenotype was different in two patients from one family with an identical mutation. The sister was blind with multiple bone fractures and was unable to move, while her brother was also blind and had osteoporosis but had never had a bone fracture. Both patients had pseudoglioma and had been blind from the beginning of their lives.

The role of *LRP5* in regulating bone density was first identified as pathogenic mutations in patients with OPPG. Subsequent studies showed that *LRP5*, as a common receptor of the WNT signaling pathway, regulated osteocyte apoptosis in addition to modulating osteoblast differentiation and proliferation (9). *LRP5* mutations are associated with pseudogliomas, hypovascularization of the retina and exudative vitreoretinopathy. WNT signaling regulates the development of retinal vasculature and regression of primary vitreous vessels in the growing eye. The range of ocular involvement of patients ranges from persistent fetal arteries to phthisis bulbi (10,11,12,13). Most patients with OPPG are congenitally blind or become blind in early childhood, and all patients will become blind by the age of 25 years (14). Although a widespread allelic heterogeneity has been reported in OPPG patients, phenotypes do not appear to have significant variation in terms of final ophthalmological outcome (15).

Mutations in *LRP5*-encoding genes can cause a variety of phenotypes, from subtle changes in bone traits to severe changes that cause multiple bone fractures. Moreover, similar gene mutation in single families can result in different phenotypes (2,16). Variable expression and diversity within the family, although usually occurring with autosomal dominant disorders, can also occur in autosomal recessive conditions. Intrafamilial variability of the OPPG disease phenotype has been reported in some families (17).

These patients may also have some degree of muscular hypotonia and ligament laxity (18). Cognitive impairment has also been reported in approximately 25% of patients with OPPG and usually the first bone fracture occurs at about two years of age. Genetic factors in combination with environmental influences may play a role in increasing cognitive dysfunction (19,20). It was notable that the younger female proband had autism and was unable to communicate verbally, while her brother with the same variant was able to continue his university education.

In 2010, Saarinen et al. (21) reported abnormal glucose tolerance test findings and hyperglycemia in patients with the *LRP5* mutation, secondary to beta-cell dysfunction. Additional findings reported by these authors included a high prevalence of hypercholesterolemia in these patients. Neither of the siblings presented herein had hypercholesterolemia or hyperglycemia.

To date, more than 70 cases of OPPG have been reported, most of them are associated with consanguineous marriages, with a prevalence of approximately 1: 2,000,000 (12,13).

In the patients presented in this case report, a novel, homozygous nonsense mutation in exon 2 of *LRP5* gene was found, and based on American College of Medical Genomics standards, there is strong evidence that this mutation is pathogenic (22). The mutation is a G to A substitution in exon 2 of *LRP5*, predicted to change tryptophan 117 to a stop codon and thus causing a modification of the *LRP5* protein sequence.

LRP5 contains 23 exons and encodes a transmembrane, low-density lipoprotein receptor that binds and internalizes ligands in the process of receptor-mediated endocytosis and plays a key role in skeletal homeostasis. The majority of mutations linked to OPPG are found in the second and third of the four YWTD b-propeller domains (23,24), coded for by exons 6 to 12. LRP5 protein consists of 1615 amino acids, of which the first 1384 amino acids form the extracellular part of this receptor. This extracellular part also includes 29 amino acid signal peptide, 20 YWTD spacer repeat domains, interspersed by four EGF-like domains and three LDL receptor-like ligand binding domains.

This variant is located in exon 2 and the extracellular region of this protein, which is indicated by an arrow. Change of tryptophan in position 117 to stop codon by this mutation leads to truncated protein and loss of function of it. On the other hand loss of function is a known mechanism of disease in *LRP5* gene (Figure 3) (25).

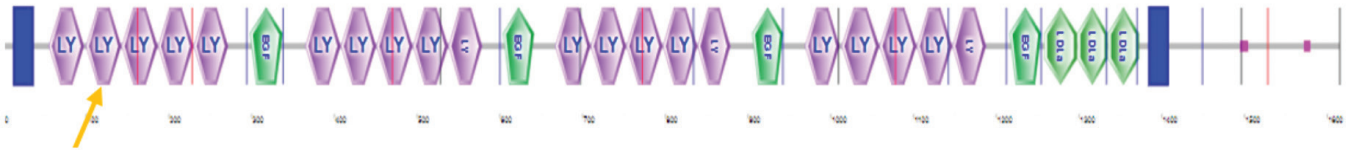

Figure 3. cDNA reference sequence for *LRP5*. The homozygous, nonsense mutation (c.351G > A) in exon 2 of *LRP5* (NM_002335)

Most of the pathogenic *LRP5* mutations are missense (67 out of 93), and there are only two nonsense and six frameshift mutations (8). *LRP5* is one of the most important factors having a remarkable effect in increasing bone mass. Other functions of LRP5 include interaction with sex hormones and growth hormone and IGF-1 (26).

Conclusion

A novel, homozygous nonsense mutation was identified in two siblings with OPPG. These siblings had remarkably different phenotype with one unable to communicate while the other was in higher education. This is the first report of a novel *LRP5* mutation in OPPG in the Iranian population. This report expands the spectrum of *LRP5* mutations that are the cause of OPPG.

Acknowledgments

We thank the patient's family for their cooperation in this study. This study was funded by Qazvin Rehabilitation Organization, Qazvin-Iran. The authors are thankful to the staff of the Clinical Research Center at Qazvin Children Hospital, affiliated to Qazvin University of Medical Sciences for their help in preparing this paper.

