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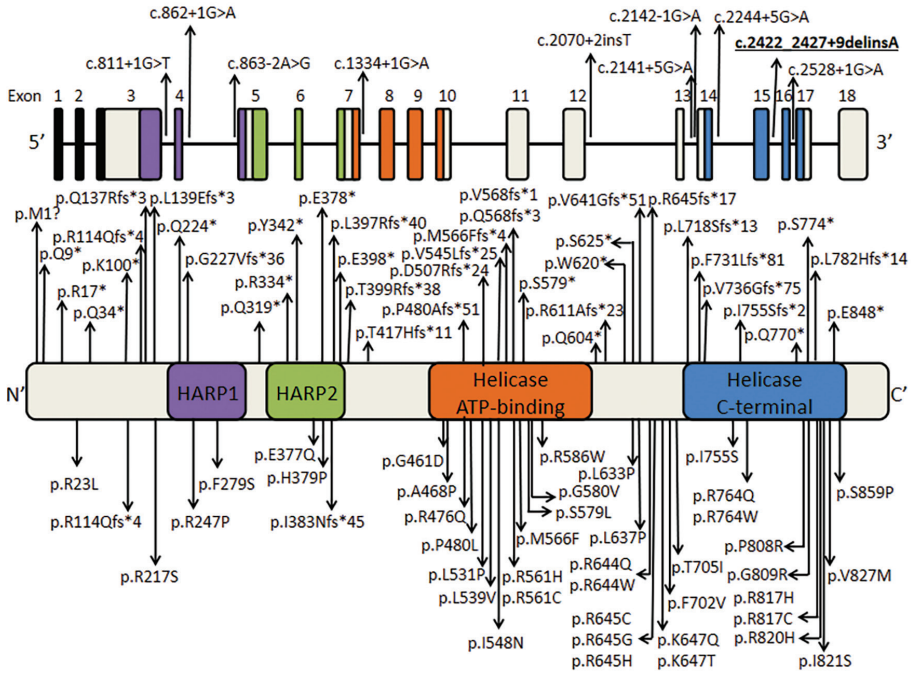

Expanding the Clinical Features of Schimke Immuno-osseous Dysplasia: a New Patient with a Novel Variant and Novel Clinical Findings

Alavanda C et al.

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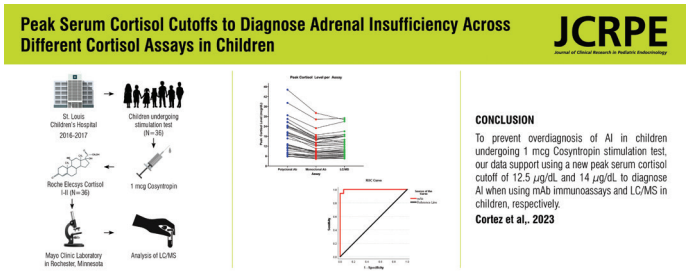

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REVIEW

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J Clin Res Pediatr Endocrinol 2025;17(2):126-135

Expanding the Clinical Features of Schimke Immuno-osseous Dysplasia: a New Patient with a Novel Variant and Novel Clinical Findings

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Abstract

Schimke immuno-osseous dysplasia (SIOD) (MIM:242900) is an ultra-rare, autosomal recessive, pan-ethnic pleiotropic disease. Typical findings of this syndrome are steroid-resistant nephrotic syndrome, cellular immunodeficiency, spondyloepiphyseal dysplasia (SED) and facial dysmorphism. Biallelic variants in the *SMARCAL1* gene cause SIOD. The five-and-a-half-year-old female patient was evaluated because of short stature, dysmorphism, hypercalcemia, hypophosphatemia, and elevated follicle-stimulating hormone (FSH) levels. Karyotype analysis and array-CGH testing were normal. Clinical exome sequencing (CES) was performed to analyze genes associated with hypophosphatemia. No pathogenic variant was detected. The subsequent detection of proteinuria during follow-up for cross-fused ectopic left kidney ultimately facilitated the diagnosis of SIOD, although no obvious SED was detected. Re-analysis of CES revealed a novel homozygous c.2422_2427 + 9delinsA pathogenic variant in the *SMARCAL1*. The literature on *SMARCAL1* gene pathogenic variants, including 125 SIOD cases from 38 articles was reviewed to investigate whether hypercalcemia, hypophosphatemia, and elevated FSH levels had been previously reported in SIOD patients. This review revealed that this was the first report of these findings in a patient with SIOD. Thus, this report expands both the phenotypic and genotypic spectrum of SIOD.

Keywords: *SMARCAL1*, hypercalcemia, hypophosphatemia, ectopic kidney, gonadal dysfunction

Introduction

Schimke immuno-osseous dysplasia (SIOD) (MIM: 242900) is an ultra-rare, autosomal recessive, pan-ethnic pleiotropic disease. The prevalence of SIOD is estimated to be 1 in 1-3 million live births in the USA (1). This syndrome was first described as chondroitin-6-sulfate mucopolysaccharidosis (2). However, after further studies, mucopolysaccharidosis was excluded (3). The main findings of this syndrome are steroid-resistant nephrotic syndrome, immunodeficiency, and spondyloepiphyseal dysplasia (SED). The short stature observed in almost all patients is due to the SED (4). Renal disease, mostly due to focal segmental glomerulosclerosis (FSGS), is progressive and eventually leads to end-stage renal failure. Defective cellular immunity is the cause of the associated immunodeficiency. Patients also exhibit typical phenotypic features, such as fine hair, a triangular face, a depressed nasal bridge, a bulbous nasal tip, microdontia, a short neck, a short trunk, hyperpigmented macules, and a protruding abdomen. In 2002, it was discovered that

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biallelic pathogenic variants in the SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, *subfamily A-like 1* gene (*SMARCAL1*) caused this syndrome (5).

Herein, we present a patient with SIOD who exhibited an atypical clinical presentation characterized by hypercalcemia and hypophosphatemia, resembling osteopenia of prematurity, along with elevated follicle-stimulating hormone (FSH) levels indicative of primary gonadal failure. Furthermore, we conducted a review of all genetically confirmed cases of SIOD, providing an occurrence ratio of clinical and laboratory findings in comparison with our patient.

Diagnostic Insights in Schimke Immuno-osseous Dysplasia

A one-and-a-half-year-old female was referred to the medical genetics outpatient clinic with disproportionate short stature and renal failure. She was the second child of a 33-year-old mother and 36-year-old father, both of whom were healthy. Although there was no consanguinity between her parents, they both came from the same small village. Family history was unremarkable. She was born at 30 gestational weeks with a birth weight of 945 g [-2.1 standard deviation score (SDS)] and birth length of 35 cm (-2 SDS), and was hospitalized in the neonatal intensive care unit (NICU) for three months due to prematurity. At that time, patent ductus arteriosus, patent foramen ovale, and atrial septal defect were detected by echocardiography. Her cranial ultrasonography (USG) was normal. She passed the hearing test and her ophthalmologic examination revealed no retinopathy of prematurity.

The timeline of the patient's medical history and diagnostic procedures, and results are shown in Figure 1. At the initial presentation, at the age of 7 months, she was referred to the pediatric endocrinology clinic for hypercalcemia. On physical examination, height was 58 cm (-3.9 SDS), weight was 4.5 kg (-4.4 SDS), and head circumference was 38 cm (-4.2 SDS), while mid-parental height was at -1 SDS. The dysmorphic examination revealed that she had fine and sparse hair, microcephaly, prominent forehead, synophrisis, upslanting palpebral fissures, malar hypoplasia, depressed nasal bridge, bulbous nasal tip, long philtrum, thin upper lip, retrognathia, everted lower lip, microdontia, posteriorly rotated and low set ears, anteverted ears, short neck, short trunk, hyperpigmented macules on the trunk, protruding abdomen, tapering fingers, and brachydactyly (Figure 2). External genitalia were normal.

The laboratory evaluation revealed the following results: calcium (Ca) of 11.6 mg/dL [normal range (NR) 8.7-11], PO_4 of 3.2 mg/dL (NR 5.0-7.8), alkaline phosphatase (ALP) of 1122 U/L (NR 116-450), 25-OH vitamin D of 24.4 µg/L (NR 30-100), parathyroid hormone of 6.2 ng/L (NR 15-65), creatinine (Cre) of 0.19 mg/dL (NR 0.0-0.42), magnesium of 2.4 mg/dL (NR 1.8-2.6), urinary Ca/Cre ratio of 0.4 mg/mg (NR 0.03-0.8), tubular phosphate resorption of 98% (NR 85-100), and tubular maximum for phosphate/glomerular filtration rate of 5.45 (NR 4.8-8). The laboratory and wrist X-ray were consistent with the osteopenia of prematurity and rickets, which were related to phosphate deficiency. Phosphate replacement therapy was initiated and continued for five months, resulting in the normalization of biochemical parameters and improvement in radiological findings (Figure 2).

The patient had not demonstrated catch-up growth and exhibited poor growth velocity. She began to sit independently at 10 months old and started walking at 18 months. She also had her first words at 18 months.

Endocrinological evaluation of short stature at the age of 19 months revealed low levels of insulin-like growth factor-1 (IGF-1) (-2 SDS) and IGF binding protein-3 (IGFBP-3) (-2.4 SDS) along with an elevated FSH level (24 U/L). Biochemical parameters, including renal function and celiac-associated antibodies showed no abnormality. The bone age was consistent with the chronological age (Figure 2a.3). Turner syndrome was considered as a potential diagnosis due to the presence of short stature, an ectopic kidney, and elevated FSH levels. However, karyotype analysis and array-CGH testing demonstrated a 46,XX karyotype with no deletion or duplication. Furthermore, a growth hormone (GH) stimulation test with glucagon showed a high basal GH level (17.6 ng/mL) and an exaggerated peak GH response (32 ng/mL) with consistently elevated GH levels throughout the test. One year after the first elevated FSH measurement, FSH was 14.1 U/L, luteinizing hormone was <0.2 U/L and estradiol was 13.3 ng/L at the age of 2.5 years. The first The first elevation in thyroid-stimulating hormone (TSH) was detected at age 3 years and had worsened by the age of 3.5 years, with levels of 6.44 mIU/L and 12.80 mIU/L (NR 0.70-5.97), respectively.

Furthermore, USG of the urinary tract showed a cross-fused ectopic left kidney at initial evaluation. The patient was referred to pediatric nephrology. At nephrological follow-up of the patient, she had experienced recurrent urinary tract infections. She was diagnosed with nephrotic syndrome at the age of 1.5 years. She was treated with albumin infusions, diuretics, angiotensin-converting enzyme inhibitors, and corticosteroids but failed to respond. The patient presented with severe decompensation of nephrotic syndrome that required peritoneal dialysis for 10 months and she received

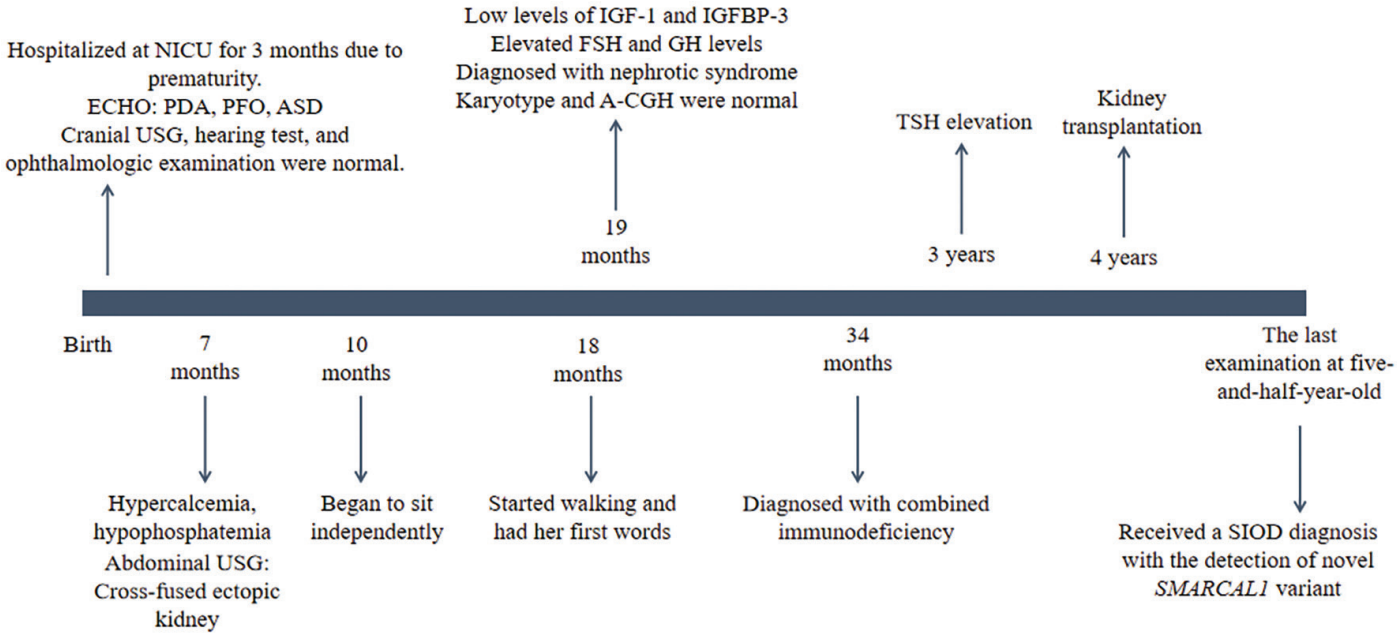

Figure 1. The timeline of the patient's medical history and diagnostic procedures, and results

NICU: neonatal intensive care unit, IGF-1: insulin-like growth factor-1, IGFBP-3: IGF binding protein-3, TSH: thyroid stimulating hormone, USG: ultrasonography

a kidney transplant from her uncle when she was 4 years old. The patient was not deemed to need a renal biopsy since she had received a genetic diagnosis. The patient's initial blood pressure measurement was 92/54 mmHg, while at the last measurement, it was 118/84 mmHg (NR 105/63).

Nephrological evaluation revealed hemoglobin 15.6 g/dL (NR 12-16), hematocrit 46.7% (NR 36-48%), mean corpuscular volume 80.9 fL (NR 80-100), white blood cell $7.3x10^3$/µL (NR 4.5-11$x10^3$), mild lymphopenia of $1x10^3$/µL (NR 2.1-7.8$x10^3$) (5), platelet count $293x10^3$/µL (NR 150-450$x10^3$), hypoalbuminemia with an albumin of 2.2 g/dL (NR 3.4-5.4), high total cholesterol level of 382 mg/dL (NR < 200) and hypertriglyceridemia of 253 mg/dL (NR < 150). Urinalysis demonstrated 3+ proteinuria. Spot urine protein was 1043 mg/dL (NR 0-10), urine Cre 37 mg/dL (NR 20-275), and the urine protein/Cre ratio was calculated as 28.1 mg/mg Cre (NR < 0.5). Serum complement C3 level was 197 mg/dL (NR 80-120) and C4 level was 59 mg/dL (NR 10-40).

Although no obvious bone dysplasia was initially detected on hand X-rays, SIOD was considered as a potential diagnosis after the development of nephrotic syndrome. However, a more comprehensive skeletal survey revealed certain skeletal abnormalities, including ovoid vertebral bodies and shallow acetabular fossae with laterally displaced femoral heads (Figure 2c, 2d). Notably, there were no epiphyseal changes observed. Subsequent X-rays taken at the age of five-and-a-half-years displayed mild platyspondyly, metaphyseal widening of the long bones, and osteopenia with metaphyseal sclerosis (Figure 2d.1).

Immunological analyses at the age of 34 months revealed mild lymphopenia of $1.3x10^3$/µL, low serum IgG of 157 mg/dL (NR 604-1921), but normal levels of IgA 58 mg/dL (NR 26-228), and IgM 167 mg/dL (NR 71-235). Serum IgE level was moderately elevated as 149 IU/mL. The patient also had anemia and thrombocytopenia. Responses to protein antigens, including hepatitis B, mumps IgG were negative, and measles, varicella, and rubella IgG were positive. Lymphocyte subgroup analysis showed severe CD3+ T, CD4+ T, and CD8+ T lymphopenia accompanied by decreased naive and increased memory CD4+ and CD8+ T cells. Elevated CD19+ B lymphocyte numbers were detected, while CD16+ CD56+ NK cell numbers were normal. Although the patient had no history of recurrent infections other than recurrent urinary tract infections, concomitant prophylactic antibiotics and immunoglobulin infusions were administered every 3 weeks with a diagnosis of combined immunodeficiency. The clinical findings of the patient are summarized in Table 1.

After obtaining informed written consent from the patient's parents, DNA was isolated from her peripheral blood using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Clinical

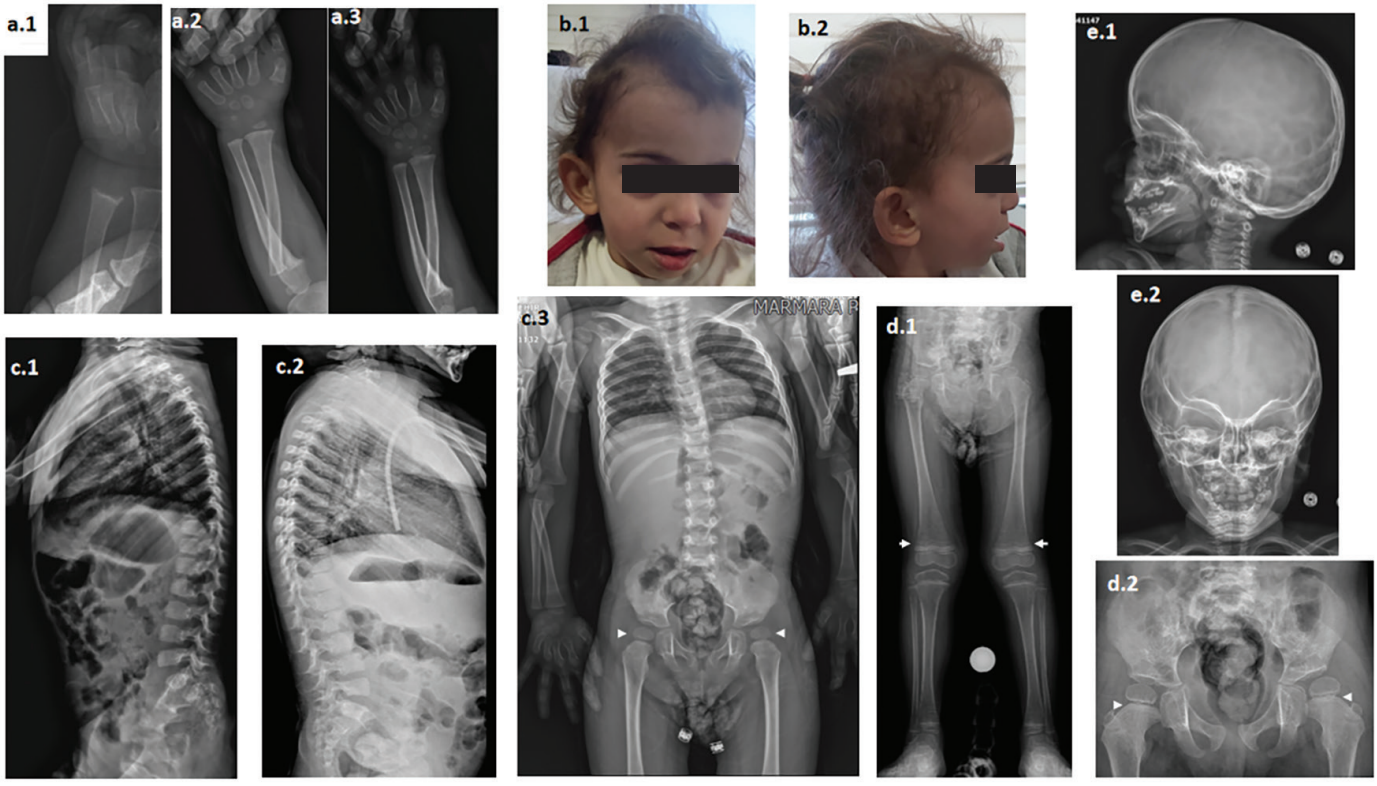

Figure 2. a) Hand radiograph at 7 months of age reveals osteopenia and cupping of distal ulna (a.1). By the age of 19 months, complete resolution of rickets findings and osteopenia, and, bone age is consistent with that of a 2-year-old (a.2). At the age of 32 months, bone age is 3-year-old (a.3). b) Facial pictures display various dysmorphic features including; fine and sparse hair, microcephaly, prominent forehead, synophrisis, upslanting palpebral fissures, malar hypoplasia, depressed nasal bridge, bulbous nasal tip, long philtrum, thin upper lip, retrognathia, everted lower lip, posterior rotated and low set, anteverted ears, short neck. c) Spinal radiographs shows mild ovoid vertebra (c.1) with mild lumbar scoliosis (c.3) at the age 33 months, and, mild platyspondyly at the age of five-and-half-years. d) At the age of five-and-half-years, long leg radiographs demonstrate mild coxa valga deformity with metaphyseal widening of long bones, resembling an Erlenmeyer flask deformity, metaphyseal sclerosis (arrow) and osteopenia (d.1), shallow acetabular fossae and lateral displacement of capital femoral epiphysis (Arrowhead) (d.2), which is more pronounce compared to the finding at 33 months of age (c.3). There is no obvious epiphyseal involvement. e) Cranial radiographs show J-shaped Sella turcica

exome sequencing (CES) was performed via next-generation sequencing (Illumina Nextseq 500) using Sophia Clinical Exome Solution V2. Data were analyzed through the Sophia DDM-V4 platform. Non-synonymous (missense, nonsense, in-frame, frameshift) variants with minor allele frequency less than 1.0% in population studies (1000 Genomes-1000G, Exome Aggregation Consortium database-ExAC, and Genome Aggregation Database-gnomAD) were filtered. American College of Medical Genetics and Genomics (ACMG) criteria were also applied. Retained variants were searched for in ClinVar and the Human Genome Mutation Database (HGMD). Segregation analyses were also performed via Illumina Nextseq 500. Molecular analysis revealed a novel homozygous c.2422_2427+9delinsA (NM_001127207) variant in the *SMARCAL1* gene (Figure 3a). It was localized at the donor site of the 15th exon. This variant was not reported in Clinvar and HGMD. According to ACMG criteria, this variant was pathogenic (PVS1, PM2, PP3). Segregation analysis revealed that her parents were heterozygous for this variant (Figure 3b, 3c). Given the presentation at the age of seven months with hypophosphatemia requiring phosphate replacement, genetic causes of hypophosphatemia were considered. No variant was detected in the customized panel containing *PHEX, DMP1, FGF23, ANKH, CYP27B1, CYP2R1, VDR, CYP3A4, CYP24A1, SLC34A1, SLC34A3, KL, GALNT3, CLCN5, SLC2A2, OCRL, FAM20C, FGFR1, ABCC6, ALPL, EXT1, SLC9A3R1, AVPR2* genes selected from CES.