Ethics

Informed Consent: Consent form was filled out by all participants (or families).

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Fatemeh Saffari, Concept: Abolfazl Heidari, Fatemeh Saffari, Design: Fatemeh Saffari, Data Collection or Processing: Ali Homaei, Analysis or Interpretation: Abolfazl Heidari, Fatemeh Saffari, Literature Search: Ali Homaei, Fatemeh Saffari, Writing: Abolfazl Heidari, Ali Homaei Fatemeh Saffari.

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CASE REPORT

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Non-hormonal Clitoromegaly due to Clitoral Priapism Caused by Appendicitis/Appendectomy

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What is already known on this topic?

Clitoromegaly is usually caused by hyperandrogenism. There are rare, non-hormonal etiologies of clitoromegaly. Penile priapism has been reported to be related to appendicitis.

What this study adds?

Prolonged clitoral priapism can present with clitoromegaly in children. Clitoral priapism may also be related to appendicitis/appendectomy. Pseudoephedrine treatment is helpful in clitoral priapism.

Abstract

Clitoromegaly usually develops due to hyperandrogenism. There are a few cases of clitoromegaly described without clinical and biochemical hyperandrogenism. Clitoromegaly due to clitoral priapism and clitoral priapism after appendectomy have not been reported previously. A 7-year-old girl was referred for enlargement of the clitoris. She reported having a mild, pulsating clitoral pain starting three days after an appendectomy operation. Subsequently, painful swelling and an increase in the size of the clitoris was observed. Her growth and physical examination were otherwise normal. Causes of the clitoromegaly due to androgen excess were excluded after a comprehensive work-up. Color Doppler ultrasound revealed a high peak systolic velocity and resistance in the cavernosal artery, consistent with clitoral priapism. The clitoromegaly and associated symptoms improved significantly with oral pseudoephedrine and intracavernosal aspiration. This unique case illustrates that clitoral priapism is a rare, non-hormonal cause of clitoromegaly and may occur after appendectomy. Pseudoephedrine treatment is helpful in alleviating the symptoms.

Keywords: Non-hormonal clitoromegaly, clitoral priapism, clitorism, acute appendicitis, pelvic inflammation, pseudoephedrine

Introduction

Clitoromegaly is an abnormal enlargement of the clitoris and is usually due to androgen excess (1). However, a few child and adult case reports have described clitoromegaly without hyperandrogenism, including neurofibromatosis, epidermoid cysts, other tumors and syndromes such as Fraser, Donohue, Seckel and Apert Syndrome (1,2,3,4,5,6).

Clitoral priapism is a rare condition that is characterized by a prolonged, painful erection of the clitoris that is not associated with sexual arousal (7). Engorgement of the clitoris and the surrounding tissues with accompanying pain is the typical presenting symptom.

In children, penile priapism usually occurs in the setting of sickle cell anemia or leukemia (8,9). Other conditions associated with priapism include local malignancies, certain medications, hematologic and thromboembolic disorders, and neurologic conditions such as transverse myelitis (8). Pelvic inflammation caused by appendicitis, can lead to the irritation of the nervi erigentes or pelvic plexus

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causing neurogenic priapism (9). The association of acute appendicitis and priapism is limited to six (four < 18 years of age) male cases reported in the literature (8,9,10,11).

Herein, we report a seven-year-old girl who was referred to a pediatric endocrinology unit due to clitoromegaly after appendectomy. We believe this is the first case of clitoral priapism associated with appendicitis.

Case Report

A 7-year-old girl presented with a progressive, painful swelling of the clitoris that had worsened for the two weeks prior to presentation. Symptoms started three months earlier after and episode of acute appendicitis requiring an appendectomy operation. She indicated that there was a mild, pulsating pain, which worsened just before voiding and decreased thereafter. In the last two weeks, she had multiple emergency visits for a stabbing pain in her vulva and a gradual increase in the size of her clitoris. With the exception of the appendicitis/appendectomy, she had no previous illness, no recent history of trauma, medication use or infection. There was no family history of sickle cell anemia or cancer. The physical examination was unremarkable except for her clitoris, which was stimulated and tender. Clitoris was enlarged and measured at 2.4 cm in length and 1.2 cm in width (normal < 1.0 cm) (Figure 1a). The urethral and vaginal orifices were normal. She had no evidence of posterior fusion. Her pubic hair and breast development were consistent with Tanner stage 1. Her growth was not accelerated [height 118.4 cm, -0.9 standard deviation score (SDS); weight 22.4 kg, -0.4 SDS]. There was no other clinical evidence of hyperandrogenism including acne, oily skin, body odor or temporal balding.

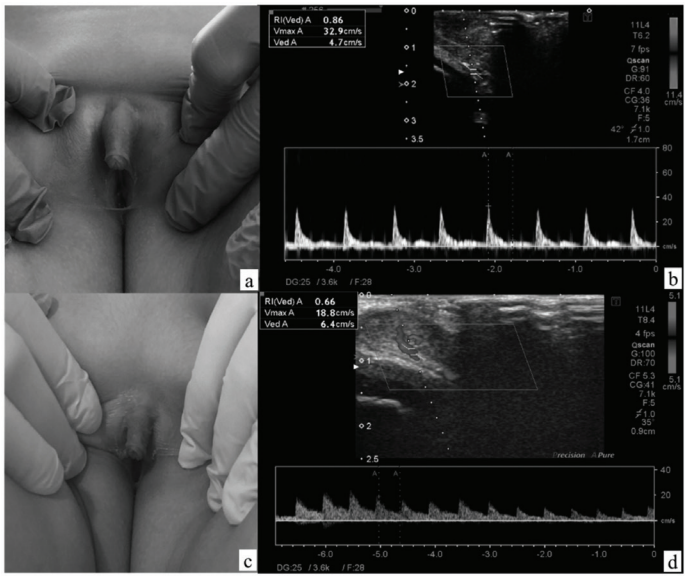

Figure 1. Non-hormonal clitoromegaly due to clitoral priapism

a) The appearance of the clitoris before treatment. b) The color Doppler ultrasound imaging of cavernosal arterial peak systolic velocity (32.9 cm/sec) and cavernosal arterial resistive index (0.86) before treatment. c) The appearance of the clitoris after treatment. d) The cavernosal arterial peak systolic velocity (18.8 cm/sec) with low resistive index (0.66) after the treatment of clitoral priapism. The imaging studies of clitoral vascularization are lacking in the literature. Although the normal values of the clitoral cavernosal arterial resistive index are not known, the decrease seen after the pseudoephedrine treatment was apparent and parallel with the clinical response

A complete blood count, sedimentation rate and prothrombin and partial thromboplastin times were normal. Hemoglobin (Hb) electrophoresis and urinalysis was normal. There was no evidence of androgen excess on biochemical evaluation (Table 1). Pelvic ultrasound was normal. Karyotype analysis was reported 46,XX.

Color Doppler ultrasound revealed high resistance flow in the cavernosal artery with a decreased venous flow, consistent with clitoral priapism. The cavernosal artery resistive index was 0.86 and peak systolic velocity was 32.9 cm/sec (Figure 1b). The patient was started on oral pseudoephedrine (Sudafed®) 15 mg every eight hours. In the following 72 hours, the clitoral swelling reduced, and the pain associated with urination only happened once when the pseudoephedrine dose was skipped. The dose of pseudoephedrine was increased to 30 mg every eight hours after the first week of treatment. However, the

Table 1. Laboratory findings of the patient at presentation

	Levels	Reference values
Total testosterone*	0.05 μg/L	(0.00-0.20)
DHEA*	0.4 μg/L	(0-3.4)
DHEAS*	155 μg/L	(440-3320)
Δ4-androstenedione*	0.17 μg/L	(0.06-1.15)
17OHP*	0.29 μg/L	(0.00-0.90)
21-deoxycortisol*	< 0.24 μg/L	(0.06-0.51)
11-deoxycortisol*	0.63 μg/L	(0.00-3.44)
FSH	3.16 U/L	(1.0-4.2)
LH	< 0.2 U/L	(0.02-0.3)
E2	< 5 ng/L	(< 15)
AMH	2.51 ng/mL	(0-8.8)
AFP	0.49 U/mL	(0.49-9.84)
HCG	< 0.5 U/L	(< 5)

*Assay method was liquid chromatography mass spectrometry.
Conversion factors for SI units are: E2, ×3.67 pmol/L; DHEAS, ×2.71 nmol/L; DHEA, ×3.44 nmol/L; Δ4-androstenedione, ×3.49 nmol/L; total testosterone, ×3.47 nmol/L; 17OHP, ×3.02 nmol/L.
DHEA: dehydroepiandrosterone, DHEAS: dehydroepiandrosterone-sulfate, 17OHP: 17α-hydroxyprogesterone, FSH: follicle stimulating hormone, LH: luteinizing hormone, E2: estradiol, AMH: anti-mullerian hormone, AFP: alpha fetoprotein, HCG: human chorionic gonadotropin

patient developed tachycardia three weeks after initiation of treatment, leading to discontinuation of pseudoephedrine. Oral ibuprofen (~15 mg/kg/day, every eight hours) treatment was then initiated due to pain associated with clitoral priapism. Due to persistence of the symptoms, intracavernosal needle aspiration and cystoscopy were performed. There was no sign of ischemia or acidosis in the blood gas analysis of cavernosal blood (pH = 7.52, PCO_2 = 27 mmHg, PO_2 = 219 mmHg). Subcutaneous injection of 0.5 mL 0.25% bupivacaine around the clitoris was performed for local anaesthesia with only limited decrease in clitoral pain. Cystoscopy was performed to rule out urologic problems that may be related to the clitoral priapism but this revealed only a mild trabeculation in the bladder. Technetium-dimercaptosuccinic acid scintigraphy and uroflowmetry were normal. Oral ibuprofen treatment was continued. One week after cystoscopy, treatment with oral pseudoephedrine 15 mg every eight hours was resumed because of persistent priapism. There was a significant improvement in the patient's complaints after restarting pseudoephedrine. She reported no pain or swelling but urgency before voiding. The clitoris size regressed to 1.9 cm (Figure 1c). Color Doppler ultrasound was repeated at the end of a month of regular pseudoephedrine treatment and this showed normal peak systolic velocity in the cavernosal artery (18.8 cm/sec) with lower resistance flow (resistive index: 0.66) (Figure 1d). She remained on pseudoephedrine treatment for another three months with reportedly less frequent and milder pain, usually before voiding. At follow-up, six months after diagnosis, the family reported a few, milder episodes of priapism despite discontinuation of pseudoephedrine. There was no further decrease in clitoral size.