Discussion

SIOD is a pleiotropic disorder with typical clinical findings of short stature, SED, immune deficiency, renal involvement, and typical dysmorphic findings. However, the diagnosis of SIOD can be challenging before the occurrence of renal

Table 1. A comprehensive overview of all reported findings with *SMARCAL1* variant in the literature along with the details of our case

	Patients in literature	Present case	Reference
Sex	71 M/54 F	F	
Age (year)	9.3 mean	5.5	
Growth and endocrine features			
Short stature, disproportionate	55/55 (100%)	+	1, 4, 7-19, 22-25, 26-41
Intrauterine growth retardation	68/72 (94.4%)	+	1, 4, 7-15, 18, 19, 22-24, 27, 30, 32, 37-39, 41, 43, 44
Elevated TSH	31/61 (50.8%)	+	1, 4, 9, 10, 14, 15, 22-24, 31-34, 42
Head and neck			
Short neck	20/22 (90.9%)	+	7, 9-11, 13, 14, 16, 18, 23, 24, 29, 30, 32, 33, 37, 39, 42, 44
Corneal opacities	4/9 (44.4%)	-	10, 24, 32
Depressed nasal bridge	48/55 (87.2%)	+	1, 6, 7, 9, 10, 12, 15, 16, 22, 30, 32, 37, 38, 40, 44
Bulbous nasal tip	48/55 (87.2%)	+	6, 7, 9, 10, 12, 14, 16, 22, 30-32, 37, 38, 40, 44
Dental anomalies (including microdontia)	13/19 (68.4%)	+	9, 10, 13, 14, 29, 31, 32, 38, 44
Hyperpigmented macules	46/55 (83.6%)	+	6, 7, 10-14, 16, 17, 23, 24, 30-32, 38, 40, 44
Fine hair	7/9 (77.7%)	+	1, 16, 30-32, 40
Abdomen			
Protruding abdomen	16/18 (88.8%)	+	7, 9, 10, 14, 16, 22-24, 30, 32, 38, 39, 42, 44
Renal			
Nephrotic syndrome	81/83 (97.5%)	+	1, 6, 8-19, 21-24, 26-31, 33, 35-39, 42, 44
Focal segmental glomerulosclerosis	55/65 (84.6%)	N/A	1, 4, 9-13, 15, 17-19, 22, 23, 28, 29, 33, 35-39, 44
Perihilar mesangial deposition of proteinaceous material	2/2 (100%)	N/A	10, 29
Renal failure	39/49 (79.5%)	+	10-15, 17-19, 21, 24, 26, 27, 31, 33–35, 37, 38, 40, 43
Hypertension	23/28 (82.1%)	+	10-16, 19, 26–29, 33, 38, 40, 42, 44
Proteinuria	84/84 (100%)	+	1, 4, 6-19, 21-24, 26-39, 42, 44
Skeletal			
Spondyloepiphyseal dysplasia	52/55 (94.5%)	-	6, 12, 13, 17, 19, 22, 23, 26, 29, 30–32, 35–39, 41–44
Osteopenia	6/8 (75%)	+	9, 12, 14, 18, 42
Lumbar lordosis	6/7 (85.7)	-	10, 13, 18, 22, 37
Platyspondyly	15/20 (75%)	+	7, 10, 12-14, 18, 22, 24, 29, 30, 32, 42, 43
Ovoid vertebral bodies	4/8 (50%)	+	7, 12, 29, 30
Thoracic kyphosis	3/6 (50%)	-	10, 23, 39
Short, broad iliac bones	3/3 (100%)	-	1, 22, 43
Slanted acetabular roofs/Shallow acetabular fossae/Small capital femoral epiphyses/Laterally displaced femoral heads/Hip dysplasia	28/30 (93.3%)	+	10, 12-15, 18, 21-24, 26, 27, 29–32, 35, 38, 42–44
Neurologic			
Normal intelligence	15/19 (78.9%)	+	1, 7, 8, 10, 11, 13, 14, 19-32, 37, 39, 43, 44
Motor delay	18/50 (36%)	-	4, 9, 16, 19, 24, 31, 41
Transient ischemic attacks	20/33 (60.6%)	-	10-15, 18, 19,27, 28, 31, 34, 38, 44
Moyamoya	4/11 (36.3%)	-	12, 13, 15, 18
Cerebral infarcts	44/94 (46.8%)	-	4, 6, 10-12, 18, 19, 24, 26, 29, 33, 34, 38, 44
Hematology			
Neutropenia	27/51 (52.9%)	-	6, 7, 10, 12, 16, 17, 19, 24, 35, 38, 40, 44
Lymphopenia	69/79 (87.3%)	+	1, 6, 7, 10-13, 16-19, 24, 28, 32, 35, 37–41, 44
Thrombocytopenia	19/46 (41.3%)	+	6, 10, 12, 16, 18, 19, 38, 40
Anemia	22/45 (48.8%)	+	6, 10-13, 16-19, 37, 38, 44

Table 1. Continued

	Patients in literature	Present case	Reference
Immunology			
Recurrent infections	65/104 (62.5%)	+	4, 6, 7, 10-13, 15-19, 21, 24, 29, 33, 36, 38, 40, 41, 44
Defective cellular immunity	8/8 (100%)	+	7, 8, 10, 16, 24, 29
T-cell deficiency	31/31 (100%)	+	1, 7-10, 13, 16, 18, 19, 23, 24, 26, 28, 29, 31–33, 35, 36, 38, 39, 41, 42
Decreased CD4+ and CD3+/CD4+ lymphocytes	24/24 (100%)	+	7-10, 13, 16-18, 23, 28, 29, 35, 36, 38, 39, 41, 42
Abnormal immunoglobulin levels	7/12 (58.3)	+	23, 24, 29, 32, 38, 41
Additional findings			
Fused crossed ectopic kidney	2/125 (1.6%)	+	19, 23
Hypercalcemia	0/125 (0%)	+	
Hypophosphatemia	0/125 (0%)	+	
Elevated FSH levels	0/125 (0%)	+	

TSH: thyroid stimulating hormone, FSH: follicle-stimulating hormone, M: F: male, F: female, N/A: non-applicable

findings, as observed in the presented case. The presented patient was born prematurely with intrauterine growth retardation (IUGR) and required prolonged hospitalization in the NICU. Subsequently, she presented with hypercalcemia related to the hypophosphatemia and needed prolonged (5 months) phosphate replacement. Genetic analysis for hypophosphatemia found no etiology, and she was accepted as having osteopenia of prematurity. Later follow-up demonstrated no catch-up growth and the presence of elevated FSH, along with an ectopic kidney, suggested Turner syndrome. However, further investigation, including a normal female karyotype and array-CGH testing did not confirm this diagnosis. Nephrological follow-up for the patient, focusing on her cross-fused ectopic left kidney and recurrent urinary tract infections, ultimately led to the early diagnosis of proteinuria and SIOD.

When we reviewed all published genetically-confirmed cases of SIOD, all cases exhibited short stature, and almost all (94.4%) had IUGR. In addition, proteinuria was a consistent feature in all cases, serving as the primary indicator leading to the diagnosis of SIOD, as observed in the presented case. Although short stature is a universal feature of SIOD, only a few patients have had their GH/IGF-1 axis evaluated. Almost all of the evaluated cases demonstrated normal GH levels on GH stimulation tests (7,8,9,10). Furthermore, when the patients were treated with GH, they responded poorly to GH treatment (4,6,11,12,13) even in the case of low GH levels at GH stimulation tests (14). The presented patient, however, showed high basal GH levels and an exaggerated peak GH response to stimulation, along with low IGF-1 and IGFBP-3 levels, indicative of GH resistance. When all reported patients were evaluated from this perspective, although most GH stimulation tests were described as normal (exact values not provided), at least one patient had GH levels similar to ours (9). However, IGF-1 and IGFBP-3 levels were not mentioned in any of the other patients. In light of the poor response to GH treatment, normal or high GH levels, and low IGF-1 and IGFBP-3 levels in our case, SIOD may be considered a condition of GH resistance related to the primary disease. Furthermore, the final height of patients with SIOD ranged from 110 to 165 cm for males and from 107 cm to 143 cm for females (6,15). Table 1 presents a comprehensive overview of all reported findings in the literature, along with the details of the presented case.

Primary gonadal failure and elevated FSH levels had not been previously reported in any SIOD patients. The FSH level was reported only once in a 14-year-old male patient and was found to be normal (9). However, cryptorchidism has been reported in five patients and may be a sign of hypogonadism (12,15,16,17,18). Furthermore, SMARCAL1 expression has been reported in fetal ovaries and testis (20). Therefore, pathogenic variants in this gene may plausibly lead to gonadal dysfunction. The reason for gonadal failure remaining unrecognized in SIOD could be due to the severity of renal or other systemic diseases and renal transplantation. More detailed laboratory studies are required in other cases of SIOD for further explanation of this issue. Unfortunately, although our patient had persistently elevated FSH levels, we did not measure anti-Mullerian hormone.

The other features we report for the first time here are hypercalcemia, hypophosphatemia and rickets in SIOD. However, these features may not be inherent to SIOD and could be a co-occurrence related to prematurity and insufficient phosphate intake.

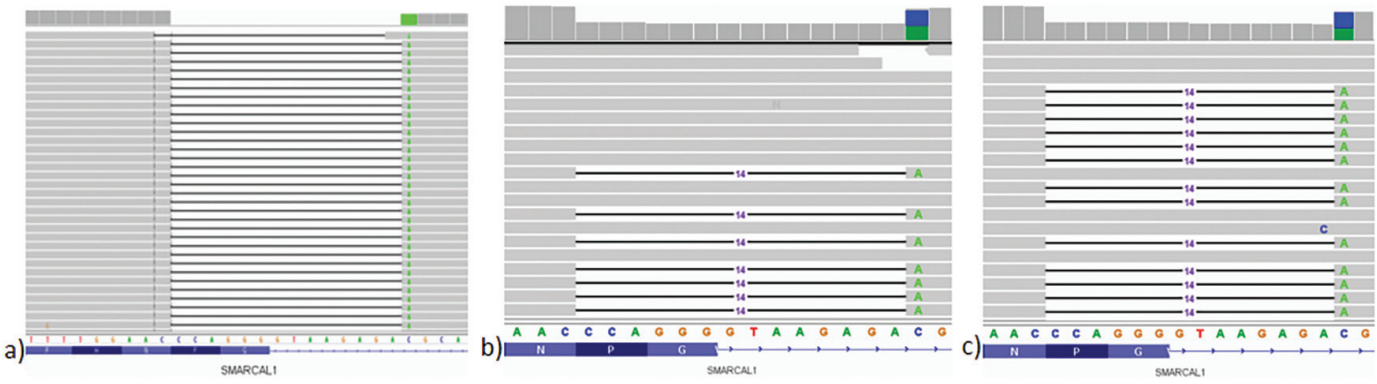

Figure 3. (a) Integrative genomics viewer visualization of novel homozygous c.2422_2427 + 9delinsA (NM_001127207) variant in the *SMARCAL1* gene detected in the patient. b, c) Her parents were heterozygous for this variant

The renal phenotype of SIOD is steroid-resistant nephrotic syndrome, which is progressive and often leads to end-stage renal failure. The main renal histopathology is FSGS; however, minimal glomerular lesions and podocytic infolding glomerulopathy have also been detected (21,22). Our patient exhibited an additional renal phenotype, namely crossed-fused ectopic kidney. Our literature review of a total of 125 genetically confirmed patients revealed crossed-fused ectopic kidney in two other SIOD patients (19,23). Furthermore, one patient with an ectopic kidney (24) and another patient with unilateral renal agenesis (10) have been reported. SMARCAL1 expression is known to be high in fetal kidneys and collecting ducts (23). Therefore, the association between *SMARCAL1* pathogenic variants and renal malformations is an expected finding. As suggested by Dekel et al. (23), this finding may have been overlooked in some reports. Evaluating SIOD patients for renal malformations will contribute to our understanding of whether renal malformations are incidental findings or a rare component of SIOD.

The clinical presentation or progression of SIOD is classified as either severe or mild (4). In severely affected patients, IUGR, neurologic manifestations such as cerebral infarction and transient ischemic attack, hypothyroidism, and bone marrow failure are observed more frequently (6). End-stage renal failure can occur at an earlier age in severely affected patients and most die within the first 15 years of life (6). The most common causes of death are severe infections, cerebral ischemia, and renal failure (4). The immune phenotype is predominantly characterized by lymphopenia and T cell failure, leading to recurrent infections, which are one of the most important causes of death (4). The presence of IUGR, early-onset renal failure, elevated TSH levels, and immunodeficiency in our patient, consistent with a severe phenotype, leads us to anticipate that the patient could experience neurological complications in the future. Moreover, the detection of a truncating variant in the presented patient indicated a severe phenotype. Patients with biallelic non-truncating variants tend to have a milder phenotype, although a strict genotype-phenotype correlation has not been found in SIOD patients. There was no significant difference observed between patients carrying truncating and non-truncating variants, particularly concerning the renal phenotype. However, it has been demonstrated that patients with biallelic truncating mutations exhibit higher mortality rates than those with biallelic missense variants (4). Furthermore, different clinical severities have been observed in two siblings carrying the same variant, indicating intra-familial variability (11).

The *SMARCAL1* gene contains 18 exons and encodes a 954 amino acid protein. SMARCAL1 protein is highly conserved and belongs to the sucrose non-fermenting 2 (SNF2) family (25). It functions as an ATP-dependent chromatin remodeling protein involved in various biological activities, such as replication, transcription, and DNA damage response. The protein contains four functionally important domains: HepA-related protein 1 (HARP1, 226-303 amino acids), HARP2 (327-398 amino acids), helicase ATP-binding domain (445-600 amino acids), and helicase C-terminal domain (716-869 amino acids). As of June 25, 2023, the HGMD lists 150 pathogenic variants in the *SMARCAL1* gene, with the majority being truncating variants. SIOD-related variants are depicted in Figure 4 with many pathogenic variants located within two helicase domains, similar to the variant detected in our report.

In conclusion, we present a patient with SIOD who exhibited an atypical clinical presentation characterized by hypercalcemia and hypophosphatemia, resembling osteopenia of prematurity, along with elevated FSH levels indicating primary gonadal failure. We identified a novel, homozygous indel variant in the donor splice site of the

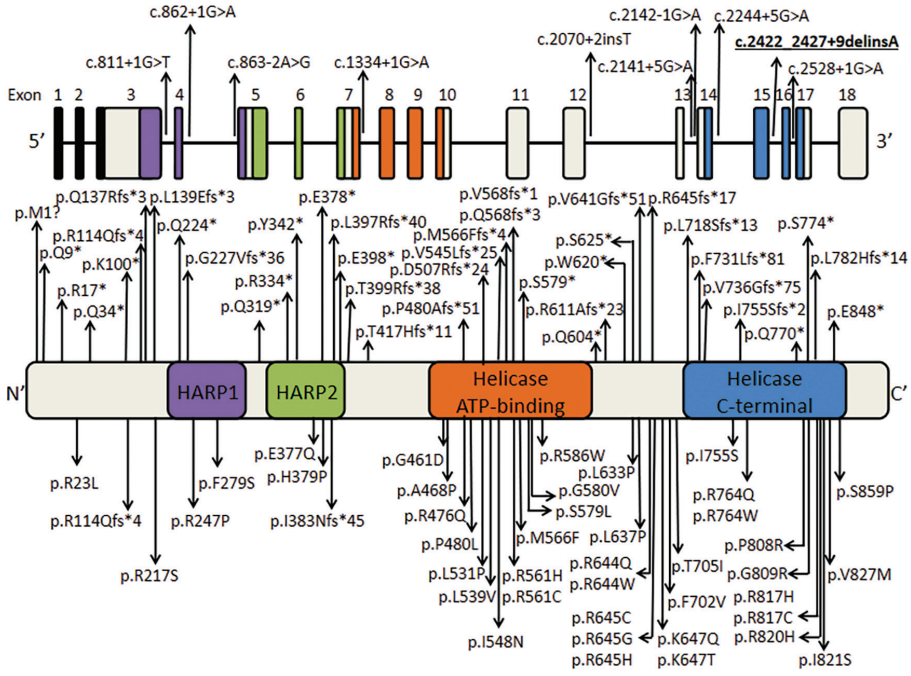

Figure 4. Schematic representation of *SMARCAL1* gene structure and its protein with the reported variants. The novel variant in the patient is indicated in bold. SMARCAL1 protein has 4 domains: HepA-related protein 1 (HARP1), HARP2, helicase ATP-binding and helicase C-terminal

SMARCAL1 gene. This report expands both the phenotypic and genotypic spectrum of SIOD.

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Footnotes

Authorship Contributions

Concept: Ceren Alavanda, Serap Turan, Design: Serap Turan, Data Collection or Processing: Serçin Güven, Mehmet Eltan, Sevgi Bilgiç Eltan, Asena Pınar Sefer, Serim Pul, Tülay Güran, Harika Alpay, Ahmet Arman, Pınar Ata, Analysis or Interpretation: Ceren Alavanda, Şenol Demir, Pınar Ata, Literature Search: Ceren Alavanda, Writing: Ceren Alavanda, Serap Turan.

Conflict of Interest: One author of this article, Serap Turan, is a member of the Editorial Board of the Journal of Clinical Research in Pediatric Endocrinology. However, she did not involved in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from different institutions.

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ORIGINAL ARTICLE

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Germ Cell Dysfunction is Universal in Adolescent Male Patients with β-thalassemia Following Earlier Successful Hematopoietic Stem Cell Transplantation

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What is already known on this topic?

Preparative conditioning regimens for hematopoietic stem cell transplantation (HSCT) which compose of primarily alkylating agents have gonadotoxic effect, potentially causing primary testicular insufficiency and abnormal spermatogenesis. However, all the studies were performed in matched-related donor and matched-unrelated donor HSCT.

What this study adds?

There are no reports on male gonadal functions following haploidentical HSCT regimen. Male patients with β-thalassemia after HSCT experienced universal spermatogenesis impairment and frequent Sertoli cell dysfunction but their Leydig cell function appears to be preserved. Comparing with match donor HSCT, frequency of impaired spermatogenesis tended to be higher in haploidentical HSCT, albeit not significant. This is likely due to limited sample size.

Abstract

Objective: To assess gonadal function in adolescent male patients with β-thalassemia who underwent earlier successful hematopoietic stem cell transplantation (HSCT).

Methods: Fifty-two male patients with β-thalassemia, aged ≥10 years, who had undergone HSCT ≥2 years previously were included. Clinical data, such as age, genital Tanner (GT) stage at HSCT and enrollment, and serum ferritin levels, were collected. Gonadal function was evaluated through measurements of serum luteinizing hormone, follicle-stimulating hormone (FSH), testosterone, inhibin B levels, and semen analysis.

Results: Age at enrollment and HSCT were 17 (10-31) and 9 (1-19) years, respectively. The duration from HSCT to enrollment was 7.5 (2-20) years. Of 52 patients, 46 (88%) exhibited Sertoli cell dysfunction. Thirty-one patients had relatively small testes for their GT stage, 34 of 44 with GT V had elevated FSH of ≥5 IU/L, and 20 of 49 with GT stages 2-5 had low serum inhibin B levels. None of the patients with GT stage 5 showed Leydig cell dysfunction or gonadotropin deficiency. Serum FSH ≥8 IU/L showed the best diagnostic accuracy for detecting oligo- and azoo-spermia. All 39 patients who underwent semen analysis had > 1 abnormal parameters. Having relatively small testes for GT stage and serum FSH ≥8 IU/L were associated with oligospermia or azoospermia ($p < 0.01$).

Conclusion: Male patients with β-thalassemia after HSCT experienced universal impaired spermatogenesis and frequent Sertoli cell dysfunction but their Leydig cell function appeared to be preserved. Male patients and/or their guardians should be informed of the high likelihood of future subfertility before HSCT.

Keywords: Gonadal function, spermatogenesis, male fertility, gonadotropin, inhibin B

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Introduction

Transfusion-dependent thalassemia (TDT) is an inherited hemolytic anemia disease, frequently found in many parts of the world. Chronic anemia necessitates repetitive red blood cell transfusions, leading to tissue iron overload. Both chronic anemia and tissue iron overload contribute to morbidities, including dysfunction of endocrine organs. The pituitary gland and testis are vulnerable to iron deposition, causing tissue damage and consequently resulting in a high prevalence of hypogonadism. Hypogonadotropic hypogonadism is commonly found in patients with TDT (1). The current curative treatment for TDT is hematopoietic stem cell transplantation (HSCT). Preparative conditioning regimens for HSCT which are composed primarily of alkylating agents, such as busulfan and cyclophosphamide, have a gonadotoxic effect, potentially causing primary testicular insufficiency and abnormal spermatogenesis (2,3,4,5,6). Recently, HSCT in patients with TDT has become more common, particularly haploidentical HSCT. Various conditioning regimens for HSCT exist, employing different types and doses of alkylating agents, which may have varying effects on male gonadal function. The selection of these regimens is based on the type of stem cell donor (matched-related, matched-unrelated and haploidentical) and the patient's age at transplantation. In previous studies, the prevalence of primary testicular dysfunction based on hormonal data in post-HSCT patients with thalassemia was 20-50%. However, all these studies were performed in matched-related donor (MRD) and matched-unrelated donor (MUD) HSCT (2,3,4,5). To the best of our knowledge, there are no reports of male gonadal function following haploidentical HSCT. Age and pubertal status at HSCT also significantly correlate with gonadal dysfunction (7,8). However, there are limited data regarding gonadal function, especially spermatogenesis, and the prognostic factors in male patients with β-thalassemia, particularly β-thalassemia/HbE, following HSCT.

Methods

Study Design and Participants

This was a cross-sectional study. All surviving male patients with β-thalassemia, aged ≥10 years, who had undergone successful HSCT at least two years earlier at the Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, were eligible (n = 75). Patients currently using medications affecting gonadal function, such as tacrolimus and systemic glucocorticoids, or who had severe systemic illness at enrollment were excluded.

The enrolled participants received hematopoietic stem cell infusion from human leukocyte antigen- (HLA-) matched or HLA-haploidentical donors. According to the current clinical practice at our institute, HLA-MRDs are the first choice, followed by HLA-MUDs from national donor registries, and HLA-haploidentical donors from patient's family members. For the conditioning regimen before stem cell infusion, a combination of chemotherapy was administered, which comprised either busulfan and cyclophosphamide or busulfan and fludarabine. Later, the patients received immunosuppressive agents, a calcineurin inhibitor and either methotrexate or mycophenolate, to prevent graft-versus-host disease (GVHD). For HLA-haploidentical donors, patients would receive cyclophosphamide post-transplant to help control GVHD.

The clinical data, including age at HSCT, serum ferritin (SF) levels prior to HSCT, post-HSCT and at the time of enrollment, type of stem cell donor, type and dose of alkylating agents used in conditioning regimens during HSCT, and any complications of HSCT, were obtained by reviewing the medical records. This study was approved by Human Research Ethics Committee, Faculty of Medicine Ramathibodi Hospital, Mahidol University (decision no: MURA2022/149, date: 01.03.2023). Written informed consent was obtained from all patients and their parents.

Cyclophosphamide Equivalent Dose

This study employed the cyclophosphamide equivalent dose (CED) to quantify the exposure to various alkylating agents commonly administered to cancer survivors, which have shown a negative correlation with spermatogenesis impairment (9,10). In the context of HSCT, the alkylating agents used were busulfan and cyclophosphamide. Therefore, the CED was calculated using the following equation: CED (g/m^2) = 1.0 [cumulative cyclophosphamide dose (g/m^2)] + 8.823 [cumulative busulfan dose (g/m^2)] (10).

Gonadal Function Assessment

All physical examinations and laboratory assessments were performed between March 2022 and February 2023. Genital Tanner (GT) stage was assessed and testicular volume was measured using a Prader orchidometer by experienced pediatric endocrinologists (N.P., P.M.). Serum luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and inhibin B levels were measured. Patients who were able to ejaculate underwent semen analysis. Semen specimens were obtained freshly and collected in sterile containers and analyzed after liquefying within 30-60 minutes. Reports of semen analysis results were

referenced using the World Health Organization (WHO) Reference Values of Human Semen Characteristics (6th edition) (11). Semen qualitative abnormalities were defined as follows: low volume (< 1.4 mL), oligozoospermia (sperm concentration < 16 million/mL), azoospermia (absence of spermatozoa), total motility < 42%, progressive motility < 30%, teratozoospermia (abnormal sperm morphology with normal forms < 4%).

Primary testicular insufficiency was defined as the presence of any dysfunction of Sertoli cells, Leydig cells or germ cells. Sertoli cell dysfunction was indicated by a relatively small testicular volume for GT stage, compared with normal testicular volume of matched healthy boys i.e. testicular volume < 4 mL for GT stage 2, < 8 mL for GT stage 3, < 15 mL for GT stage 4 or < 20 mL for GT stage 5 (12,13), or elevated serum FSH ≥5 IU/L (95th percentile of normal) for GT stage 5 (14) or low serum inhibin B ≤60 pg/mL (5th percentile of normal) for GT stages 2-5 patients (15). Normal ranges of serum inhibin B for adolescent boys with GT stages 2-5 were from 60-330 pg/mL (5th to 95th percentile). The ranges were similar between GT stages 2 to 5 (15). Leydig cell dysfunction for GT stage 5 was defined as elevated serum LH ≥6.3 IU/L (95th percentile of normal) (14) with low testosterone < 326 ng/dL (5th percentile of normal) for GT stage 5 (14). Compensated Leydig cell dysfunction was defined as serum LH ≥6.3 IU/L with normal testosterone > 326 ng/dL. Germ cell dysfunction was defined as presence of at least one abnormal parameter according to the WHO criteria for semen analysis (16).

Patients with low serum FSH, LH, and testosterone levels were suspected of having gonadotropin deficiency. To confirm this, a gonadotropin-releasing hormone analog test (2-hour test) was performed using a subcutaneous injection of 0.1 mg triptorelin and serum LH and FSH obtained every 30 minutes during the test (17). Gonadotropin deficiency was diagnosed if peak serum LH response during the 2-hour test was lower than 9.74 IU/L (18). This cut-off value was shown to distinguish hypogonadotropic hypogonadism from constitutional delayed growth and puberty with sensitivity and specificity at approximately 80%.

Serum LH, FSH and testosterone levels were analyzed by chemiluminescent microparticle immunoassay, using Alinity i analyzer (Abbott, IL, USA). The lower limits of detection were 0.04 IU/L for LH, 0.02 IU/L for FSH, and 1.44 ng/dL for testosterone. Intra-assay and inter-assay coefficients of variation (CV) were 2.0-4.3% and 2.8-4.7% for LH, 1.8-2.2% and 1.9-2.7% for FSH, and 2.3-3.5% and 2.6-8.7% for testosterone, respectively. Serum inhibin B levels were measured by in-house sandwich enzyme-linked immunosorbent assay using inhibin β_B polyclonal antibody (Invitrogen™, MA, USA). Recombinant human inhibin β_B protein (Abcam, Cambridge, UK) served as the standard, with concentrations ranging from 4.88 to 5,000 pg/mL. The lower limit of detection was 5 pg/mL. Intra-assay CV was 2.0% (based on a single run in this study).

Statistical Analysis

Continuous variables are summarized as median (range). For comparisons, chi-squared tests were used for dichotomous outcomes, while t-tests and Mann-Whitney U tests were used for continuous outcomes. Bivariate analysis was performed using regression analysis. Spearman's correlation analysis was used to assess correlations between spermatogenesis impairment and contributing factors. A receiver operating characteristic (ROC) curve was generated to determine the area under the curve (AUC) for serum FSH level as a predictor of impaired spermatogenesis. All statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA). A p value of less than 0.05 was considered statistically significant.

Results

Clinical Characteristics

A total of 52 patients were enrolled in the study. Their median (range) age at enrollment was 17 (10-31) years, and median (range) age at HSCT was 9 (1-19) years. The median (range) duration from HSCT to enrollment was 7.5 (2-20) years. Fifty of 52 patients (96%) had β-thalassemia/HbE, while the remaining two patients had ß-thalassemia major. Both patients with β-thalassemia major had clinical characteristics comparable with those patients with β-thalassemia/HbE. Nearly half (n = 25) of the patients underwent haploidentical HSCT. Three patients (6%) have had chronic cutaneous GVHD at enrollment but none required systemic treatment. Seven of 52 patients (13%) had SF levels ≥1,000 ng/mL at enrollment, indicating moderate to severe iron overload (19). All patients were pubertal; 44 of 52 (85%) patients had GT stage 5, 6 of 52 (11%) had GT stage 4, and only 1 patient was in each GT stage 2 and 3. None had received testosterone replacement therapy (Table 1).

Gonadal Function (Sertoli Cell and Leydig Cell Function)

Sertoli cell dysfunction was identified in 88% (46 of 52 patients), as indicated by either testicular volume smaller than expected for their GT stage, or elevated serum FSH levels (≥ 5 IU/L) for patients with GT 5, or low serum inhibin B levels (≤60 pg/mL) for patients with GT stages 2-5. Leydig cell dysfunction for patients with GT stage 5 was not

identified in any patients. However, compensated Leydig cell dysfunction was found in 4 of 44 (9%) patients with GT stage 5. Gonadotropin deficiency was not identified in any patients (Table 1).

Semen Quality Assessment

Of 39 semen analysis specimens, all exhibited at least one abnormality in semen parameters. The most commonly affected parameters were sperm concentration and abnormal morphology (teratozoospermia). About half (51%)

Table 1. Clinical and hormonal characteristics and semen characteristics of 52 enrolled patients

Characteristics of 52 enrolled patients	Median (range) or n (%)[a]
Age at enrollment, years	17 (10-31)
Age at HSCT, years	9 (1-19)
Pubertal	52 (100)[a]
- GT 2	1 (2)[a]
- GT 3	1 (2)[a]
- GT 4	6 (11)[a]
- GT 5	44 (85)[a]
Duration from HSCT to enrollment, years	7.5 (2-20)
Type of thalassemia	
- ß-thalassemia major	2 (4)[a]
- ß-thalassemia/hemoglobin E	50 (96)[a]
Donor type	
- Matched-related donor	15 (29)[a]
- Matched-unrelated donor	12 (23)[a]
- Haploidentical	25 (48)[a]
CED, g/m^2	
- Matched-related donor	4.0 (3.4-4.6)
- Matched-unrelated donor	3.9 (3.2-4.6)
- Haploidentical	4.6 (3.5-6.0)
Chronic GVHD at enrollment	3 (6)[a]
Pre-HSCT SF, ng/mL	1,500 (49-7,166)
Post-HSCT SF, ng/mL	809 (110-5,510)
SF at enrollment > 1,000 ng/mL	7 (13)
Clinical and hormonal characteristics of testicular dysfunction	**n (%)**
Sertoli cell dysfunction	46 of 52 (88)
- Relatively small testicular volume for genital Tanner stage	31 of 50 (62)
- Serum FSH > 5 IU/L (GT 5)	34 of 44 (77)
- Serum inhibin B < 60 pg/mL (GT stages 2-5)	20 of 49 (41)
Leydig cell dysfunction	
- Serum LH > 6.3 IU/L with serum testosterone < 326 ng/dL (GT stage 5)	0 of 44 (0)
Compensated Leydig cell dysfunction	
- Serum LH > 6.3 IU/L with serum testosterone > 326 ng/dL (GT stage 5)	4 of 44 (9)
Gonadotropin deficiency	0 of 51 (0)
- Low serum LH, FSH and testosterone with low LH and FSH response to GnRHa stimulation test	
Semen characteristics of 39 enrolled patients	**n (%)**
Germ cell dysfunction	
- ≥ 1 abnormal semen analysis parameter	39 of 39 (100)
Low volume (< 1.4 mL/ejaculate)	20 of 39 (51)
Sperm concentration	
- Azoospermia	7 of 39 (18)
- Oligozoospermia	17 of 39 (44)
- Oligo- and azoospermia	24 of 39 (62)
- Normal sperm concentration (≥ 16 million/mL)	15 of 39 (38)
Teratozoospermia (normal forms < 4%)	27 of 29 (93)
Abnormal sperm motility	
- Total motility < 42%	2 of 32 (4)
- Progressive motility < 30%	3 of 32 (6)

[a]: number (%).

HSCT: hematopoietic stem cell transplantation, CED: cyclophosphamide equivalent dose, GT: genital Tanner, GVHD: graft-versus-host disease, SF: serum ferritin, LH: luteinizing hormone, FSH: follicle stimulating hormone, GnRHa: gonadotropin releasing hormone analog

had low semen volume. Azoospermia was identified in 18%, oligozoospermia in 44% and normal sperm concentration in 38%. Of the specimens with detectable spermatozoa, 93% exhibited teratozoospermia (Table 1).

When comparing patients with normal sperm concentration to those with oligo- and azoospermia, no significant differences were observed in age at HSCT, CED, and pre-HSCT and post-HSCT SF levels. While there were more patients with SF levels ≥1,000 ng/mL at enrollment in the oligo- and azoospermia group compared to the normal sperm concentration group, this difference was not significant. The median (range) duration from HSCT to enrollment appeared longer in the normal sperm concentration group compared to the oligo- and azoospermia group, but this difference did not reach statistical significance (Table 2). However, significant differences were found in serum FSH and LH levels. Patients with oligo- and azoospermia had significantly higher FSH and slightly higher LH levels compared to those with normal sperm concentration [11.5 (3.2-22.9) vs. 4.8 (1.5-22) IU/L, $p < 0.001$ and 3.7 (1.9-11.8) vs. 3.2 (1.3-5.0) IU/L, $p = 0.04$, respectively]. There were no significant differences in serum testosterone and inhibin B levels between the two groups (Table 2). Interestingly, a high proportion of patients in these two groups had low serum inhibin B levels, 5 of 15 (33%) in normal sperm concentration and 9 of 24 (38%) in oligo- and azoospermia groups. Even among those with normal inhibin B levels, their values ranged within the lower quartile of the normal range (Figure 1).

Comparing the clinical, hormonal, and semen characteristics among different types of HSCT donors, no significant differences were found in patient age at enrollment among the three groups (Table 3). Patients who underwent haploidentical HSCT were the oldest compared to MRD and MUD. They also had had the shortest duration from HSCT to enrollment compared to MRD and MUD. Among the three groups, patients who underwent haploidentical HSCT received the highest CED compared to those who underwent MRD and MUD. There was no difference in median pre-HSCT SF levels among groups, but the haploidentical HSCT group had significantly higher post-HSCT SF levels. This group also had the highest number of patients with SF levels >1,000 ng/mL at enrollment compared to MRD and MUD. No significant differences were found in serum FSH, LH, testosterone, and inhibin B levels, even though the haploidentical HSCT group tended to have higher FSH and lower inhibin B levels (Table 3).

ROC curve analysis was used to determine the optimal serum FSH level for predicting oligo- and azoospermia. The AUC was 0.815 indicating good discriminatory power. A serum FSH level at 8 IU/L provides 73% sensitivity and 93% specificity for predicting oligospermia or azoospermia. Therefore, serum FSH 8 IU/L appears to be the optimal cut-off for identifying patients with these abnormalities (Figure 2).

Among patients with GT stage 5 who underwent semen analysis (n = 37), those with small testicular volume (<15 mL, 20 of 37 patients) had significantly higher frequency of oligo- and azoospermia, (80%, 16 of 20) compared to those with a testicular volume of >15-25 mL, (47%, 8 of 17), $p = 0.006$ (Figure 3).