Discussion

Clitoromegaly is generally associated with conditions of androgen excess, such as virilizing forms of congenital adrenal hyperplasia, and virilising adrenal and gonadal tumors. Exhaustive hormonal work-up and the absence of other signs of hyperandrogenism in the presented case excluded androgen excess. Priapism of the clitoris is a rare condition associated with prolonged erection of the clitoris causing engorgement, swelling, and pain to the clitoris and immediate adjacent area. Prolonged penile priapism is known to cause penile enlargement (12). Although not reported previously, we believe that clitoral priapism in our case caused clitoral enlargement similar to that previously only reported in males. All of the patients with clitoral priapism reported so far were adults and the typical initial symptoms were vulvar and clitoral pain with swelling and tenderness (13,14,15,16,17,18,19). Most reported cases of clitoral priapism were drug-induced, all having alpha-adrenergic blocking potential. The most commonly reported drug associated with clitoral priapism was trazodone (14,16,17).

The presented case, to the best of our knowledge, is the first prepubertal case of clitoral priapism with clitoromegaly as a presenting symptom and is also the first case of clitoral priapism observed after appendicitis/appendectomy, a known etiology for penile priapism. The main physiologic mechanism of priapism consists of impaired outflow from the corpora cavernosa through direct venous obstruction or failure of the alpha-adrenergic relaxation system, resulting in prolonged relaxation of corporal smooth muscle (17,20). There are three types of priapism: ischaemic (low-flow or veno-occlusive) (LFP), stuttering (intermittent or recurrent) and non-ischaemic (high-flow) (20). The high-flow type is characterized by an increase of arterial inflow and does not appear to occur in women (7,16,17). LFP occurs because of venous outflow obstruction, usually seen in the setting of sickle cell anemia or leukemia in children (20). Inflammation is an uncommon etiology of LFP. It has been reported in systemic infections such as mumps, Rocky Mountain spotted fever, tularemia, undulant fever, *Mycoplasma pneumoniae*, *Coxsackie B* and Echovirus 14 (8,10,21,22,23). Appendicitis, Crohn disease, ulcerative colitis and localized urogenital infections, such as chronic prostatitis and urethritis can cause pelvic inflammation that can lead to irritation the nervi erigentes or pelvic plexus causing a neurogenic LFP (9,24,25). There are six reported cases of LFP associated with appendicitis appearing in the literature to date and all of them are males (9). In three of the reported cases, priapism was the main complaint leading to the diagnosis of appendicitis and in the other three cases priapism developed as a postoperative complication (8,9,10,11,26,27), similar to the process seen in our patient.

The intermittent nature of the symptoms and shorter, self-limiting episodes of clitoral priapism in our patient were consistent with stuttering priapism that has a similar etiology with LFP. Sickle cell anemia is the most common cause of stuttering priapism (28). However, priapism secondary to sickle cell anemia has not been reported in females. Furthermore, Hb electrophoresis in our patient was normal. Although the underlying mechanism of stuttering priapism is still unknown, it is thought that neurogenic or endothelial dysregulation of smooth muscle relaxation is involved in the pathophysiology (20,28). In this context, therapeutic agents that help detumescence of erectile tissue by increasing smooth muscle tone of the corpus cavernosum can be used in the treatment.

Pseudoephedrine, more commonly used as an oral decongestant, is often used as a first-line treatment for stuttering priapism (28). Pseudoephedrine acts on two receptors in bronchial smooth muscle to induce relaxation through cAMP and vasoconstriction via α-adrenergic receptors in the respiratory mucosa. Although smooth muscle contraction is possibly mediated by the effects of pseudoephedrine on α-adrenergic receptors, its effects on smooth muscle have not yet been studied. In studies conducted in a small number of cases in stuttering priapism due to sickle cell anemia, a variable response to prophylactic treatment with pseudoephedrine has been observed (29,30).

Intracavernosal injections to cavernosaphenous shunts had been performed in other reported cases with appendicitis related LFP (8,9,10,11). Intracavernosal needle aspiration, and local anaesthesia with bupivacaine, resulted in partial benefit in our patient. Unlike appendicitis associated with LFP and other cases of LFP, cavernosal blood gas analysis did not suggest ischemia or acidosis. In the literature, there is no case in which clitoral cavernosal blood gas analysis was performed. Pseudoephedrine treatment, which has been shown to work in cases of stuttering pediatric male priapism (29) and in one case of persistent symptomatic female priapism (17) was also successful in our case. Besides the significant decrease in the patient's complaints and clitoral size, we were able to objectively demonstrate a significant decrease in the cavernosal artery resistive index compared to pre-treatment measurement by Doppler ultrasound imaging.

Conclusion

Clitoral priapism should be kept in mind as a rare non-hormonal cause of clitoromegaly. Priapism associated with acute appendicitis can also occur in females. Oral pseudoephedrine treatment is helpful in alleviating the symptoms.

Ethics

Informed Consent: The parents gave their written informed consent to publish the data and pictures of the patient.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices - Concept - Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: Büşra Gürpınar Tosun, Ahsen Karagözlü Akgül, Eda Almus, Sadık Abidoğlu, Serap Turan, Abdullah Bereket, Tülay Güran.

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CASE REPORT

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Metreleptin Treatment in a Boy with Congenital Generalized Lipodystrophy due to Homozygous c.465_468delGACT (p.T156Rfs*8) Mutation in the *BSCL2* Gene: Results From the First-year

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What is already known on this topic?

Congenital generalized lipodystrophy is a rare, autosomal recessive disorder characterized by an almost complete absence of body fat. Metreleptin, a preparation of recombinant human leptin, has been suggested as an effective treatment option.