Table 2. Characteristics of patients with normal sperm concentration and oligo- and azoospermia

Characteristics	Sperm concentration		p
	Normal (n = 15)	Oligo- and azoospermia (n = 24)	
Age at enrollment, years	21 (13-28)	17 (13-31)	0.07
Age at HSCT, years	9 (4-19)	9.5 (1-16)	0.63
Duration from HSCT to enrollment, years	10 (3-20)	7 (2-20)	0.07
CED, g/m^2	4.3 (3.8-5.2)	4.4 (3.3-6.0)	0.92
Pre-HSCT SF, ng/mL	1,432 (49-3,700)	1,656 (500-7,100)	0.50
Post-HSCT SF, ng/mL	660 (110-3,056)	943 (113-5,510)	0.71
Δ Pre-post HSCT ferritin, ng/mL	313 (64-2,426)	313 (-2,478-1,666)	0.95
SF >1,000 ng/mL at enrollment, n (%)	1 (6.6%)	5 (20.8%)	0.23
FSH, IU/L	4.8 (1.5-22)	11.5 (3.2-22.9)	<0.001
LH, IU/L	3.2 (1.3-5.0)	3.7 (1.9-11.8)	0.04
Testosterone, ng/dL	638 (291-1,406)	588 (350-1,369)	0.93
Inhibin B, pg/mL	99 (29-210)	77 (26-205)	0.52

Data were expressed as median (range).
HSCT: hematopoietic stem cell transplantation, CED: cyclophosphamide equivalent dose, SF: serum ferritin, FSH: follicle stimulating hormone, LH: luteinizing hormone

Table 3. Clinical, hormonal and semen characteristics among different donor-types HSCT

Characteristics	HSCT donor-types			
	MRD (n = 15)	MUD (n = 12)	Haploidentical (n = 25)	p
Clinical characteristics, median (range)				
Age at enrollment, years	18 (11-31)	18 (12-24)	17 (10-28)	0.08
Age at HSCT, years	6 (1-18)	6.5 (3-11)	11 (3-19)	0.003
Duration from HSCT to enrollment, years	10 (3-20)	9.5 (6-20)	5 (2-9)	< 0.001
CED, g/m^2	4.0 (3.4-4.6)	3.9 (3.2-4.6)	4.6 (3.5-6.0)	0.004
Pre-HSCT SF, ng/mL	1,374 (49-3,700)	2,053 (1,170-3,208)	1,500 (500-7,166)	0.44
Post-HSCT SF, ng/mL	630 (110-3,056)	557 (117-2,250)	1,176 (384-5,510)	0.02
Pre-post HSCT SF, ng/mL	314 (-91 to 2,210)	313 (43 to 1,968)	414 (-2,475 to 1,910)	0.77
SF at enrollment > 1,000 ng/mL, n (%)	1 (6.6)	0 (0)	7 (28)	0.04
Hormonal characteristics, median (range)				
Serum FSH, IU/L	7.4 (1.5-22.9)	5.0 (1.6-18.6)	10.7 (1.5-21.7)	0.20
Serum LH, IU/L	3.8 (1.3-9.8)	2.9 (1.4-5.8)	3.5 (0.4-11.8)	0.41
Serum testosterone, ng/dL	505 (267-1,406)	440 (118-1,140)	666 (39-1,369)	0.43
Serum inhibin B, pg/mL	90 (29-205)	84 (26-210)	61 (23-142)	0.07
Semen characteristics, n (%)				
Patients with semen analysis	14 (93)	8 (67)	17 (68)	-
Low volume (< 1.4 mL/ejaculate)	6 (43)	4 (50)	7 (41)	0.67
Sperm concentration				0.37
- Azoospermia	2 (14)	2 (25)	3 (18)	
- Oligozoospermia	4 (29)	3 (37.5)	10 (59)	
- Oligo- and azoospermia	6 (43)	5 (62.5)	13 (77)	
- Normal sperm concentration (≥ 16 M/mL)	8 (57)	3 (37.5)	4 (23)	
Sperm concentration, M/mL, median (range)	17.1 (0-136)	6 (0-63)	1.3 (0-149)	0.66
Teratozoospermia (normal forms < 4%)	12 of 12 (100)	3 of 4 (75)	12 of 13 (92)	0.23
Abnormal sperm motility				
- % motility, median (range)	59.5 (32-88)	69 (13-91)	65 (47-87)	0.66
- Total motility < 42%	1 of 12 (8)	1 of 6 (17)	0 of 14 (0)	0.34
- % progressive motility, median (range)	49 (27-85)	64 (13-89)	58 (43-75)	0.65
- Progressive motility < 30%	2 of 12 (17)	1 of 6 (17)	0 of 14 (0)	0.27

MRD: matched-related donor, MUD: matched-unrelated donor, HSCT: hematopoietic stem cell transplantation, CED: cyclophosphamide equivalent dose, SF: serum ferritin, FSH: follicle stimulating hormone, LH: luteinizing hormone, M: million

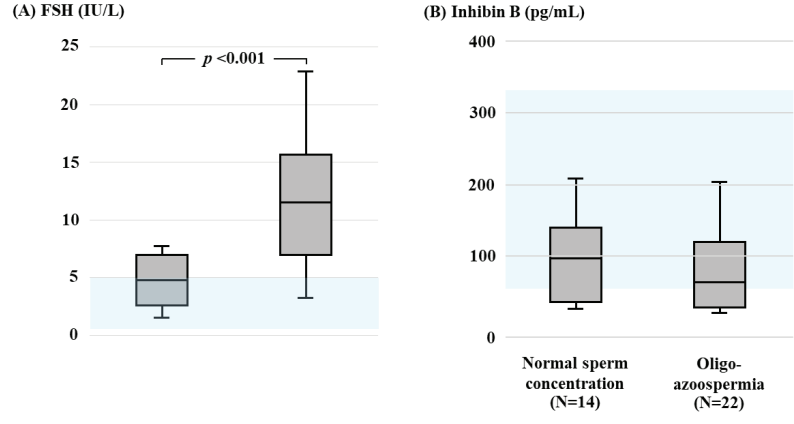

Figure 1. Comparison of serum FSH and inhibin B levels between GT stage 5 patients with normal sperm concentration and oligo- and azoospermia. In patients with oligo- and azoospermia, serum FSH levels were significantly higher than those with normal sperm concentration [11.5 (3.2-22.9) vs. 4.8 (1.5-22) IU/L, p < 0.001, respectively] (A). Serum inhibin B levels were not different between the two groups [77 (26-205) vs. 99 (29-210) pg/mL, p = 0.52, respectively]. In these 2 groups, about 35% of patients had low serum inhibin B levels (< 60 pg/mL) and those with normal serum inhibin B, their levels fell in the lower quartile of normal (B). Shaded areas represent normal ranges of serum FSH (0.6-5 IU/L) (A) and serum inhibin B levels (60-330 pg/mL) levels (B) in healthy males with GT stage 5

FSH: follicle stimulating hormone, GT: genital Tanner

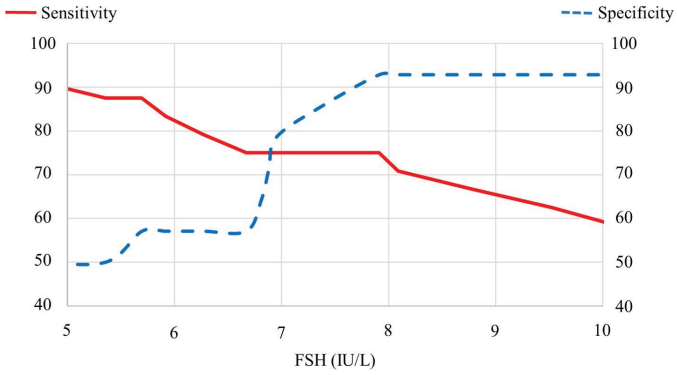

Figure 2. Sensitivity and specificity of serum FSH levels for predicting oligozoospermia and azoospermia. Serum FSH level at 8 IU/L gives the optimal cutoff for predicting oligozoospermia and azoospermia with sensitivity of 73% (solid line) and specificity of 93% (dash line)

FSH: follicle stimulating hormone

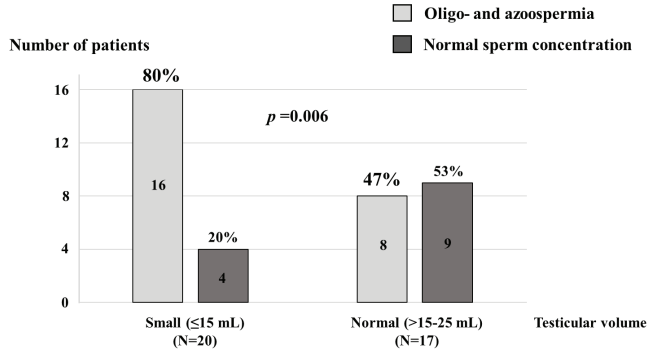

Figure 3. Compare spermatogenesis between patients with small and normal testicular size for genital Tanner stage 5. Patients with small testicular volume (≤15 mL) had a significantly higher frequency of oligo- and azoospermia than those with normal testicular volume (> 15-25 mL)

Discussion

This study provided comprehensive gonadal function assessment, including spermatogenesis, in a group of male patients with β-thalassemia/hemoglobin E who had undergone HSCT, and in particular, haploidentical HSCT. The main findings were that small testicular volume ≤15 mL among GT 5 patients and an FSH cut-off value (≥8 IU/L) were predictive factors for oligo- and azoospermia.

Germ cells are highly vulnerable to HSCT conditioning regimens as evidenced by all semen analysis specimens with at least one abnormal parameter and a high frequency of impaired spermatogenesis (62% oligo- and azoospermia) in 100% of the specimens. It is well-established that alkylating agents exhibit gonadotoxic effects and have the potential to disrupt normal spermatogenesis. In the present study, patients were administered a relatively low dose of alkylating agents (CED 3.3-6 g/m²), which contrasts with the higher doses typically used in cancer treatment (20). Consequently, the majority of patients in this study exhibited detectable sperm in their semen. However, it is noteworthy that almost all (93%) of the detectable sperm displayed abnormal morphology. This finding is consistent with a previous study in cancer survivors who underwent alkylating agent therapy without radiation exposure, where higher CED was linked to an elevated risk of azoospermia. Specifically, individuals who received CED > 10 g/m² were more likely to experience azoospermia, whereas those who received CED < 6 g/m² retained varying degrees of detectable sperm (19).

In recent years, haploidentical HSCT has been increasingly used due to limited availability of matched-donors. Haploidentical HSCT usually requires higher cumulative doses of busulfan than matched-donor HSCT (approximately 520 vs. 400-500 mg/m², respectively) whereas cumulative doses of cyclophosphamide are about 100 mg/kg in haploidentical HSCT and 0-200 mg/kg in matched-donor HSCT (21). Since there is greater gonadal toxicity when using busulfan than cyclophosphamide, CED in haploidentical HSCT was significantly higher than matched-donor HSCT. Thus, the frequency of impaired spermatogenesis tended to be higher in haploidentical HSCT, albeit not significantly. This is likely due to the small number of patients.

Impaired spermatogenesis has been documented in patients with TDT who exhibit intact hypothalamic-pituitary-gonadal (HPG) axis function (1,6). Chen et al. (1) highlighted that abnormal semen analysis findings in these patients were associated with the presence of iron deposits in the testes (22). In recent decades, T2-weighted magnetic resonance imaging (T2*-MRI) has become a key tool for diagnosing tissue iron overload in patients undergoing chronic blood transfusions (23). Despite its efficacy, SF, a conventional biomarker of iron deposit, is frequently used as a cost-efficient and readily available screening tool for assessing the likelihood of developing iron overload. A previous study demonstrated that SF levels ≥1,000 ng/mL displayed a high sensitivity (92%) and negative predictive value (91%) in discriminating between moderate to severe and mild iron overload as determined by T2*-MRI (2). In the present study, no significant association was found between oligo- and azoospermia and iron overload identified by SF levels ≥ 1,000 ng/mL. This absence of a significant association may be attributed to the limited number of patients, which may have constrained the statistical power of the study.

Previous studies have indicated that iron overload in β-thalassemia predisposes sperm to oxidative injury, leading to sperm DNA damage and subsequent subfertility (1,24). Interestingly, while pituitary function remains unaffected by iron loading, the testes are vulnerable and impacted by chronic iron overload. Rostami et al. (6) observed a rise in the frequency of oligo- and azoospermia in patients with TDT, increasing from 40% to 63% following HSCT. This finding aligns with our study, where the observed frequency was 62%. The higher occurrence of oligo- and azoospermia in patients with TDT following HSCT suggests an additional risk factor from exposure to alkylating agents during the transplantation procedure.

A greater frequency of normal sperm concentration was observed in post-HSCT patients following longer duration after HSCT (20). Our study also observed a trend towards a higher frequency of normal sperm concentration in post-HSCT patients with a longer duration since the procedure. This finding suggests the potential for spermatogenesis recovery, which aligns with a retrospective study reporting an 80% recovery rate in post-HSCT cancer survivors at seven years (25). However, our study was cross-sectional and cannot definitively assess the reversibility of spermatogenesis.

Spermatogenesis can be directly assessed by semen analysis. However, obtaining ejaculates from adolescents and young adults can sometimes be challenging. Patients frequently decline to masturbate in a private room during hospital visits. Therefore, predictive factors for oligo- and azoospermia obtained from physical examination and biochemical tests play a crucial role in clinical assessment. GT stage during pubertal progression relies primarily on testosterone effects, whereas testicular volume is mainly influenced by germ cell maturation. Consequently, patients with impaired spermatogenesis but relatively preserved Leydig cell function may exhibit smaller testes relative to their GT stage. This clinical observation is essential for predicting spermatogenesis impairment. A relatively small testicular volume for GT stage and an elevated FSH (≥8 IU/L) were identified as predictors for oligo- and azoospermia. Inhibin B, a hormone produced by Sertoli cells and post-pubertal germ cells (26), was not found to be associated with impaired spermatogenesis. This lack of association could be attributed to the low to low-normal serum inhibin B levels observed in the majority of patients.

Germ cells and Sertoli cells exhibited high vulnerability to HSCT treatment, while Leydig cell function remained comparatively preserved. Gonadotropin deficiency, a common occurrence in patients with TDT primarily caused by hypothalamic-pituitary hemochromatosis (27), was not observed in the present study. This finding could be attributed to either optimal iron chelation therapy or the possibility of reversible HPG function following HSCT. However, since we did not evaluate HPG function before HSCT, the reversibility of this function cannot be definitively proven by our results.

Study Limitations

We acknowledge several limitations in this study. First, a relatively small number of patients were recruited. Second, pre-HSCT data on puberty and serum gonadotropins to compare with post-HSCT data were unavailable. Third, healthy controls for comparison were not used. Fourth, the inability to evaluate semen analysis in adolescents who are uncomfortable or unable to masturbate significantly limited the study. Fifth, the absence of sequential semen analysis prevents us from demonstrating reversibility of spermatogenesis. Finally, there was a lack of longitudinal data on pubertal progression in patients. To enhance the robustness of future research in this area, a prospective study with a larger sample size would be beneficial. This design would provide more comprehensive information. A longitudinal study with sequential semen analysis would enable the assessment of spermatogenesis reversibility. In addition, collecting data on sequential pubertal progression and testicular maturation would improve our understanding of the impact of various factors on reproductive health outcomes.

Conclusion

Male patients with β-thalassemia/hemoglobin E who underwent HSCT during childhood or adolescence exhibited universal germ cell abnormalities and a high frequency of impaired spermatogenesis. Sertoli cell dysfunction was also frequent, while Leydig cell function remained preserved. Given these findings, patients or their guardians should be informed about the high likelihood of future subfertility and counseled on sperm cryopreservation prior to HSCT.

Ethics

Ethics Committee Approval: This study was approved by Human Research Ethics Committee, Faculty of Medicine Ramathibodi Hospital, Mahidol University (decision no: MURA2022/149, date: 01.03.2023).

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

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Footnotes

Authorship Contributions

Surgical and Medical Practices: Nuttha Piriyapokin, Pat Mahachoklertwattana, Preamrudee Poomthavorn, Usanarat Anurathapan, Concept: Pat Mahachoklertwattana, Design: Nuttha Piriyapokin, Pat Mahachoklertwattana, Preamrudee Poomthavorn, Wararat Chiangjong, Data Collection or Processing: Nuttha Piriyapokin, Pat Mahachoklertwattana, Preamrudee Poomthavorn, Usanarat Anurathapan, Wararat Chiangjong, Analysis or Interpretation: Nuttha Piriyapokin, Pat Mahachoklertwattana, Preamrudee Poomthavorn, Usanarat Anurathapan, Wararat Chiangjong, Literature Secrh: Nuttha Piriyapokin, Pat Mahachoklertwattana, Preamrudee Poomthavorn, Writing: Nuttha Piriyapokin, Pat Mahachoklertwattana, Preamrudee Poomthavorn.

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ORIGINAL ARTICLE

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Evaluation of Arrhythmia Risk in Children with Type 1 Diabetes Mellitus

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What is already known on this topic?

Children with type 1 diabetes mellitus (T1DM) are susceptible to cardiac arrhythmias and even sudden cardiac death.

What this study adds?

This study demonstrated that early identification of risk of arrhythmia in children with T1DM is achievable through routine electrocardiography. This technique is a cost-effective, non-invasive method compatible with daily activities. Implementing this approach may reduce mortality and morbidity in this high-risk, vulnerable population.

Abstract

Objective: Children with type 1 diabetes mellitus (T1DM) are susceptible to cardiac arrhythmias and even sudden cardiac death. The aim of this study was to explore the risk of arrhythmia among children with T1DM by assessing electrocardiographic (ECG) parameters.
Methods: Children diagnosed with T1DM, aged 10-18 years, and healthy children matched for age and gender were included. The ECG ventricular depolarization-repolarization parameters of both groups and the correlation of these parameters with length of time since diagnosis of T1DM, markers of metabolic control, and the presence of additional complications were evaluated.
Results: There were 165 children with type 1 diabetes and 154 controls in the groups, which were similar in for age, gender, weight, height, and body mass index. The median length of time since diagnosis of diabetes was 5 years. QT (maximum), QTc (minimum and maximum), QT and QTc dispersion, Tp-e (minimum and maximum), Tp-e dispersion, and Tp-e/QTc-maximum values were significantly higher in the diabetic group compared with controls, although QTc intervals were within normal ranges. No significant correlation was observed between ECG findings and length of time since diagnosis of T1DM, HbA1c levels, or complications.
Conclusion: As children with T1DM are at high risk of impaired ventricular depolarization and repolarization, they should undergo cardiac assessment and regular annual ECG monitoring.
Keywords: Children, diabetes, dispersion, arrhythmia, sudden death

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Introduction

Type 1 diabetes mellitus (T1DM) is a common metabolic disorder of childhood and as of 2021, there are more than 1.5 million children with T1DM worldwide (1,2). As the incidence of diabetes increases, the complications associated with the disease are also becoming more apparent. Young people with diabetes mellitus have been found to have higher risk for sudden cardiac death compared to those without diabetes (3). Cardiac autonomic neuropathy is one of the common complications of T1DM ranging from 12% to 76% in childhood and youth, contributing significantly to both mortality and morbidity (1,2,3,4). This dysfunction can adversely affect the regulation of heart rate, blood pressure, and other cardiovascular functions, leading to increased risks of life-threatening events, such as arrhythmias and sudden cardiac death but underlying mechanisms are still underdiagnosed (4,5,6,7). Recognizing and addressing cardiac risk factors early in the disease course would be important so that appropriate management strategies and interventions can be started that will reduce mortality and improve overall patient outcomes.

In the present study, the aim was to evaluate the arrhythmia risk in diabetic children by assessing electrocardiographic (ECG) ventricular depolarization and repolarization parameters compared to healthy matched peers. In addition, it was planned to investigate any correlation of these parameters with length of time since diagnosis of T1DM, hemoglobin A1c (HbA1c) levels (a recognized marker of metabolic control in T1DM), and the presence of additional diabetic complications.

Methods

This prospective, cross-sectional, controlled study was conducted between May 2023 and October 2023 in the Department of Pediatric Cardiology and Pediatric Endocrinology of Ankara Bilkent City Hospital. The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Human Ethics Committee of Ankara City Hospital, University of Health Sciences of Türkiye, with the decision numbered E2-23-3979, dated 25.04.2023. Written informed consent was obtained from all the participants.

Study Population and Inclusion and Exclusion Criteria

Children diagnosed with T1DM and aged 10-18 years, who were admitted to the outpatient clinics during the study period, were eligible to be included in T1DM group. Healthy age- and gender-matched children admitted for innocent murmur and without any known chronic disease were included as the control group. Diabetic patients with chronic systemic disease including systemic hypertension defined as previously described (8,9), chronic renal failure, congestive heart failure, thyroid disease, Cushing syndrome, and/or celiac disease, congenital or acquired heart disease (cardiomyopathy, operated or unoperated atrial septal defects, ventricular septal defects, patent ductus arteriosus, bicuspid aortic valve, pulmonary hypertension) which may lead ventricular hypertrophy or/and dilatation, atrioventricular conduction disorders and bundle branch blocks, and atrial-ventricular extrasystoles were excluded (n = 26, Figure 1). All patients' blood electrolyte levels and blood gas values were normal.

Office blood pressure measurements (OBPMs) were recorded for all children included during the study period. Measurements were conducted with patients seated, feet flat on the floor, arm supported at heart level, following a 5-minute rest period, using appropriately sized cuffs, in alignment with guideline recommendations (8,9,10,11,12). Using OBPMs, children eligible for the control group could be diagnosed as hypertensive if systolic blood pressure or diastolic blood pressure was at the 95th percentile or greater for age, sex, and height, measured on at least three separate measurements. Children diagnosed in this fashion were excluded (n = 4). Diabetic children whose OBPMs exceeded the 90th percentile for age, sex, and height, as measured on at least three separate measurements using automated devices, were excluded from the study and referred to pediatric nephrology for further evaluation (n = 3).

All participants were evaluated using transthoracic echocardiography. Age, gender, weight, height, body mass index (BMI), and for those with T1DM length of time since diagnosis, glycated HbA1c levels, and average HbA1c levels over the last two years were noted. Microalbuminuria was screened for using 24-hour urine collection and was defined as 30-300 mg/day (6). All children diagnosed with diabetes were evaluated for, diabetic retinopathy and other ocular complications through fundus examination. In the patient group, burning, tingling sensation and/or paresthesia, numbness, fatigue, cramping or pain in their lower extremities were investigated in order to assess evidence of peripheral neuropathy. Warmth and pinprick sensation in the feet were evaluated as physical examination (13).

Electrocardiography

All ECG were analyzed from the medical records of the patients with 12-lead at a speed of 25 mm/s and amplitude of 10 mm/mV with the patient lying down after at least five minutes of rest. The high-resolution computer software program (Adobe Photoshop CS2, Adobe System Incorporated,

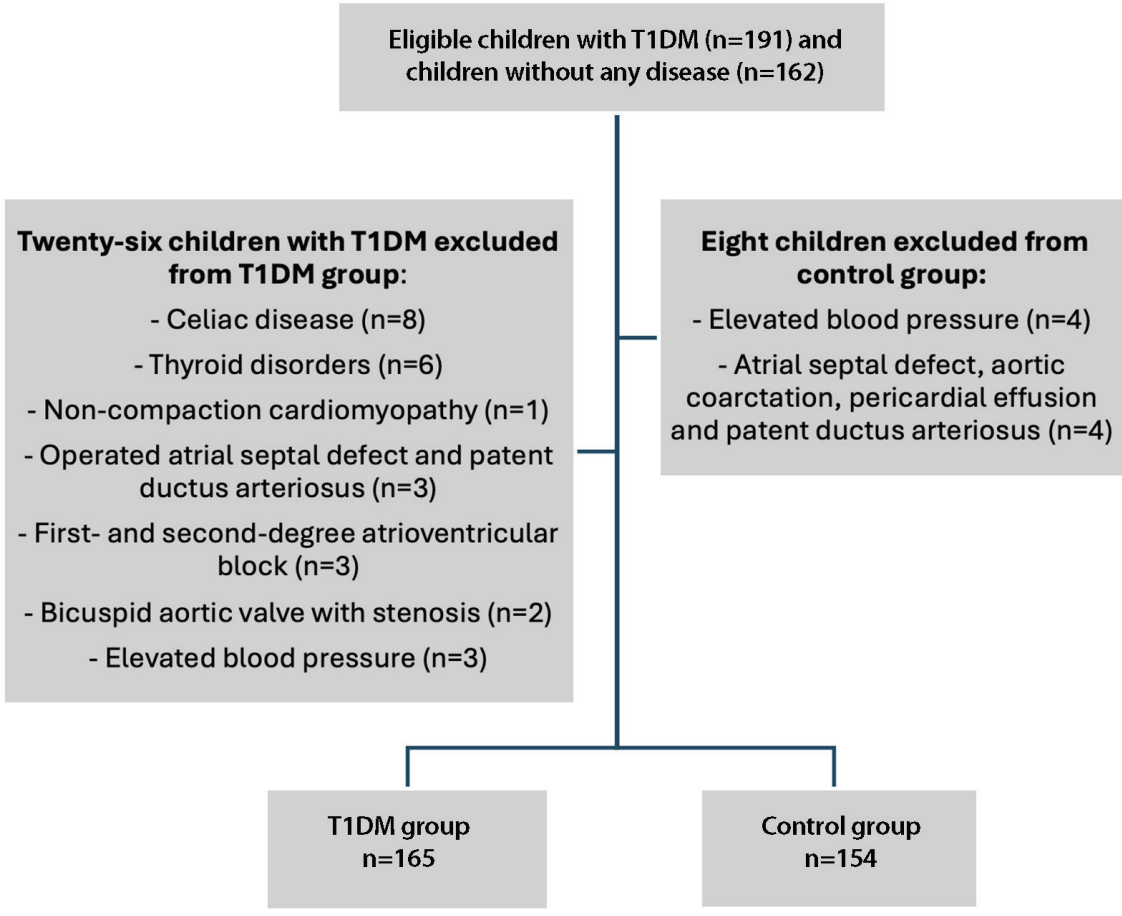

Figure 1. Flow chart of study population

T1DM: type 1 diabetes mellitus

San Jose, CA, USA) was used for the investigation of ECG results by a single blinded pediatric cardiologist. The measurement of the QT interval started from the onset of the QRS complex until the end of the T-wave. A discrete U-wave after the T-wave was excluded from measurement. The QT corrected for heart rate (QTc) duration was calculated using Bazett's formula ($QTc = QT/\sqrt{RR}$). QT and QTc dispersion (QTd, QTcd) was calculated as the difference between the maximum and minimum QT and QTc duration. Measuring from the peak of the T-wave to the end of the T-wave provided the Tp-e interval, which was defined as the intersection of the isoelectric line with the tangent to the downslope of the T-wave in precordial leads (14). The Tp-e duration was calculated by measuring the distance between the two points in the isoelectric line. The difference between the maximum and the minimum Tp-e in the precordial leads was the Tp-e dispersion (Tpe-d). Based on these measurements, Tp-e, Tp-e dispersion, and Tp-e/QTc ratio were calculated.

Statistical Analysis

Before the study, a power analysis was performed using the G*power program 3.1.9.4 version. Power analysis showed that 139 patients should be included in both groups at the 0.300 effect size with α: 0.05 and 80% power based on the comparison of QT (ms) between T1DM patients and controls in the study of Bezen et al. (15).

The data of from the present study were analyzed using Statistical Package for the Social Sciences, version 25.0 (IBM Inc., Armonk, NY, USA). Data are expressed as frequency and percentages. Normality analysis was carried out using the Kolmogorov-Smirnov test. The variables with or without normal distribution are presented as mean ± standard deviation or median (interquartile range; with 25-75th percentiles), respectively. Categorical variables were compared with the chi-square test. Numerical variables with and without normal distribution were compared using the independent samples t-test or Mann-Whitney U, respectively. Pearson and Spearman correlation analysis

was used to investigate possible correlations between ECG intervals and clinical variables. The statistical significance was set at $p < 0.05$.

Results

There were 165 children in the T1DM group and 154 matched individuals in the control group. The demographic characteristics of the participants of the study are shown in Table 1. The groups were similar in terms of age and gender.

Table 1. The demographic features of the patient and control groups

		T1DM group (n = 165) (mean ± SD)	Control group (n = 154) (mean ± SD)	p
Age (years)		13.72 ± 2.64	13.18 ± 2.41	0.522*
Gender, n (%)	Female	85 (50.1)	80 (51.9)	0.654[b]
	Male	80 (48.5)	74 (48.1)	
Weight (kg)		48.15 ± 14.59	46.5 ± 14.24	0.296*
Height (cm)		155.15 ± 15.86	153.37 ± 17.69	0.348*
BMI (kg/cm²)		20.57 ± 3.62	18.99 ± 2.93	0.056*
BMI SDS		1.01 ± 0.09	1.03 ± 0.13	0.17*
Length of time since diagnosis of T1DM [a]Median (IQR)		5.0 (3.2-14.1)	-	

*Student's t-test, [a]Data are expressed as median with interquartile range in parentheses, [b]Fisher's exact test.
BMI: body mass index, IQR: interquartile range, T1DM: type 1 diabetes mellitus, SDS: standard deviation (SD) score

Table 2. The comparison of electrocardiographic findings in the T1DM and control group

Electrocardiographic measurements (ms) (mean ± SD)	T1DM (n = 165)	Control group (n = 154)	p*
Heart rate (/min)	92 ± 16	89 ± 17	0.675
QT maximum	361.27 ± 33.60	351.38 ± 30.2	**0.006**
QT minimum	306.66 ± 28.39	312.16 ± 22.7	0.058
QTd	54.017 ± 16.74	39.22 ± 19.40	**< 0.001**
QTc maximum	410.44 ± 20.45	386.82 ± 22.18	**< 0.001**
QTc minimum	382.96 ± 19.98	375.60 ± 20.87	**0.011**
QTc-d	27.50 ± 9.61	11.21 ± 7.75	**< 0.001**
Tp-e maximum	68.11 ± 9.52	61.27 ± 9.02	**< 0.001**
Tp-e minimum	46.39 ± 8.33	42.63 ± 9.27	**0.017**
Tpe-d	23.91 ± 7.32	14.89 ± 5.32	**< 0.001**
Tp-e/QTc-maximum	0.19 ± 0.03	0.16 ± 0.03	**< 0.001**

*Student's t-test.
Tp-e: T-peak-to-end, Tpe-d: Tp-e dispersion, QTc: corrected QT interval, QT-d: QT interval-dispersion, T1DM: type 1 diabetes mellitus

The weight, height, heart rate, and BMI of the groups also did not differ significantly. The median length of time since diagnosis of T1DM was five years. None of the patients had cardiac complaints. Microalbuminuria was present in 7/165 (4.3%) of the T1DM patients. No one had retinopathy as target organ damage. There were no peripheral neuropathic symptoms and findings on physical examination in the patient group. The mean HbA1c level over the past two years was 8.75 ± 1.59%. Moreover, 59 (35.7%) of the patient group had an HbA1c level exceeding 9%.

The ECG findings of the groups are summarized in Table 2. All ECG intervals associated with depolarization and repolarization were notably higher in the T1DM group compared to the control group ($p < 0.05$).

Spearman correlation analysis between clinical variables and ECG intervals is presented in Table 3. A weak positive correlation was observed between Tp-e maximum and Tp-e minimum when the patient group was stratified based on HbA1c levels into HbA1c < 9 and HbA1c > 9 subgroups. (respectively; Rho = 0.205, p = 0.015; Rho = 0.206, p = 0.014). No correlation was found between other variables and the ECG intervals ($p > 0.05$).

Discussion

Young individuals with diabetes have a two to tenfold increased risk of sudden cardiac death compared with people without diabetes. Underlying mechanisms are multifactorial (3,7,16). Although numerous studies have focused on cardiovascular risk in adolescents and young adults with T1DM, the etiology of sudden cardiac death remains underdiagnosed in childhood, despite the heightened risks of mortality and morbidity.

The prolongation of QT and QTc intervals serves as independent predictors of high cardiovascular mortality in the general population (17,18). Children with T1DM have a sixfold increased risk for QT and QTc prolongation (19). Prolonged QTc interval and ventricular arrhytmias (VAs) have been identified as predictors of increased mortality in individuals with T1DM (3,20). In a study with a large number of people with T1DM (855 patients, 1710 controls), depolarization parameters were observed to be higher in people with T1DM, particularly among the youth. The increase was negatively correlated with the age (21). QTd and QTcd are the markers positively related to arrhythmogenic events and are associated withall-cause mortality in patients who have congestive heart failure (22). QTd has been recognized as a potential marker for increased risk of VAs and adverse cardiovascular events (22,23). People with T1DM exhibit alterations in electrophysiological parameters, including

Table 3. Correlation analysis of electrocardiographic intervals with clinical variables in the patient group (n = 165)

		QT-max	QT-min	QTd	QTc-max	QTc-min	QTcd	Tp-e max	Tp-e min	Tpe-d	Tp-e/QTc-max
LOT	Rho	-0.029	0.066	-0.171	0.005	-0.001	-0.026	-0.042	-0.087	-0.056	0.036
	p	0.732	0.438	0.053	0.949	0.995	0.761	0.621	0.308	0.509	0.676
HbA1c < 9 or > 9	Rho	0.037	0.125	-0.085	-0.045	-0.090	0.064	**0.205**	**0.206**	0.046	0.168
	p	0.666	0.141	0.317	0.596	0.289	0.450	**0.015**	**0.014**	0.593	0.058
HbA1c levels*	Rho	-0.022	0.125	-0.198	0.028	-0.003	0.003	-0.012	0.020	-0.037	-0.019
	p	0.797	0.141	0.19	0.741	0.972	0.975	0.888	0.813	0.660	0.823
MAU	Rho	-0.132	-0.126	-0.026	-0.057	-0.080	0.063	-0.136	-0.175	0.121	-0.084
	p	0.121	0.138	0.762	0.502	0.345	0.461	0.108	0.039	0.154	0.326

*The mean HbA1c of the previous two years.
HbA1c: hemoglobin A1c, Tp-e: T-peak-to-end, Tpe-d: Tp-e dispersion, QTc: corrected QT interval, QT-d: QT interval-dispersion, LOT: length of time since diagnosis of type 1 diabetes mellitus, MAU: microalbuminuria, max: maximum, min: minimum

QTd, which is indicative of ventricular depolarization and repolarization variability (24). Studies conducted with adult diabetic patients have shown an association between prolonged QTc interval and increased QTd with mortality (25,26). In pediatric patients with T1DM, certain studies have shown an elevation in QTcd and QTd, consistent with our findings (15,19,27). Although within normal limits, prolonged values of QT maximum, and QTc minimum and maximum were found in the T1DM group compared to the controls in the present study. In addition, when compared to the control group, the increased QTc and QTcd values in the patient group may indicate a predisposition to arrhythmia in these children. Certain studies involving pediatric patients with T1DM have reported elongation of atrial and ventricular depolarization parameters, irrespective of history of diabetic ketoacidosis (DKA) occurrence, length of time since diagnosis of T1DM, and metabolic status (15,27). Similarly, our findings revealed that these parameters associated with ventricular depolarization remained independent of length of time since diagnosis of T1DM, HbA1c levels, and diabetic complications, such as microalbuminuria, aligning with existing literature (24,27). This suggests that even in the early stages of T1DM during childhood, there may be a predisposition to arrhythmias, and this appears to be independent of metabolic status. Closer cardiac monitoring should be provided to this vulnerable group with a high risk of arrhythmia. Diabetic complications are less commonly observed in the pediatric age group. Therefore, it is imperative to conduct further research in order to elucidate this relationship and provide a more comprehensive understanding of the cardiac health of pediatric patients with diabetes.

The Tp-e and Tp-e/QTc ratios are valuable markers demonstrating transmural repolarization and prolongation of the Tp-e indicates risk for VAs, even in people with normal QTc (28,29). Elevated Tp-e/QT ratios are regarded as arrhythmogenic indices (14,30). In a recent study, depolarization parameters were found to be increased in T1DM patients of any age but repolarization parameters were only increased in young people with T1DM and this may be related to sudden cardiac death and the "dead in bed" syndrome (21). Furthermore, Eğil et al. (31) reported elevated Tp-e values in children with DKA. In the present study, even in the absence of ketoacidosis, Tp-e values in diabetic children were higher compared to non-diabetic peers. In a study with adult T1DM patients, repolarization parameters were found to be related to length of time since diagnosis of T1DM and HbA1c levels (32). In another study with adult patients with type 2 diabetes mellitus, Tp-e interval, and Tp-e/QTc ratio were found to be associated with severity of microvascular complications. Similar to the literature, we found higher Tp-e (minimum and maximum), Tpe-d and Tp-e/QTc-maximum values in the T1DM group. To the best of our knowledge, our study represents the most comprehensive research to date with the largest number of children with T1DM and largest control group size. However, direct comparison was not feasible as our patient cohort comprised pediatric individuals, with only microalbuminuria noted as a complication. Furthermore, the correlation between Tp-e values and HbA1c levels was notably weak, even given the relatively poor mean HbA1c in our cohort.

Study Limitations

One limitation of the study was the lack of long-term follow-up of patients in terms of arrhythmia. Another limiting aspect was the lack of correlation with 24-hour rhythm and blood pressure Holter monitoring in terms of atrial or VAs and blood pressure variability. HbA1c is the most pragmatic marker for assessing overall glycemic control in patients with type 1 diabetes; however, it does not reflect acute glucose excursions or indicate the severity of hypo/hyperglycemia (33). Another limitation of our study is the lack of continuous glucose monitoring for assessing glycemic control.

Conclusion

Considering that pediatric patients with T1DM often have a longer life expectancy and will therefore be expected to live with a greater length of time since diagnosis of T1DM compared to adults, and given their heightened susceptibility to impaired ventricular depolarization and repolarization along with associated cardiac arrhythmias, we assert that meticulous cardiological surveillance is essential. We advocate for routine ECG for all children diagnosed with T1DM, and annual ECG follow-ups during outpatient clinic visits, even in the absence of cardiac symptoms. This proactive approach would aim to mitigate the cardiac risks associated with T1DM in children. In addition, in cases of inadequate metabolic control, which is often a large proportion of the pediatric and especially adolescent population with T1DM, we recommend routine 24-hour rhythm Holter monitoring to facilitate the early detection of VAs, thereby potentially averting adverse outcomes and preserving lives.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Human Ethics Committee of Ankara City Hospital, University of Health Sciences of Türkiye, with the decision numbered E2-23-3979, dated 25.04.2023.

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

Footnotes

Authorship Contributions

Surgical and Medical Practices: Mehmet Boyraz, İbrahim İlker Çetin, İbrahim Ece, Concept: Yasemin Özdemir Şahan, Gönül Büyükyılmaz, Mehmet Boyraz, İbrahim İlker Çetin, İbrahim Ece, Design: Yasemin Özdemir Şahan, Gönül Büyükyılmaz, Mehmet Boyraz, İbrahim İlker Çetin, İbrahim Ece, Data Collection or Processing: Oğuzhan Doğan, Analysis or Interpretation: Oğuzhan Doğan, Literature Search: Yasemin Özdemir Şahan, Gönül Büyükyılmaz, Writing: Yasemin Özdemir Şahan.

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Is Automated Insulin Delivery System Therapy Safe and Effective in Children Under Seven Years Old?

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What is already known on this topic?

The evidence, experience and knowledge about the use of automated insulin delivery systems in patients under seven years of age are currently inadequate.

What this study adds?

The present study demonstrated for the first time that the MinimedTM 780G may be used in patients with type 1 diabetes mellitus under seven years of age by comparing the Minimed™ 780G with the Minimed™ 640G and multiple daily dose therapy.

Abstract

Objective: To evaluate the off-label use of the MiniMed™ 780G system in children under seven years old, as clinical outcomes in this age group are less well-established, despite the improvements in glycemic control seen with MiniMed™ 780G therapy.

Methods: Children under seven years old with type 1 diabetes using MiniMed™ 780G pump therapy were compared with children of similar age and gender using MiniMed™ 640G insulin pump therapy and multiple-dose insulin therapy with continuous glucose monitoring systems (CGMs). CGM metrics, total daily insulin (TDI) dose, and hemoglobin A1c (HbA1c) levels were evaluated retrospectively at baseline and at the 3rd, 6th, and 12th months.

Results: At the initiation of MiniMed™ 780G therapy, the mean age was 5.25 ± 1.22 years (range: 2.8-6.8 years), and the mean TDI was 10.12 ± 4.34 U/day (range: 4.5-17.0 U/day). The glucose management indicator and HbA1c remained lower in the MiniMed™ 780G group at the 3rd, 6th, and 12th months compared to baseline ($p = 0.009$ and $p < 0.001$, respectively). In the MiniMed™ 780G group, time above range (TAR) was significantly lower at the 3rd, 6th, and 12th months ($p = 0.018$, $p = 0.017$ and $p = 0.04$, respectively) while time in range (TIR) was higher at the 3rd, and 12th months ($p = 0.026$ and $p = 0.019$, respectively) compared to other groups. The coefficient of variation (CV) of the sensor glucose and HbA1c were lower at the 12th month ($p = 0.008$ and $p = 0.015$, respectively) compared to both other groups. No instances of ketoacidosis or severe hypoglycemic events were observed in any of the children during the follow-up period.

Conclusion: The absence of significantly higher levels of hypoglycemia compared to other groups at any time point, along with a significant decrease in TAR across all time points, a significant increase in TIR at the 3rd and 12th months, and a significant decrease in HbA1c and CV suggests that the MiniMed™ 780G system is both safe and effective for children under seven years old.

Keywords: Automated delivery system, diabetes, diabetes mellitus, endocrinology, predictive low glucose suspension

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Introduction

The incidence of type 1 diabetes (T1D) continues to rise, with 18% of new diagnoses occurring in children aged nine and younger (1). Treatment of T1D in young children is challenging since they often experience marked day-to-day and within-day variability in glucose levels and high variability in insulin requirements compared with older children with T1D (2). Current glycemic goals issued by the American Diabetes Association and the International Society of Pediatric and Adolescent Diabetes (ISPAD) recommend that young children maintain a hemoglobin A1c (HbA1c) level < 7.0% when possible but without risk of severe hypoglycemia (3). However, recent data from the SWEET study found that 69% of children with T1D under six years old have HbA1c higher than 7%, suggesting this age group would benefit from increased attention and interventions to support diabetes management (4). Diabetes management is complicated by rapid physical and neurological development, difficulty verbalizing thoughts and feelings, frequent and unpredictable physical activity, picky eating, and behavioral challenges and fears (5). The fear of nighttime hypoglycemia is common, and only a minority of young children's hypoglycemia appears to be recognized with self-monitoring blood glucose (BG) measurements (6). Apart from hypoglycemia, a 6-year longitudinal study suggested that gray and white matter volumes and cognitive scores are affected by hyperglycemia in early-onset T1D (7).

Diabetes technology, insulin pumps, and continuous glucose monitoring systems (CGMs) are evolving tools for diabetes management, and the use of such technologies in young children has increased markedly in recent years (8). Recent data from the T1D exchange indicate that CGM use in children under 6 years old has increased by 45% from 2016 to 2022 (9), and insulin pump use nearly doubled, with the highest use rates in the youngest patients (10). Hybrid closed-loop systems, which automatically adjust insulin delivery according to glucose levels aside from mealtime boluses, are relatively novel in young children. There are results from observational and randomized studies for MiniMed™ 780G systems in children over seven years old suggesting that an algorithm that automatically doses basal insulin based on sensor glucose (SG) levels improved time in range (TIR) without increasing or even decreasing the time spent below range (TBR) (11,12,13). Use of the MiniMed™ 780G improved glycemic control safely in a 12-week study period in toddlers and preschoolers, while simultaneously diminishing parental diabetes distress (14). In another study involving 11 patients aged between 2 and 6 years, the use of MiniMed™ 780G for 6 months resulted in an increase in TIR without any risk of hypoglycemia (15).