What this study adds?

The presented case is currently the youngest patient from Turkey who was successfully managed using metreleptin treatment. Initiation of metreleptin treatment in the early period may be beneficial in reducing mortality and morbidity and increasing quality of life in patients with congenital generalized lipodystrophy

Abstract

Congenital generalized lipodystrophy (CGL) is a rare, autosomal recessive disorder characterized by an almost complete absence of body fat. In CGL, patients may have hyperphagia due to leptin deficiency. Recombinant human leptin (metreleptin) has been suggested as an effective treatment option. We present successful treatment with metreleptin in a boy with CGL and results from the first year of follow-up. An eight-month-old boy presented with excessive hair growth and a muscular appearance. On examination he had hypertrichosis, decreased subcutaneous adipose tissue over the whole body and hepatomegaly. Laboratory investigations revealed hypertriglyceridemia, hyperinsulinemia, elevated liver transaminases and low leptin levels. Molecular genetic analysis detected a homozygous, c.465_468delGACT (p.T156Rfs*8) mutation in the *BSCL2* gene. A diagnosis of CGL type 2 was considered. Despite dietary intervention, exercise, and treatment with additional omega-3 and metformin, the hypertriglyceridemia, hyperinsulinemia, and elevated liver transaminase levels worsened. Metreleptin treatment was started and after one year hyperphagia had disappeared, and there was dramatic improvement in levels of insulin, hemoglobin A1c, triglycerides and liver transaminases. Hepatosteatosis was lessened and hepatosplenomegaly was much improved. Metreleptin appears to be an effective treatment option in children with CGL that remarkably improved metabolic complications in the presented case. Initiation of metreleptin treatment in the early period may decrease mortality and morbidity, and increase the quality of life in children with CGL.

Keywords: Congenital generalized lipodystrophy, *BSCL2* gene, metreleptin treatment

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Introduction

Congenital generalized lipodystrophy (CGL), also known as Berardinelli Seip syndrome, is a rare, autosomal recessive disorder, usually presenting in the first year of life and characterized by near-total fat atrophy, muscular hypertrophy, and prominent veins (1,2,3,4,5). The prevalence of CGL is estimated as 1:10,000,000 (6). However, the prevalence of CGL in Turkey has been estimated to be much higher at 1:2,000,000 (5). In CGL, hyperphagia associated with leptin deficiency, rapid growth, umbilical hernia, hepatosplenomegaly and acromegalic appearance may be present (1,2,3,7,8).

CGL may cause hyperinsulinemia, hypertriglyceridemia, insulin resistance, diabetes, polycystic ovary syndrome, non-alcoholic fatty liver disease, proteinuria and cardiomyopathy (1,2,3,4,5,6,7). In addition to phenotypic characteristics, evaluation of body fat percentage and distribution, dual-energy X-ray absorptiometer (DXA) and whole-body magnetic resonance imaging may be helpful for the diagnosis of CGL (1,2,7). Low serum leptin levels support the diagnosis, and this finding is also important from the point of view of clinical management (5). Genetic testing is required to confirm the diagnosis and genetic counseling should be given (7). Pathogenic variants in the genes *AGPAT2, Berardinelli-Seip congenital lipodystrophy 2 (BSCL2), CAV1* and *PTRF* cause CGL type 1, type 2, type 3 and type 4, respectively (1).

The goal of the treatment is to prevent metabolic complications and restore cosmetic appearance. Lifestyle changes, including dietary intervention and physical exercise, are the cornerstones of the initial treatment of CGL (1,2,7). When achieving target triglyceride levels with appropriate dietary intervention and exercise is unsuccessful, lipid-lowering drugs may be used (1,2,7). Metformin is the first choice drug for pharmacotherapy when there is insulin resistance. In patients who develop diabetes, insulin may be used in conjunction with metformin treatment (2,7). Many studies have reported the efficacy of recombinant human leptin (metreleptin) in CGL (9,10,11,12). Metreleptin reduces appetite through leptin receptors in the hypothalamus (13) and improves peripheral and hepatic insulin sensitivity (14). Metreleptin reduces hyperphagia, triglyceride, and hemoglobin A1c (HbA1c) levels and improves hepatic steatosis. In the present case report, presenting features, diagnosis and the first-year results of a male with CGL type 2 due to a homozygous pathogenic variant in the *BSCL2* gene and treated with metreleptin are presented.

Case Report

The eight-month-old boy presented with excessive hair growth and a muscular appearance over the whole body. He was born to first cousins after a term, uneventful gestation via spontaneous vaginal delivery. His birth weight was 3100 g. On physical examination performed at the time of presentation his weight was 9 kg [0.09 standard deviation score (SDS)], height was 73.8 cm (0.92 SDS), body mass index was 16.5 kg/m^2 (-0.64 SDS) and blood pressure was 80/50 mmHg (Table 1). Remarkable hypertrichosis was present on the extremities, waist and hips. The muscular appearance was prominent on his arms, and reduced subcutaneous adipose tissue was observed in the face and whole body. The abdomen was distended with enlarged liver (5-6 cm palpable), spleen (3-4 cm palpable) and umbilical hernia. Testicular volume was 2/2 mL, pubic and axillary hair were at Tanner stage 1, and stretched penile length was 6 cm. Diagnosis and follow-up images are presented in Figure 1. On laboratory investigation, glucose was 95 mg/dL, insulin 36.5 mIU/mL, HbA1c 5.9%, triglyceride 218 mg/dL, total cholesterol 162 mg/dL, and liver transaminases

Figure 1. Images of the case before (a, b) and first year (c, d) treatment with metreleptin

Table 1. The follow-up data before and after metreleptin treatment

	At diagnosis	F/up visit 1	F/up visit 2	F/up visit 3	F/up visit 4 (metreleptin initiated)	F/up visit 5	F/up visit 6	F/up visit 7	F/up visit 8 (first year of metreleptin treatment)	% difference (after 1 year treatment)
Age (years)	0.75	3.75	4.75	5.25	5.5	5.75	6	6.25	6.5	
Height SDS	0.92	2.42	2.88	3.04	3.2	3.1	3.3	3.13	3.06	-4.3
BMI SDS	-0.64	2.75	2.52	2.4	2.3	1.63	1.53	0.9	1	-56
Fasting glucose (mg/dL)	95	92.5	105	68	135	78	81	70	85	-37
Fasting insulin (mIU/mL)	36.5	54.2	65	151	185	2.42	21	10	12	-93
HbA1c (%)	5.9	6.2	6.7	7.6	6.7	5.6	5.1	5.1	5.2	-22
Triglyceride (mg/dL)	218	192	248	208	197	38.3	88		85	-66
Total cholesterol (mg/dL)	162	119	131	136	162	131	118		120	-25
LDL cholesterol (mg/dL)	104	68	85	81	100	69	60		70	-30
HDL cholesterol (mg/dL)	7	21.3	25	41	26	50	42		41	+36
ALT (U/L)	309	257	380	747	482	45	42	18	31	-93
AST (U/L)	267	147	254	342	306	33	41	21	29	-90
Liver size (mm)	101	137	195		195	170			167	-14
Spleen size (mm)	70	124	130		142	134			121	-15
Bone age (years)					8.5					
Echocardiography	Normal				Normal				Normal	
Treatment	Diet	Diet, exercise, metformin	Diet, exercise	Diet, exercise	Diet, exercise, metreleptin	Diet, exercise, metreleptin	Diet, exercise, metreleptin	Diet, exercise, metreleptin	Diet, exercise, metreleptin	

F/up: follow-up, BMI: body mass index, HbA1c: glycated hemoglobin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, LDL: low-density lipoprotein, HDL: high-density lipoprotein, SDS: standard deviation score

were elevated with an alanine aminotransferase of 309 U/L and an aspartate aminotransferase of 267 U/L. His leptin level (0.19 ng/mL) was low (Table 1).

Molecular Genetic Analysis

Genomic DNA was isolated from peripheral blood cells using standard techniques. Mutation analyses were performed by bidirectional sequencing of the coding exons and the exon-intron boundaries of the *AGPAT2* and *BSCL2* genes. Polymerase chain reaction primers used in order to amplify the regions of interest are available upon request. Sequencing was performed with Miseq V2 chemistry on Illumina MiSeq Sequencer (Illumina, CA, USA). Analysis was performed with IGV software. Molecular genetic analysis unveiled a homozygous, c.465_468delGACT (p.T156Rfs*8) pathogenic variant in the *BSCL2* gene, and a diagnosis of CGL type 2 (Berardinelli-Seip syndrome) was considered (Figure 2). This pathogenic variant has been previously reported (5).

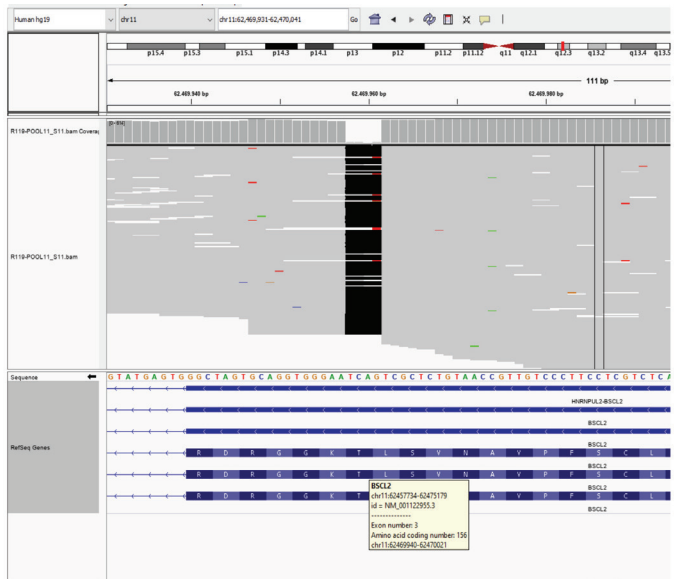

Figure 2. Molecular genetic analysis of the case *BSCL2* c.465_468del (p.T156Rfs*8)

Treatment and Follow-up Characteristics

Initial management was attempted with lifestyle modification. However, dietary adherence was poor due to hyperphagia and his clinical (marked hepatosplenomegaly and hepatosteatosis) and biochemical (increase in triglyceride levels, insulin resistance, and HbA1c levels) features had worsened. Metreleptin treatment was considered at this time but was not commenced due to rejection of social security institution rules for reimbursement. Therefore, metformin treatment was delayed for several years. During this time, and despite dietary intervention, exercise, omega-3 supplementation and metformin treatments, hypertriglyceridemia, insulin resistance, liver transaminase levels and hepatosteatosis did not improve. Eventually, when he was five and a half years-old, metreleptin treatment was initiated at a subcutaneously administered dose of 0.06 mg/kg/day. Metreleptin treatment improved his sense of satiety, and he achieved weight loss, thereby restoring metabolic parameters including normal triglyceride, insulin, HbA1c and liver transaminase levels. The size of the liver and the spleen and abdominal distension decreased. The follow-up data after metreleptin treatment are presented in Table 1. The patient did not develop cardiomyopathy, arrhythmia, or proteinuria although neurodevelopmental delay was present. Informed consent was obtained from the patient's parents for publication and the use of photos.