This is the first safety study comparing the off-label use of MiniMed™ 780G in children aged 2-7 years, diagnosed with T1D for at least one year and comparing with in patients using MiniMed™ 640G pump or multiple daily insulin (MDI) therapy plus CGM and comparing outcomes quarterly over a period of one year.

Methods

This retrospective, non-randomised study reviewed children between 2 and 7 years of age diagnosed with T1D for at least one year and who were on MiniMed™ 780G insulin pump, MiniMed™ 640G insulin pump or MDI + CGM therapy at least 12 months. HbA1c, insulin dose and CGM metrics of all the patients were downloaded from patient charts and Medtronic Carelink Personal Software, Libreview, and Dexcom Clarity Diabetes Management Software reports retrospectively. Clinicians and diabetes nurses monitored the safety of the treatment on a weekly basis (via phone call and WhatsApp), and pump settings [target glucose, insulin carbohydrate ratio, active insulin time (AIT)] were adjusted as required in the first month of pump initiation and monthly thereafter.

In our T1D clinic, all patients receive standardized training. T1D patients who start on MiniMed™ 780G or MiniMed™ 640G pump therapy receive complete carbohydrate counting training standardised according to ISPAD guidelines (16,17). In patients under 7 years of age the MiniMed™ 780G insulin pump is initially used in manual mode for 2 weeks followed by auto mode. The target BG is set to 100 mg/dL, and the active insulin time to 3 hours initially.

In MiniMed™ 640G insulin pump therapy, target BG is set to 100 mg/dL, low glucose suspend to 60 mg/dL, low and high alarm to 60 mg/dL and 180 mg/dL, and active insulin time to 3 hours at the beginning.

MDI + CGM patients receive the standardised education for CGM including the use of arrows, alarm settings and target glucose levels according to the CGMs consensus (18).

Outcomes measured included CGMs metrics according to the international recommendations (19). Safety endpoints included serious adverse events, such as severe hypoglycemia and diabetic ketoacidosis. Clinical and glycemic data are reported using descriptive statistics, expressed as mean (standard deviation) and/or median (interquartile range).

Ethical committee approval was obtained from the Ethics Committee of the Ege University (decision date: 24-5.1T/24, date: 23.05.2024). The study was conducted in accordance with the Helsinki Declaration, which was revised in October

2013. Informed consent was obtained from all parents or caregivers of children recruited in the study.

Statistical Analysis

Statistical Package for the Social Sciences statistics for Windows, version 28.0. was used for statistical analysis (IBM Inc., Armonk, NY, USA). A normality test was performed for the distribution. Shapiro-Wilk test was used in groups that included 30 or fewer children; otherwise, Kolmogorov-Smirnov test was used to determine distribution. One-way ANOVA test was used to compare MiniMed™ 780G, MiniMed™ 640G, and MDI + CGMs therapy groups in normally distributed variables. Kruskal-Wallis test was used to compare three groups' variables which were not distributed normally. Wilcoxon test was used in variables that were not distributed normally to compare pre-treatment (baseline) variables with the same at 3, 6 and 12 months. These were TAR, TIR, TBR, HbA1c, and other variables in the same group, and a paired t-test was used as a parametric equivalent. A $p < 0.05$ was accepted as statistically significant.

Results

Thirty-three children with T1D, eleven using the MiniMed™ 780G insulin pump, eleven MiniMed™ 640G insulin pump, and eleven using MDI + CGMs, were retrospectively analyzed. Among the 33 participants, 14 (42%) were female, the mean age was 98 ± 1.39 (2-6,8) years at the time of CGMs/pump initiation, and the duration of diabetes was 3.51 ± 1.54 years.

The mean age at the initiation of the MiniMed™ 780G, MiniMed™ 640G insulin pump was 5.25 ± 1.22 (2.8-6.8) years and 4.1 ± 2.13 (2.0-6.5) years, respectively. In the MDI + CGMs group, the mean age was 5.59 ± 1.19 (3.3-6.7) years.

In the MiniMed™ 780G group, SmartGuard™ usage in all children exceeded 85% after the initial two weeks of use in manual mode, as intended (93.73%, 96.45%, and 87.91% at 3, 6 and 12 months, respectively). The glucose management indicator (GMI) and HbA1c remained significantly lower within this group over time ($p = 0.01$ and $p < 0.001$, respectively); of note, marked decreases were observed within three months after auto-mode was switched on (Table 1).

Initially mean TDI dose was 10.6 ± 4.34 (4.5-17.6) U/day in the MiniMed™ 780G group, 13.9 ± 6.0 (3.5-24.2) in the MiniMed™ 640G group, and 14.8 ± 6.72 (4.5-25.0) in the MDI + CGMs group. In the MiniMed™ 780G group, TAR was lower at the 3rd, 6th, and 12th months ($p = 0.02$, $p = 0.02$ and $p = 0.04$, respectively) while TIR was higher at the 3rd and 12th months ($p = 0.03$ and $p = 0.02$, respectively). TIR increased by 8.4% (70% to 75.9%), TAR decreased by 10.4% (23.67% to 21.2%), and TBR decreased by 12.1% (3.3% to 2.9%) in twelve months of MiniMed™ 780G use

Table 1. Comparison between 0-3-6-12. month values extracted from MiniMed™ 780G

	MiniMed™ 780G				p
	0. month initiation "Manuel Mode"	3. month	6. month	12. month	
TAR (%)	23.67 ± 12.72	18.44 ± 7.33	20 ± 5.92	21.2 ± 8.93	0.91
180-250	17.64 ± 7.02	15.91 ± 5.13	17.18 ± 6.1	18.09 ± 5.43	0.56
> 250	4.73 ± 6.77	3.55 ± 2.70	6.82 ± 11.5	3.55 ± 3.39	0.41
TIR (%)	**70.00 ± 16.01****	**76.67 ± 7.11****	72.45 ± 15.61	75.90 ± 7.71	0.891
TBR (%)	4.67 ± 3.14	4.78 ± 2.86	3.64 ± 2.42	5.46 ± 2.13	0.27
54-70	2.82 ± 2.4	3.27 ± 2.01	2.91 ± 1.81	2.45 ± 1.29	0.50
< 54	0.45 ± 0.69	1.27 ± 2.1	0.64 ± 0.81	0.45 ± 0.52	0.42
CV (%)	36.13 ± 5.62	37.13 ± 4.35	36.46 ± 3.58	34.3 ± 2.14	0.38
GMI (%)	7.27 ± 1.19	6.56 ± 0.22	6.64 ± 0.21	6.71 ± 0.38	**0.01**
HbA1c (%)	8.8 ± 1.7	6.64 ± 0.47	6.71 ± 0.4	6.51 ± 0.38	**< 0.001**
SmartGuard™ (%)	-	93.73 ± 12.96	96.45 ± 3.45	87.91 ± 29.3	
TDI (U/day) (min-max)	4.5-17.6	8.2-20.3	7.7-25.9	9.3-33.2	0.08
AIT (hours)	3	3	3	3	
Meal per day	4.4 ± 1.1	4.8 ± 2	5.9 ± 1.4	6.1 ± 2.1	0.08
Amount of carb	128.6 ± 33.5	136.1 ± 45.4	154.8 ± 28.6	154.5 ± 33.2	0.09

Significant difference regarding GMI and HbA1c was observed during the one-year follow-up. The most remarkable improvement was between 0 to 3 months.
**Although TIR didn't show any significant increase when 12 months statistically examined together. It was significantly changed between initial time to 3rd month ($p < 0.001$).
AIT: active insulin time, CGMs: continuous glucose monitoring system, CV: coefficient of variation, GMI: glucose management indicator, MDI: multiple dose insulin treatment, TAR: time above range, TBR: time below range, TDI: total daily insulin dose, TIR: time in range, HbA1c: hemoglobin A1c, min-max: minimum-maximum

(Figure 1). The coefficient of variation (CV) of the SG and HbA1c were lower at 12 months ($p = 0.01$ and $p = 0.02$, respectively) (Figure 2). Moreover, average BG was lower at the 6th and 12th months ($p = 0.02$ and $p = 0.01$, respectively) compared to the other groups (Table 2). The other MiniMed™ 780G, MiniMed™ 640G, and CGM data are also shown in Table 2.

Discussion

T1D is associated with numerous morbidities that may significantly impact the lives of children. Initiating the most effective therapy as early as possible can mitigate complications (20). The MiniMed™ 780G insulin pump appears to be the most effective therapy for achieving this goal (21). However, there is a notable lack of studies investigating the effectiveness and safety of such devices

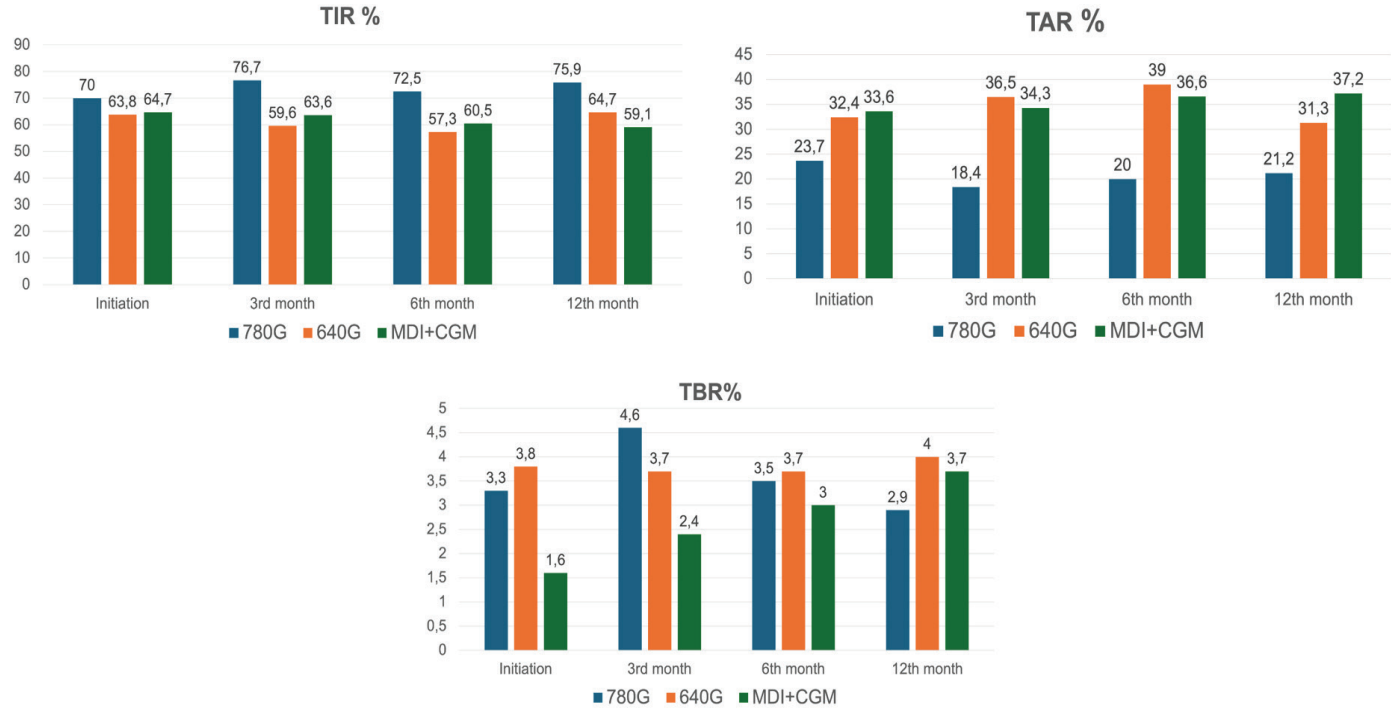

Figure 1. TIR, TAR, and TBR changes of the groups

780G: minimed™ 780G, 640G: minimed™ 640G, MDI + CGM: multiple dose insulin + continuous glucose monitoring, TAR: time above range, TBR: time below range, TIR: time in range

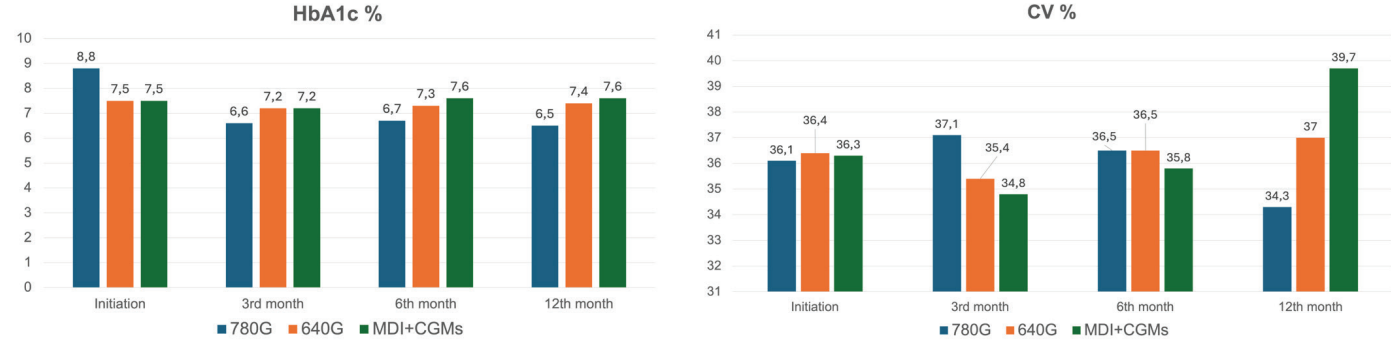

Figure 2. HbA1c and CV changes of the groups

780G: minimed™ 780G, 640G: minimed™ 640G, CV: coefficient of variation, MDI + CGM: multiple dose insulin + continuous glucose monitoring, HbA1c: hemoglobin A1c

Table 2. Comparison between MiniMed™ 780G and MiniMed™ 640G and CGMs + multi-dose SC insulin users

	0-month			p*	3-month		
	MiniMed ™ 780G	MiniMed ™ 640G	MDI + CGMs		MiniMed™ 780G	MiniMed™ 640G	MDI + CGMs
TAR (%)	23.7 ± 12.7	32.4 ± 12.7	33.6 ± 19.8	0.11	18.4 ± 7.3	36.5 ± 17.9	34.3 ± 15.5
180-250	17.6 ± 7	25.6 ± 10.5	21.6 ± 9.5	0.14	15.9 ± 5.1	28.1 ± 14.4	25.5 ± 7.6
> 250	4.7 ± 6.8	6.8 ± 5.3	12.3 ± 11.7	0.11	3.6 ± 2.7	8.4 ± 5.8	8.6 ± 8.8
TIR (%)	70 ± 16	63.8 ± 13.8	64.7 ± 19.7	0.24	76.7 ± 7.1	59.6 ± 17.5	63.6 ± 15.1
TBR (%)	4.7 ± 3.1	3.8 ± 2.4	1.6 ± 1.1	0.06	4.8 ± 2.9	3.7 ± 2.8	2.4 ± 2.3
54-70	2.8 ± 2.4	3.1 ± 1.9	2.4 ± 2.5	0.75	3.3 ± 2	3.1 ± 2.3	2.3 ± 2.1
< 54	0.5 ± 0.7	0.7 ± 0.8	0	**0.03***	1.3 ± 2.1	0.6 ± 0.9	0.1 ± 0.3
CV (%)	36.1 ± 5.6	36.4 ± 5.3	36.3 ± 5.5	0.98	37.1 ± 4.4	35.4 ± 5.7	34.8 ± 5.1
GMI (%)	7.3 ± 1.2	6.4 ± 2.2	5.2 ± 3.4	0.15	6.6 ± 0.2	7.1 ± 6.4	6.4 ± 2.4
HbA1c (%)	8.8 ± 1.7	7.5 ± 1.1	7.5 ± 1.3	0.22	6.6 ± 0.5	7.2 ± 0.8	7.2 ± 1.3
Average BG (mg/dL)	161.3 ± 25.8	166.4 ± 27.6	-	0.91	152.4 ± 24.5	173.7 ± 27.9	-
TDI (U/day) (min-max)	10.6 (4.5-17.6)	13.9 (3.5-24.2)	14.8 (4.5-25)	0.25	12.8 (8.2-20.3)	15.1 (4-25.6)	15.7 (5-26)
Amount of bolus insulin (U)	6.7 ± 2.9	9.2 ± 4.5	-	0.18	8 ± 2	10.4 ± 4.9	-
Auto-correction insulin (U)	-	-	-	-	1.1 ± 0.9	-	-
Basal insulin (U)	4 ± 2.7	4.2 ± 2	-	0.55	4.8 ± 3	4.5 ± 2.4	-
Meal per day	4.4 ± 1.1	6.6 ± 1.9	-	**0.01***	4.8 ± 2	5.9 ± 1.2	-
Amount of carb	128.6 ± 33.5	144.9 ± 38.2	-	0.28	136.1 ± 45.4	143.7 ± 42.9	-
Average SG (mg/dL)	145.1 ± 20	153.7 ± 15.1	162.7 ± 32.4	0.27	139.8 ± 13	158.5 ± 21.1	161.7 ± 23.6

Table 2. Continued

	p*	6-month			p*	12-month			p*
		MiniMed™ 780G	MiniMed™ 640G	MDI + CGMs		MiniMed™ 780G	MiniMed™ 640G	MDI + CGMs	
TAR (%)	**0.02***	20 ± 5.9	39 ± 18.7	36.6 ± 19.4	**0.02***	21.2 ± 8.9	31.3 ± 10.7	37.2 ± 19.4	**0.04***
180-250	**0.02***	17.2 ± 6.1	28.7 ± 13.7	25.1 ± 11.3	0.05	18.1 ± 5.4	23.5 ± 6.8	20.7 ± 6.6	0.15
> 250	0.12	6.8 ± 11.5	10.3 ± 7.7	11.5 ± 11.7	0.56	3.6 ± 3.4	7.82 ± 4.22	16.5 ± 18.5	**0.03***
TIR (%)	**0.03***	72.5 ± 15.6	57.3 ± 17.9	60.5 ± 19.1	0.12	75.9 ± 7.7	64.7 ± 9.7	59.1 ± 18.7	**0.02***
TBR (%)	0.18	3.6 ± 2.4	3.7 ± 2.6	3 ± 2.2	0.75	5.5 ± 2.1	4 ± 3.1	3.7 ± 3	0.94
54-70	0.51	2.9 ± 1.8	3 ± 1.8	2.9 ± 2.2	0.99	2.5 ± 1.3	3.2 ± 1.9	3.6 ± 2.9	0.48
< 54	0.13	0.6 ± 0.8	0.7 ± 0.9	0.1 ± 0.3	0.1	0.5 ± 0.5	0.8 ± 1.5	0.2 ± 0.4	0.29
CV (%)	0.7	36.5 ± 3.6	36.5 ± 5.4	35.8 ± 5.5	0.92	34.3 ± 2.1	37 ± 3.8	39.7 ± 4.9	**0.01***
GMI (%)	0.47	6.6 ± 0.2	7.3 ± 0.5	6.6 ± 2.3	0.46	6.7 ± 0.4	6.1 ± 2.1	6.5 ± 2.2	0.63
HbA1c (%)	0.68	6.7 ± 0.4	7.3 ± 0.7	7.6 ± 1.6	0.18	6.5 ± 0.4	7.4 ± 0.5	7.6 ± 1.3	**0.02***
Average BG (mg/dL)	0.16	151.6 ± 30.7	180.9 ± 21.9	-	**0.02***	152.5 ± 28.2	179.6 ± 14.6	-	**0.01***
TDI (U/day) (min-max)	0.55	13.9 (7.7-25.9)	16 (6.2-24.9)	18.9 (5.2-22)	0.2	15.9 (9.3-33.2)	17.6 (5.8-29.4)	21.4 (13-34)	0.26
Amount of bolus insulin (U)	0.28	8.8 ± 2.3	11 ± 4.2	-	0.18	9.9 ± 2.8	11.8 ± 5.1	-	0.39
Auto-correction insulin (U)	-	2.1 ± 1.6	-	-	-	2.6 ± 2.2	-	-	-
Basal insulin (U)	0.97	5.1 ± 2.9	5 ± 2.3	-	0.53	6.1 ± 4.1	5.9 ± 2.2	-	0.55
Meal per day	0.12	5.9 ± 1.4	6.1 ± 1.7	-	0.97	6.1 ± 2.1	5.9 ± 1.5	-	0.77
Amount of carb	0.62	154.8 ± 28.6	149.2 ± 35.7	-	0.6	154.5 ± 33.2	158.6 ± 38.8	-	0.67
Average SG (mg/dL)	0.78	141.3 ± 13.7	164.7 ± 22.4	163.5 ± 29	**0.02***	142.6 ± 14.2	156.9 ± 16.3	169.6 ± 42.9	0.83

*p < 0.05: Statistically significant.
BG: blood glucose, carb: carbohydrate, CGMs: continuous glucose monitoring system, CV: coefficient of variation, GMI: glucose management indicator, SC: subcutaneous, SG: sensor glucose, MDI: multiple dose insulin treatment, TAR: time above range, TBR: time below range, TDI: total daily insulin dose, TIR: time in range, HbA1c: hemoglobin A1c, min-max: minimum-maximum

in children under seven years old. Additionally, glucose control in this age group is challenging due to the variability of insulin requirements (2). This paper aimed to show the effectiveness and reliability of the MiniMed™ 780G insulin pump in children aged 2 to 7 years.

Pulkkinen et al. (14) investigated 35 children aged between 2 to 6 years old receiving MiniMed™ 780G treatment. In their study, TIR showed an 8.3% increase with an 8.6% decrease in TAR during the 12 weeks under MiniMed™ 780G treatment. Similar results were reported in their extended follow-up study, though they focused on time in tight range. TIR increased from 58.3% initially to 66.2% in the sixth month, and these values were sustained during an 18-month follow-up. However, TIR remained below 70% throughout the investigation, with the most significant increase observed in the first three months. They concluded that TIR values below 70% might be attributable to the younger age group and lower baseline TIR values compared to other studies (22). Tornese et al. (23) also investigated MiniMed™ 780G in a similar age group, showing an 8.5% increase in TIR along with a significant decrease in TAR. A further study conducted by Abraham et al. (15) found that TIR increased from 64.1% at baseline to 74.7% in the fifth week. In our study, similar to the aforementioned studies, TIR increased by 6.67% in the third month, which remained consistent throughout the 12 months. It demonstrated statistically significantly higher values than the MiniMed™ 640G and MDI + CGMs groups in the third and sixth months, and this difference persisted during the follow-up period.

TAR and TBR serve as additional indicators of treatment success. Similar to studies conducted by Pulkkinen et al. (22) and Tornese et al. (23), TAR showed a significant decrease during follow-up in our study. Additionally, TAR was significantly lower than in the other treatment groups, except initially. However, TBR did not significantly decrease in MiniMed™ 780G compared to MiniMed™ 640G and MDI + CGMs. Furthermore, no instances of severe hypoglycemia or ketoacidosis were observed in any case. This suggests that the MiniMed™ 780G insulin pump is as safe as the MiniMed™ 640G insulin pump and MDI + CGMs, as indicated by TBR and TAR in this vulnerable age group.

Pulkkinen et al. (22) showed that CV didn't decrease significantly during the follow-up period. In contrast to Pulkkinen et al. (22), Tornese et al. (23) found a significant decrease in CV during their study period. Our study is the first study that compares CV between three different treatment groups. Similar to Pulkkinen et al. (22), CV didn't change during the follow-up in our research but was significantly lower in the MiniMed™ 780G group compared to the other treatment groups.

Pulkkinen et al. (22) found that HbA1c decreased significantly over 18 months. However, during the follow-up period, they observed a temporary increase in HbA1c between the sixth and twelfth months, which was attributed to the lifting of COVID-19 restrictions, particularly an increase in infections during that period. In our study, HbA1c decreased significantly during the 12-month follow-up in the MiniMed™ 780G group. It was significantly lower in the MiniMed™ 780G group, with the most remarkable change observed in the third month compared to the other treatment modalities. GMI, derived from the term of estimated A1c, had been created to assess more accurately and make more personalized glucose management (24). Tornese et al. (23) investigated the GMI and found that the change in the GMI was insignificant. Seget et al. (25) also published their 2023 study with a significant decrease in the GMI. Unfortunately, numerous studies have indicated that the GMI alone might not be used in this regard. Instead, it is advised to be used with HbA1c value to estimate hypoglycemia risk. An increased gap between HbA1c and GMI is associated with an increased risk of hypoglycemia (25). Moreover, if higher HbA1c values persist despite lower GMI, the risk of diabetes-associated complications will increase (26). Although a larger gap between GMI and HbA1c was observed in the MiniMed™ 780G and MiniMed™ 640G groups initially, it decreased during follow-up in our study. However, in the MDI + CGMs group, this gap persisted over time. HbA1c levels in the MiniMed™ 780G group significantly decreased during follow-up, reaching even lower levels than GMI in the twelfth month. In contrast, in the MiniMed™ 640G group, HbA1c did not differ over time. Considering that lower HbA1c values than GMI and lower HbA1c indicate lower diabetes-associated complications, the MiniMed™ 780G insulin pump is more effective and safe than the MiniMed™ 640G insulin pump and MDI + CGMs in this age group.

The instructions for determining minimum and maximum total daily insulin (TDI) doses are outlined in the MiniMed™ 780G insulin pump manual. The manufacturer has set the minimum TDI at eight daily units (27). In the study by Pulkkinen et al. (22), TDI was a minimum of 8 U/day. In Tornese et al.'s (23) study, the minimum TDI was 6 U initially under manual mode, 6.6 U after auto-mode, and 7.2 U in the 3rd month. In our study, the minimum TDI was under 8 U (4.5 U), initially in manual mode. It reached 8.2 U in the third month and decreased to 7.7 U in the sixth month.

Study Limitations

Low number of patients; more patients are necessary to make more accurate decisions. Retrospective study design.

Conclusion

In our study, we observed that the MiniMed™ 780G was superior to both the MiniMed™ 640G and MDI + CGMs in terms of metabolic control (achieving HbA1c < 7% and TIR > 70%) over a one-year follow-up period in children 2-7 years.

Ethics

Ethics Committee Approval: Ethical committee approval was obtained from the Ethics Committee of the Ege University (decision date: 24-5.1T/24, date: 23.05.2024).

Informed Consent: Informed consent was obtained from all parents or caregivers of children recruited in the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Nihal Gül Uslu, Deniz Özalp Kızılay, Günay Demir, Yasemin Atik Altınok, Şükran Darcan, Samim Özen, Damla Gökşen, Concept: Deniz Özalp Kızılay, Şükran Darcan, Samim Özen, Damla Gökşen, Design: Nihal Gül Uslu, Şükran Darcan, Samim Özen, Damla Gökşen, Data Collection or Processing: Nihal Gül Uslu, Günay Demir, Yasemin Atik Altınok, Damla Gökşen, Analysis or Interpretation: Nihal Gül Uslu, Damla Gökşen, Literature Search: Nihal Gül Uslu, Damla Gökşen, Writing: Nihal Gül Uslu, Damla Gökşen.

Conflict of Interest: Two authors of this article, Samim Özen, Damla Gökşen, are members of the Editorial Board of the Journal of Clinical Research in Pediatric Endocrinology. However, they were not involved in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from different institutions.

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ORIGINAL ARTICLE

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Long-term Impact of Continuous Glucose Monitoring Assistance on Glycemic Control in Children and Adolescents with Type 1 Diabetes Following the 2023 Kahramanmaraş Earthquake

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What is already known on this topic?

Natural disasters, like earthquakes, can negatively impact glycemic control in people with diabetes. Continuous glucose monitoring (CGM) aids individuals with diabetes in maintaining improved glycemic control.

What this study adds?

This study reports the impact of CGM support on glycemic control in children and adolescents with diabetes following the 2023 Kahramanmaraş earthquake. Despite the negative impacts of the earthquake, there was no change in hemoglobin A1c (HbA1c) levels among those who did not benefit from CGM support, while a decrease in HbA1c was observed in those who did, and this reduction was sustained over a 9-month follow-up period. In children and adolescents benefiting from CGM support, an increase in active CGM use and a decrease in the frequency of hypoglycemia were observed in follow-up.

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Abstract

Objective: To evaluate the impact of continuous glucose monitoring (CGM) assistance on glycemic control in children with type 1 diabetes (T1D) in earthquake-affected regions, comparing those who benefited from CGM with those who did not. Additionally, the study assessed changes in CGM metrics over nine months of CGM use.

Methods: A multicenter, cross-sectional study was conducted across 11 centers in Türkiye. Children with T1D were divided into two groups: those who received CGM support (CGM+) and those who continued with finger-stick glucose monitoring (CGM-). Hemoglobin A1c (HbA1c) levels were measured at four intervals: pre-earthquake, 3-6 months, 6-9 months, and 9-12 months post-earthquake. In the second phase, CGM metrics were analyzed over 90-day intervals in the CGM+ group with at least 85% sensor usage.

Results: A total of 532 children were included. Median HbA1c levels decreased from 9.1% pre-earthquake to 8.8% 3-6 months post-earthquake ($p = 0.027$). In the CGM+ group, HbA1c levels significantly decreased from 8.8% to 8.3% ($p < 0.001$), while no significant change was observed in the CGM- group. Of the 412 subjects with access to CGM reports, 105 (25.4%) had less than 85% sensor usage and were excluded. In the remaining 307 patients, there was a significant increase in active sensor time and daily glucose measurements, along with a reduction in hypoglycemia frequency over the 90-day intervals ($p < 0.001$ for all three).

Conclusion: CGM assistance improved glycemic control in children with T1D, even under the challenging conditions following a devastating earthquake. These findings highlight the need for broader access to CGM devices to enhance diabetes management.

Keywords: Continuous glucose monitoring, diabetes, earthquake, glucose sensor

Introduction

On February 6, 2023, Kahramanmaraş, Türkiye, was struck by two major earthquakes with magnitudes of 7.7 Mw and 7.6 Mw, occurring nine hours apart. Just two weeks later, a significant aftershock measuring 6.4 Mw hit Hatay on February 20, 2023. Official reports indicate that at least 50,000 people died, and 9.1 million people, one-tenth of Türkiye's population, were affected. In the disaster area, approximately 300,000 homes were destroyed. The World Health Organization declared a Level 3 emergency, and a state of emergency was declared in the ten affected provinces (1,2). Following the earthquakes, 94 hospitals sustained light damage, while 42 hospitals were moderately to severely damaged. To mitigate the impact on the healthcare system, volunteer healthcare personnel were deployed, pharmaceuticals and medical supplies were delivered, and 35 field hospitals were established (3).

It is well known that social stressors, including natural disasters, can negatively impact glycemic control in individuals with diabetes (4). Children and adolescents with diabetes were among the groups most significantly impacted by this disaster. Those living closer to the epicenters, where homes were destroyed or severely damaged, faced substantial disruptions in their care. In contrast, those in regions further from the epicenters, which were less affected, experienced disruptions in their daily routines and dietary habits due to challenges that included fear of entering homes and relocation, particularly in the early post-earthquake period. In response, the Turkish Society for Pediatric Endocrinology and Diabetes coordinated the deployment of volunteer pediatric endocrinologists, as well as the distribution of insulin pens, fingerstick blood glucose meters, and blood glucose test strips to the affected regions. They also organized a network of voluntary pediatric endocrinology specialists from across Türkiye to provide daily consultations for doctors in the disaster areas, sharing their contact information with local medical teams. In addition, medical device companies and pharmaceutical companies independently made donations. During this period, in order to facilitate access to medication, patients with type 1 diabetes (T1D) were allowed to obtain their insulin directly from the pharmacy without a prescription. Finally, and most notably, one month after the earthquake, in March 2023, the government took a significant step by distributing free continuous glucose monitoring (CGM) devices and compatible mobile phones to all children and adolescents with diabetes under the age of 22 (5). All who applied during the announced application period received these devices for a two-year period.

We previously reported a study conducted in Adana province, where we compared hemoglobin A1c (HbA1c) levels before and after the earthquake and demonstrated a significant decrease in HbA1c levels among patients who benefited from CGM support (6). Building on these findings, we sought to replicate the study on a larger, multicenter scale while also incorporating longitudinal follow-up data. The primary aim of this study was to evaluate the impact of this intervention on glycemic control in patients living in earthquake-affected regions by assessing changes in HbA1c levels before and after the earthquakes and analyzing CGM parameters over time in those who benefited from CGM device support.

Methods

Study Design

This study was conducted as a multicenter, cross-sectional analysis involving 11 centers across seven provinces significantly affected by the earthquake (Figure 1). The study population consisted of children and adolescents under the age of 18 years diagnosed with T1D. Exclusion criteria included patients in the honeymoon phase, those using insulin pumps, and those already using CGM before the earthquake.

Study Population and Data Collection

The study protocol was shared with all centers, and data on eligible patients, including demographic information and HbA1c levels, were requested to be entered into a standardized data form. Patients who did not initially use CGM but later received CGM support (Freestyle Libre 2, Abbott Diabetes Care Inc, California, USA) were classified as the CGM (+) group, while those who either missed the opportunity for CGM support or chose not to apply, thereby continuing with finger-stick glucose measurements, were categorized as the CGM (-) group.

In the first phase of the study, HbA1c levels were evaluated at four specific time intervals: the three months preceding the earthquake (November 2022-February 2023), three to six months post-earthquake (June-August 2023) (to reflect at least three months of sensor use for those who benefited from CGM support), six to nine months post-earthquake (September-November 2023), and nine to twelve months post-earthquake (December 2023-February 2024). Only patients who regularly attended follow-ups every three months and had complete HbA1c data across all four time intervals were included in this analysis. Patients whose initial HbA1c measurement was taken at the time of diagnosis of diabetes were excluded.

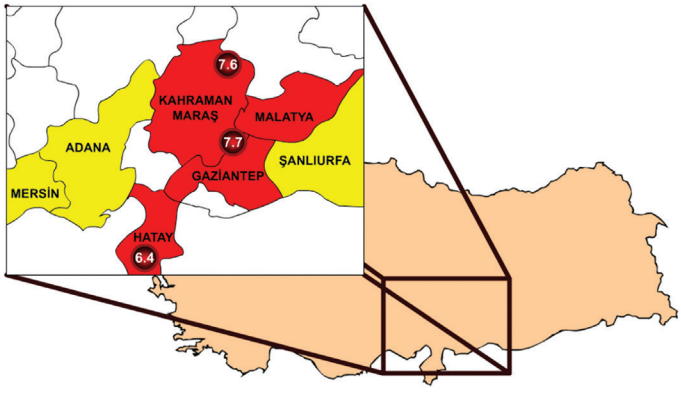

Figure 1. Map of the earthquake-affected region and epicenters with event magnitudes. The colored areas are the provinces with participating centers. Provinces shown in red are severely affected by the earthquakes, while those in yellow are relatively less affected

The second phase of the study focused on CGM usage metrics in patients who received CGM support. Sensor parameters (percentage of days with sensor data, active sensor time, number of low glucose events, average daily scan frequency, coefficient of variation, glucose management indicator, and percentage of time spent in different glucose ranges) were accessed through the Libreview.com remote access system. To standardize the evaluation, patients with less than 85% active sensor use during any 90-day period were excluded. CGM metrics were assessed in 90-day intervals starting from May 2023 to evaluate trends in sensor use and its impact on glycemic control.

Ethical Considerations

This study was approved by the Adana City Training and Research Hospital Scientific Research Ethics Committee with approval dated: 30.05.2024, and decision number: 35.

Statistical Analysis

All statistical analyses were performed using Statistical Package for the Social Sciences version 27 (IBM Inc., Armonk, NY, USA). To assess whether the data followed a parametric distribution, histogram curves were examined, and the Kolmogorov-Smirnov test was applied. Parametric data are presented as mean ± standard deviation, while non-parametric data are expressed as median (minimum-maximum). Comparisons between two independent variables were made using the T-test for parametric data and the Mann-Whitney U test for non-parametric data. For comparisons involving more than two dependent variables, repeated measures analysis of variance (ANOVA) was used for parametric data, with Tukey's test applied for *post-hoc* pairwise comparisons if significance was found. For nonparametric data, the Friedman test was used, and *post-hoc* comparisons were conducted using the Wilcoxon test with Bonferroni correction. A two-way repeated measures ANOVA was conducted to analyze the interaction effect between the earthquake and CGM usage on the change in HbA1c levels, as well as to analyze the interaction effect between the earthquake and age groups on the change in HbA1c levels only in the CGM (+) group. Since both pre- and post-earthquake HbA1c levels exhibited a nonparametric distribution, logarithmic transformation was applied to achieve a Gaussian distribution, and the transformed data were used in the ANOVA analysis. A p value below 0.05 was considered statistically significant.

Results

Hemoglobin A1c Levels Before and After the Earthquake

In the analysis examining HbA1c trends, data from 532 patients [male/female (M/F): 256/276] were included. The mean age was 12.4 ± 3.5 years, ranging from 2.5 to 18 years. The baseline HbA1c levels prior to the earthquake were significantly higher in the CGM (-) group compared to the CGM (+) group ($p = 0.019$). Across the entire cohort, the median HbA1c level decreased from 9.1% pre-earthquake to 8.8% three to six months post-earthquake, showing a significant improvement ($p = 0.027$). In the CGM (+) group, the median HbA1c decreased from 8.8% to 8.3% ($p < 0.001$), while no statistically significant change in HbA1c was observed in the CGM (-) group. This trend was consistent across both the primarily and secondarily affected provinces (Table 1). In addition, as shown in Figure 2, when analyzing HbA1c trends in the CGM (+) and CGM (-) groups, it was found that in the CGM (+) group, median HbA1c level significantly decreased after the earthquake and then remained stable in subsequent measurements.

In the two-way repeated measures ANOVA analysis for the change in HbA1c levels between the two time points (before and after the earthquake), a significant effect of CGM usage on changes in HbA1c levels ($F = 11{,}063$, $p < 0.001$), indicating that the change in HbA1c levels between the two time points varied significantly based on whether participants were using CGM.

When the CGM (+) group was divided into two subgroups according to age < 12 years (n = 130) and ≥12 years (n = 133), the two-way repeated measures ANOVA analysis showed that there was no significant interaction effect between the earthquake and age group ($F = 0.370$, $p = 0.544$), indicating that the impact of the earthquake on HbA1c levels did not differ based on the age groups within the CGM (+) group.

Continuous Glucose Monitoring Use and Glycemic Control Trends

In the analysis of sensor parameters in children and adolescents who benefited from CGM support, data from a total of 412 individuals were collected from all centers. However, 105 were excluded due to less than 85% sensor data capture during any 90-day period, resulting in a final analysis of 307 children and adolescents (M/F: 166/141). The average age in this group was 11.5 ± 3.5 years (2-18). Over the nine-month follow-up period, active sensor use steadily increased, hypoglycemia events decreased, and the average number of daily glucose measurements rose (Table 2). Also, as seen in Figure 3, the time when the patient was hypoglycemic consistently decreased across the three time intervals.

Discussion

In this study, the impact of the devastating Kahramanmaraş earthquakes and glucose sensor assistance on glycemic

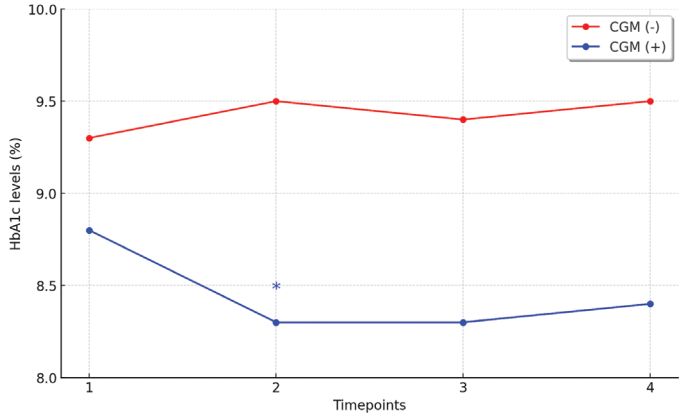

Figure 2. Trends in the median HbA1c levels in the CGM (+) and CGM (-) groups

*Statistically significant with the prior median HbA1c level after Bonferroni correction.