Discussion

We report successful management of metabolic complications in a child with CGL2 due to a homozygous c.465_468delGACT (p.T156Rfs*8) mutation in the *BSCL2* gene. CGL2 (OMIM #269700) is caused by pathogenic variants of the *BSCL2* gene (1), which encodes for the transmembrane protein seipin. This protein is involved in the fusion of small lipid droplets in adipocytes and also in adipocyte differentiation (1,2). Low serum leptin levels may help to confirm the diagnosis and determine the management strategies (5). A relatively high concentration of adiponectin is a differential feature of CGL2, while serum leptin levels are extremely low in all subtypes of CGL (5). In our case, the leptin level was low and the response to metreleptin treatment was excellent.

Early-onset hyperphagia associated with leptin deficiency, rapid growth, advanced bone age, umbilical hernia, hepatosplenomegaly, and acromegalic appearance are among the clinical features of CGL (1,2,3,7,8). The present case had excessive growth of body hair, a muscular appearance, prominent veins due to absence of subcutaneous fat, hepatosplenomegaly and umbilical hernia at the time of the diagnosis. Hyperphagia appeared after the age of one year (Figure 1).

However, avoiding excess calorie intake, adjusting dietary fat content to manage hypertriglyceridemia, and increasing monounsaturated fat and long-chain fatty acids and in selected cases lipid-lowering drugs may be part of the treatment (1,2,7). The present case had insulin resistance, hypertriglyceridemia and hepatosteatosis at diagnosis. He was poorly adherent to the nutrition plan because of his hyperphagia. Metformin is the first choice drug in patients with severe insulin resistance, but it is not yet been approved for children under the age of 10 years. In patients with CGL who develop diabetes, insulin in combination with metformin, may be required. Moreover, the insulin therapy may need to be extremely high-dose (>100 units/day) because of the severity of insulin resistance in these patients (2,7). In the present case, an off-label use of metformin was done with consent family, while it was stopped due to lack of a clinical or biochemical improvement. Metreleptin regulates appetite and also improves peripheral and hepatic insulin sensitivity, partially independent of food intake (13,14,15,16). It also normalizes gonadotropin secretion, reduces proteinuria, improves immune function and lowers androgen levels, particularly in lipodystrophic females with polycystic ovary syndrome (17).

In this case, metreleptin treatment was initiated at the age of five and a half, which successfully decreased hyperphagia with a dramatic improvement in metabolic parameters, including insulin resistance, and triglyceride and liver transaminase levels, while HbA1c level reduced to within normal ranges. Hepatosteatosis improved with a remarkable decrease in the size of the liver and the spleen (Table 1). Furthermore, no side effects associated with metreleptin treatment were observed.

Conclusion

To the best of our knowledge, the present case is the youngest patient with CGL2 in Turkey to be successfully managed using metreleptin treatment. In children with CGL, metreleptin seems to be the most effective treatment option for preventing the development of metabolic complications. We suggest that initiation of metreleptin treatment in the early period may decrease mortality and morbidity due to the dramatic improvement seen in markers of metabolic derangement, as well as improvement of the quality of life in CGL patients.

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Ethics

Informed Consent: Informed consent was obtained from the patient's parents for publication and the use of photos.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Şervan Özalkak, Meliha Demiral, Edip Ünal, Funda Feryal Taş, Hüseyin Demirbilek, Concept: Şervan Özalkak, Funda Feryal Taş, Hüseyin Onay, Mehmet Nuri Özbek, Design: Şervan Özalkak, Funda Feryal Taş, Hüseyin Onay, Mehmet Nuri Özbek, Data Collection or Processing: Şervan Özalkak, Meliha Demiral, Edip Ünal, Funda Feryal Taş, Hüseyin Onay, Hüseyin Demirbilek, Mehmet Nuri Özbek, Analysis or Interpretation: Şervan Özalkak, Hüseyin Onay, Hüseyin Demirbilek, Mehmet Nuri Özbek, Literature Search: Şervan Özalkak, Hüseyin Demirbilek, Writing: Şervan Özalkak, Hüseyin Onay, Hüseyin Demirbilek.

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Letter to the Editor Regarding "Effect of Propolis on Precocious Puberty in Female Rats" - Does Propolis Induce Thelarche and Gynecomastia in Prepubertal Children?

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Keywords: Premature thelarche, prepubertal gynecomastia, propolis

Dear Editor,

Propolis is a product obtained from plants by honeybees and has been reported to have various biological effects such as anti-inflammatory, anti-microbial, anti-viral, and anti-oxidative. It consists of a mixture of various chemicals such as flavonoids, coumaric acid, and caffeic acid, some of which have phytoestrogenic effects (1). Phytoestrogens are substances that are structurally and functionally like estrogens. In vivo experiments on rats have shown that phytoestrogens bind to estrogen receptors and cause ductal cell proliferation in the uterus and mammary glands without an increase in endogenous estrogen production (1,2,3). Okamoto et al. (1) reported that propolis induced the expression of estrogen-responsive genes in their study on ovariectomized rats.

In this context, we read the article "Effect of Propolis on Precocious Puberty in Female Rats" recently published in your journal, with interest (3). We aimed to contribute to this article by reporting 4 girls and 4 boys who presented to our outpatient clinic with premature thelarche and prepubertal gynecomastia and had a history of propolis use.

Four girls aged between 4.9 and 8.2 years presented with breast development that had been present for an average of two months. They did not describe a growth spurt or a family history of precocious puberty. Their height and body mass index (BMI) standard deviation score (SDS) ranged between -0.17 – 0.67 SDS and -0.16 – 1.2 SDS respectively. Three girls had breast budding which is consistent with Tanner stage 2, and one girl had breast development consistent with Tanner stage 3. None of them did have pubic hair. All of them had luteinizing hormone (LH) levels <0.1 IU/L and E2 levels <5 pg/mL, and their FSH levels were between 0.3-2.8 IU/L. Uterus, ovary, and endometrial thickness in ultrasonography were compatible with the prepubertal period. Bone ages were compatible with their own ages. Prepubertal LH response was obtained in the LHRH stimulation test.

The ages of boys presenting with prepubertal gynecomastia ranged from 2.4 to 10 years. They did not describe a growth spurt. Their family history was unremarkable for gynecomastia. Only one had bilateral gynecomastia, while the others had unilateral gynecomastia. All had bilateral prepubertal testicular sizes (below 4 cc) and they had no pubic hair. Their height and BMI SDS ranged between -0.97 – 1.21 and -1.3 – 0.17 2 SDS respectively. Serum FSH, LH, total testosterone levels were prepubertal. All had normal serum estradiol and prolactin levels. All were reported to take oral propolis drop for a mean of 3.9 months (2-12 months). After the cessation of propolis intake, breast development resolved in all cases at the mean follow-up visit 3 months later.

Premature thelarche is characterized by isolated breast development in girls without other pubertal signs and

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prepubertal gynecomastia is a rare condition characterized by the presence of unilateral or bilateral breast tissue in boys without other pubertal signs. The use of drugs and herbal products containing phytoestrogens can cause premature thelarche and prepubertal gynecomastia. Ramsey et al. (4) reported three cases of premature thelarche and one case of prepubertal gynecomastia with the use of fragrances containing lavender, a phytoestrogen-containing product. In the article published in your journal (3), it was shown that propolis, probably triggers precocious puberty in female rats by interacting with estrogen receptors, and the regression of breast development in our patient group with the discontinuation of propolis intake suggested that propolis may trigger mammary duct proliferation with its phytoestrogenic effects in prepubertal children.

Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: Elif Eviz, Gül Yeşiltepe Mutlu, Şükrü Hatun, Design: Elif Eviz, Gül Yeşiltepe Mutlu, Şükrü Hatun, Data Collection or Processing: Elif Eviz, Gül Yeşiltepe Mutlu, Sebahat Yılmaz Ağladıoğlu, Şükrü Hatun, Literature Search: Elif Eviz, Gül Yeşiltepe Mutlu, Şükrü Hatun, Writing: Elif Eviz, Gül Yeşiltepe Mutlu, Şükrü Hatun.

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Letter to the Editor Regarding "Comparison of Commonly Used Methods to Predict the Final Height in Constitutional Tall Stature"

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Keywords: Early puberty, growth, paediatric endocrinology

Dear Editor,

We read with interest the article titled "Comparison of Commonly Used Methods to Predict the Final Height in Constitutional Tall Stature" by Matias et al. (1) published in the Journal of Clinical Research in Paediatric Endocrinology (Volume 15, Issue 1).

The article highlights prediction of adult height in children with constitutional tall stature. The investigators did an excellent job by including only children with constitutional tall stature and excluding other aetiologies of tall stature. They have done a tedious job at collecting all the records of growth and final height. We compliment the authors for addressing this highly contentious issue. The study concluded that Bayley-Pinneau chart approximates the final height better than Tanner-Whitehouse I method in children with constitutional tall stature. Investigators in the past also support using the Bayley-Pinneau method in this scenario (2).

However, there are a few issues we wish to raise. The first issue pertains to the definition of tall stature. Tall stature is taken as height beyond 97th percentile or more than 2 standard deviation (SD) above the mean height for age and sex in a defined population (3). The present study has adopted 90th percentile height as cut off to define tall stature. We believe that lowering the cut-off may increase the number of individuals with tall stature who may not require evaluation. de Waal et al. (2) have used 90th percentile to define tall stature in their study adopted from a Netherland data (4). We would like to know if any such data is available to apply the 90th percentile cut-off on Israeli population.

Second, the investigators have shown that the mean height SD score for boys returned to normal at the end of 17 years although for girls it remains elevated. This seems discrepant and a possible explanation for this would be welcome.

While we raise these issues, we once again congratulate the authors for analysing the height prediction methods and comparing them head-to-head. We believe this seminal work will open avenues for further research in this area and address more issues.

Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Subhankar Roy, Concept: Subhankar Roy, Abhranil Dhar, Pankaj Singhania, Tapas Chandra Das, Design: Subhankar Roy, Abhranil Dhar, Pankaj Singhania, Analysis or Interpretation: Abhranil Dhar, Tapas Chandra Das, Literature Search: Subhankar Roy, Pankaj Singhania, Tapas Chandra Das, Writing: Subhankar Roy, Abhranil Dhar, Pankaj Singhania.

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