CGM: continuous glucose monitoring, HbA1c: hemoglobin A1c

Table 1. Changes in HbA1c levels before and after the earthquakes

	n	HbA1c level before earthquakes (%)	HbA1c levels after earthquakes (%)	p
All subjects	532	9.1 (4.9-16.6)	8.8 (5.1-16.4)	**0.027**
CGM (+)	263	8.8 (4.9-15.6)	8.3 (5.1-15.0)	**< 0.001**
CGM (-)	269	9.3 (5.6-16.6)	9.5 (5.7-16.4)	0.203
Primary affected region				
CGM (+)	124	8.7 (4.9-15.6)	8.4 (6.0-15.0)	**0.006**
CGM (-)	103	9.7 (5.6-16.6)	9.4 (5.7-15.5)	0.588
Secondary affected region				
CGM (+)	139	8.8 (5.4-14.7)	8.5 (5.1-12.9)	**< 0.001**
CGM (-)	166	9.3 (5.8-16.0)	9.5 (6.0-16.4)	0.211

CGM: continuous glucose monitoring, HbA1c: hemoglobin A1c

control in children and adolescents with T1D was investigated across a broad region, including the provinces affected by the earthquake. The main finding was that CGM support after the earthquake significantly improved glycemic control in children with diabetes, whereas those who did not receive this support did not exhibit any notable changes in their glycemic control. Previous research on the impact of earthquakes on glycemic control in individuals with diabetes has largely been conducted in Japan (7,8,9). These studies have generally reported an increase in HbA1c levels post-earthquake, with peaks observed around the 3rd and 5th months, followed by a decrease in the months thereafter. Almost all studies have been conducted in adults, except for a study examining the effects of the 1999 Marmara earthquake, which included adolescents over the age of 14 years and demonstrated a similar rise in HbA1c at the third month post-earthquake, followed by a subsequent decline (10). Only in the 2016 Kumamoto earthquake, HbA1c levels remained stable after the earthquake, which was attributed to the effective management strategies implemented by patients, who used social network platforms to exchange information on insulin dosing, carbohydrate counting, and dietary management in the post-earthquake period (9). The present study, the first to examine this issue in the pediatric population, found no significant increase in HbA1c levels after the earthquake among those who did not benefit from CGM support, regardless of whether they were in the primarily or less affected regions. This contrasts with findings in adult studies and may be due to the successful efforts of both the association and the government to ensure easy access to essential diabetes supplies. Moreover, considering that insulin therapy in children is often closely monitored by parents, it is possible that stricter adherence to glycemic control contributed to maintaining stable HbA1c levels.

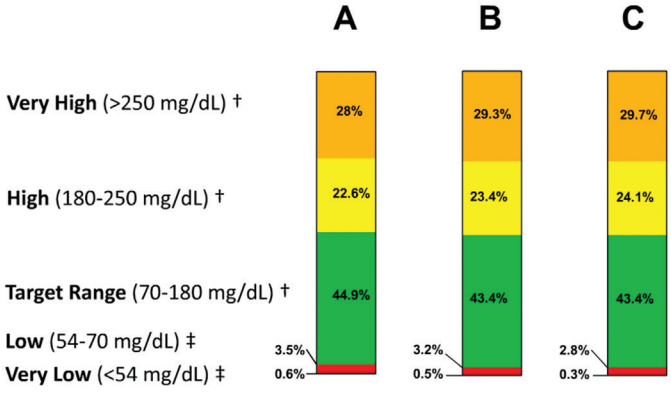

Figure 3. Comparison of the percentage of time spent in different glucose ranges across three time intervals

†Indicates a significant difference between A-B and A-C after Bonferroni correction. ‡Indicates a significant difference among A-B, B-C, and A-C after Bonferroni correction

Disasters, such as earthquakes or hurricanes, can disrupt access to medications and healthcare services, as pharmacies and clinics may be forced to close. Furthermore, medications might become damaged or inaccessible, leaving individuals without sufficient supplies, even temporarily. The lack of access to healthy food options and the interruption of regular physical activity routines can also create significant challenges, particularly for those managing diabetes (4). Although this was largely true for the regions most affected by the earthquake, in the areas that were less severely impacted, the primary challenges stemmed from people relocating to different homes, either due to ongoing damage assessments or out of fear. These disruptions in living arrangements led to significant disturbances in daily routines. The present study revealed a significant reduction in HbA1c levels among patients who received CGM assistance, which aligns with the existing literature that highlights the considerable enhancement in glycemic control facilitated by CGM use (11,12). What sets our study apart is the ability of CGM to improve even severe conditions and reverse negative trends in glycemic control, achieving positive outcomes even in challenging circumstances, both in regions severely impacted by the earthquake and in those less affected.

In the current study, after demonstrating that CGM usage effectively reduced HbA1c levels, we sought to determine whether this benefit varied between different age groups or was specific to a particular age group. To explore this, we divided the participants into two categories: children under 12 years and adolescents aged 12 and above. Our previous study in the Adana region showed that CGM use significantly

Table 2. Comparison of sensor parameters over three-month intervals

	First three months	Second three months	Third three months	p
Active sensor time (%)	89 (31-100)	92 (38-100)	94 (57-100)	**<0.001***
Number of hypoglycemia events	40 (0-203)	35 (0-192)	34 (0-165)	**<0.001***
Average daily scan frequency	10 (2-56)	12 (2-92)	23 (3-163)	**<0.001***
Coefficient of variation (%)	43.6 ± 6.8	42.7 ± 6.6	42.5 ± 7	**<0.001****
Glucose management indicator (%)	8 (6-12.8)	8.1 (6.2-13.1)	8.1 (6.2-12.8)	**<0.001****

*Statistically significant among all three pairwise comparisons. **Statistically significant between the first and second, and the first and third three-month intervals

improved glycemic control, particularly in adolescents (6). However, in this larger-scale study, we observed comparable benefits in both age groups. Notably, there is a lack of studies that have examined the effectiveness of CGM by categorizing children based on age (12). The improvement in glycemic control associated with CGM use in the pediatric population has been attributed to age-specific factors: younger children often resist finger-stick glucose monitoring, while adolescents may face challenges in maintaining consistent monitoring as they begin to take over diabetes management (13,14). Despite the negative effects of the earthquake, CGM use in this study showed consistent benefits by helping both groups overcome these age-related challenges.

In the second phase of the study, an analysis of sensor parameters over the 9-month follow-up period revealed a gradual increase in active sensor usage and the frequency of blood glucose measurements, accompanied by a decrease in the frequency of hypoglycemic events. When examining trends within specific glucose ranges, a similar reduction in the duration of hypoglycemia was observed over time. Despite a significant decrease in time in range and an increase in both the hyperglycemic range and the glucose management indicator, these changes were minor and not clinically significant, whereas the decrease in hypoglycemia was considered clinically valuable. Studies conducted on patients using CGM have also highlighted that the reduction in HbA1c levels and hypoglycemic events observed after the initial transition to CGM is sustained over the long term (15,16). The decrease in hypoglycemia frequency can be largely attributed to the ability to monitor blood glucose more comfortably and to the alerts from the hypoglycemia alarm. Furthermore, in our patients, the observed increase over time in the frequency of blood glucose measurements and active sensor usage may be related to the fact that, although technical aspects such as sensor placement were taught during the initial distribution, these patients had not been seen by a clinician at that time. As time progressed, regular hospital follow-ups likely provided additional information on sensor usage and further encouragement from health care staff contributing to this increase.

Study Limitations

This study has some limitations. As a retrospective study, it lacked data on the frequency of blood glucose measurements before the earthquake for patients using CGM. Consequently, we could not establish a link between the observed HbA1c improvement and potential changes in blood glucose monitoring frequency following CGM use. Furthermore, we were unable to assess changes in patients' dietary habits and carbohydrate intake before and after the earthquake, factors that may have directly impacted glycemic control.

Conclusion

This study provided a comprehensive overview of the impact of the Kahramanmaraş earthquake on children with diabetes, emphasizing the effectiveness of CGM in improving glycemic control, despite the challenging circumstances caused by the disaster. Importantly, this improvement was not transient; it persisted throughout long-term follow-up, underscoring the sustained benefits of CGM. These findings strongly support the argument for making CGM devices freely accessible to all individuals with diabetes in Türkiye.

Ethics

Ethics Committee Approval: This study was approved by the Adana City Training and Research Hospital Scientific Research Ethics Committee with approval dated: 30.05.2024, and decision number: 35.

Informed Consent: Retrospective study.

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Footnotes

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ORIGINAL ARTICLE

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Could MOTS-c Levels in Children with Type 1 Diabetes Mellitus Be an Indicator for Early Diabetic Kidney Disease?

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What is already known on this topic?

Vascular complications associated with diabetes are not commonly observed in children and young people. However, structural abnormalities may manifest a few years after the onset of the disease, usually starting from age 11 years with between two and five years of diabetes duration. Intensive education and treatment during childhood can help prevent or delay the onset and progression of diabetic complications, including diabetic kidney disease (DKD), retinopathy, and neuropathy. Renal failure and hypertension may develop due to DKD. Hyperglycemia in diabetic patients leads to an increase in reactive oxygen species (ROS). This increase in oxidative stress and ROS is a critical factor in the development of diabetic vascular complications.

What this study adds?

The findings of this study suggest that the onset of oxidative damage and mitochondrial dysfunction in type 1 diabetes mellitus is independent of DKD. Furthermore, the results suggest that levels of glycated hemoglobin A1c, commonly used as a pragmatic marker of glycemic control in patients with diabetes, and duration of disease are significant risk factors for oxidative stress and tissue damage, while changes in estimated glomerular filtration rate and microalbuminuria continue to serve as indicators for DKD.

Abstract

Objective: To compare serum mitochondrial open reading frame of 12S rRNA-c (MOTS-c) levels, a new potential biomarker for oxidative stress, in children with type 1 diabetes mellitus (T1DM) and healthy children. A further aim was to investigate serum MOTS-c levels as a potential early indicator of diabetic kidney disease (DKD) by correlating levels with changes in glomerular filtration and microalbuminuria.

Methods: Patients with a diagnosis of T1DM and healthy controls were recruited. MOTS-c, urinary albumin excretion, estimated glomerular filtration rate (eGFR), and hemoglobin A1c (HbA1c) were evaluated and clinical features and anthropometric measurements were collected. Patients were stratified according to diabetes duration, presence of albuminuria, glomerular hyperfiltration, eGFR decline and metabolic control.

Results: The T1DM group included 82 [female:male (F:M) 1:1.64] patients while the controls numbered 61 (F:M 1:0.97), with respective mean ages of 14.3 ± 3.3 and 10.6 ± 4.2 years ($p < 0.01$). MOTS-c levels were significantly lower in the T1DM group than controls (76.2 ± 1.3 vs 105.2 ± 7.0, $p < 0.001$). No difference was found in MOTS-c levels between patient subgroups categorized by diabetes duration, obesity, metabolic control, hypertension, hyperlipidemia, glomerular hyperfiltration, decline in eGFR, and presence of microalbuminuria. Simple linear regression indicated that MOTS-c was not predictive for DKD.

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Conclusion: MOTS-c levels were lower in children with T1DM than in healthy children. However, the lack of association of MOTS-c with renal biomarkers suggested that it is not an effective early marker for DKD. However, this finding suggests that the onset of oxidative damage and mitochondrial dysfunction in T1DM is independent of DKD. In addition, the results suggests that HbA1c and duration of diabetes are significant risk factors for development of microalbuminuria, while changes in eGFR and microalbuminuria continue to serve as indicators of DKD.
Keywords: Children, diabetes mellitus, diabetic kidney disease, MOTS-c, oxidative stress

Introduction

Vascular complications associated with diabetes are not commonly observed in children and young people. However, structural abnormalities may manifest a few years after the onset of the disease, usually starting from the age of around 11 years and between two and five years of diabetes duration (1). Intensive education and treatment during childhood may help prevent or delay the onset and progression of diabetic complications, including diabetic kidney disease (DKD) retinopathy, and neuropathy. Renal failure and hypertension may develop due to DKD (2). Risk factors for the development of DKD in children and adolescents include poor metabolic control, long-term diabetes, dyslipidemia, obesity, smoking, and family history of DKD (3). Urinary albumin excretion (UAE) and changes in glomerular filtration rate (GFR) remain important diagnostic tools for DKD (4). However, indicators that detect DKD earlier, before albuminuria develops and GFR declines, are needed.

Many structural and functional changes in DKD are believed to be due to a chronic inflammatory insult to the kidney. Chronic inflammation activates apoptosis, causes podocyte foot-process effacement, alters glomerular hemodynamics, increases vascular endothelial permeability leading to glomerular sclerosis, tubulointerstitial fibrosis and increased oxidative stress (5). Hyperglycemia in diabetic patients leads to an increase in reactive oxygen species (ROS). This increase in oxidative stress and ROS is a critical factor in the development of diabetic vascular complications (6,7,8,9,10).

Mitochondria are organelles that play a key role in regulating cellular metabolism and are sensitive to oxidative stress. Oxidative stress can cause damage to mitochondrial DNA, lipids, and proteins, leading to mitochondrial damage and apoptosis. Mitochondrial-derived peptides (MDPs) are a family of peptides encoded by the mitochondrial genome that regulate mitochondrial function, gene expression and metabolic homeostasis in the body (8). A new member of the MDPs, mitochondrial open reading frame of 12S rRNA-c (MOTS-c), is a peptide hormone that has been shown to exert positive effects on obesity, improve muscle function, promote bone metabolism, enhance immune regulation, inhibit inflammation, block cellular apoptosis, delay aging and reduces aging related disorders (9,11). MOTS-c is present in skeletal muscle and in organs, such as the brain, testis, kidney, liver and circulates in plasma, but MOTS-c levels decline with age. Under oxidative stress MOTS-c translocates to the nucleus, stimulating antioxidant pathways by interacting with nuclear factor erythroid 2-related factor 2, inhibits mitochondrial oxidative stress, promotes the clearance of damaged mitochondria, and improves mitochondrial biogenesis (12). MOTS-c has been shown to regulate metabolic homeostasis through AMP-activated protein kinase (AMPK) and thus modify glutathione production, prevent insulin resistance, and have favorable effects in diabetes mellitus (9,11). It has been suggested that the reduction in MOTS-c may also exert an effect on age-related diseases, such as Alzheimer's, cardiovascular disease, osteoporosis and diabetes, and experimental studies continue to investigate the benefits of MOTS-c treatment for these diseases (9,11).

The aim of the present study was to compare serum MOTS-c levels in children with type 1 diabetes mellitus (T1DM) to those of healthy children. Considering that the increase in oxidative stress and ROS, as well as mitochondrial dysfunction, are likely related to the development of diabetic vascular complications, the second aim was to investigate whether MOTS-c has a potential role in diabetic nephropathy. There have been several studies on type 2 diabetes mellitus (T2DM), but we believe our study is the first to investigate the association of DKD with serum MOTS-c levels in children and youth with T1DM.

Methods

Study Design, Subjects, and Definitions

A prospective, cross-sectional study involving children with T1DM was conducted in 2021-2022 at a tertiary care referral hospital. Patients who were being treated for insulin-dependent diabetes at the outpatient pediatric endocrinology clinic were eligible for inclusion. The study involved patients who were at puberty or at least 11 years old, whichever came first, and with 2-5 years diabetes duration. A group of normotensive children with normal body mass index (BMI) who visited the outpatient pediatric clinic for minor issues were enrolled as the control group.

Patients with chronic inflammatory diseases, chronic kidney disease, hypertension and, acute infection, as well as those taking medication other than insulin, were excluded.

Weight (kg), height (cm) and manual blood pressure were measured, and BMI was calculated. The standardized method of Tanner staging was used to assess pubertal status (13). Standard techniques were used to measure systolic blood pressure and diastolic blood pressure. BP was calculated in accordance with the National High Blood Pressure Education Program Working Group Report on High Blood Pressure in Children and Adolescents (14). Demographic and clinical data including age, sex, diabetes duration and diabetes treatment were collected from patients' medical records.

Laboratory Assessments

Serum creatinine, cystatin C, triglyceride and lipid levels were measured using the electrochemiluminescence method on the Cobas 702 systems (Roche Diagnostics, Mannheim, Germany). Hemoglobin A1c (HbA1c) was measured by high-pressure liquid chromatography using Tosoh G8 instruments (Tosoh Bioscience, Japan).

Blood and urine samples were collected on the same day following an overnight fast. Urinary albumin was measured using a solid-phase competitive chemiluminescent immunoassay on Cobas 702 systems (Roche Diagnostics, Mannheim, Germany). To measure of MOTS-c in human serum, approximately 5 mLs of venous blood was collected into a serum separator tube. The samples were allowed to stand at room temperature for approximately 15 minutes and then centrifuged at 3500 rpm for 10 minutes. Human MOTS-c levels were measured using commercial kits from BT Lab (Bioassay Technology Laboratory, Shanghai, China) using an enzyme-linked immunosorbent assay. Values for MOTS-c levels are given in ng/mL.

Mean HbA1c levels above 7% during follow-up were taken to indicate poor metabolic control (15). The averages of at least three HbA1c levels in the previous year for all patients were used. Patients with ≤2 HbA1c results within the preceding year were excluded from the metabolic control subgroup.

The degree of albuminuria was expressed as urinary albumin-to-creatinine ratio in mg/g or UAE in mg/L. ACR values of less than 30 mg/g was defined as normal, and 30 to 299 mg/g were defined as microalbuminuria (16). Patients with albumin excretion >30 mg/g at baseline had two additional samples repeated over 3-6 months to ensure albuminuria was persistent (1). Patients without albuminuria at baseline were asked to provide a urine sample every six months. The GFR was calculated using creatinine-based estimated GFR (eGFR) ($eGFR_{cr}$) (17). The eGFR of the T1DM group at the start of the study was recorded and compared with the data from at least 1 year of follow-up. The formula [(baseline eGFR - final eGFR) x 100 / baseline eGFR] was used to calculate the estimated percentage change in GFR. Progressive decline was defined as an eGFR decline of 3.3% (+1 standard deviation) or more per year (18). Glomerular hyperfiltration was defined as an eGFR of more than 120 mL/min per 1.73 m^2 (19).

Patients were categorized into five subgroups according to the presence of microalbuminuria, glomerular hyperfiltration, eGFR decline, metabolic control, and diabetes duration.

Statistical Analysis

As there was no similar published study to use as a reference, we conducted the power analysis in line with the expectations and information obtained from the literature. Assuming that the effect size of the difference between the groups was moderate (d = 0.5), it was calculated that 80% power could be obtained with a 95% confidence level when at least 128 people (at least 64 people for each group) were included.

The Kolmogorov-Smirnov analysis was used to test central tendency and variability in data. If the data were normally distributed, mean and standard deviation are given. Continuous variables without normal distribution are presented as medians and interquartile range (Q1-Q3, 25^{th}-75^{th} percentile values). Categorical variables are expressed as numbers and percentages. The independent samples t-test was used for comparisons between groups when parametric test conditions were met. The Mann-Whitney U test was used for comparisons between groups when parametric test conditions were not met. Chi-squared analysis was used to investigate differences between categorical variables. The ANOVA test was used to determine differences between three or more unrelated samples or groups. Simple linear regression analysis was used to investigate whether MOTS-c predicted DKD. The Statistical Package for Social Sciences (SPSS) for Windows, version 27.0 (SPSS Corp., Chicago, IL, USA) was used for statistical analysis. A $p < 0.05$ was considered statistically significant.

Results

There were 82 participants with T1DM (31 girls and 51 boys) and 61 healthy children (31 girls and 30 boys) in the T1DM and control groups, respectively. In terms of gender distribution, there was no difference between the groups ($p = 0.12$). However, the T1DM group's mean age was 14.3 ± 3.3 (5.5-20) years, significantly older than the control

group with a mean age of 10.6 ± 4.2 years (p < 0.01). Table 1 presents the descriptive data and laboratory results of the patient group. Eight (9.8%) patients were obese, and 16 (19.5%) had hyperlipidemia.

Nine (11%) patients were prepubertal and 73 were pubertal. Upon comparing the pubertal and prepubertal patients, no significant differences were found between the two groups in terms of HbA1c levels, MOTS-c levels, eGFR decline, frequency of hyperfiltration, or the presence of microalbuminuria.

Based on mean HbA1c available in 72 (87.8%) of the T1DM group, 12 (16.7%) patients had good metabolic control and 60 (83.3%) had poor metabolic control. No significant differences were found between these two groups in terms of laboratory data and MOTS-c levels (Tables 2, 3). However, mean HbA1c was correlated with UAE and $eGFR_{cr}$ decline (Table 4).

The duration of diabetes was less than 5 years in 23 (28%) patients and more than 5 years in 59 (72%) patients. Diabetes duration was correlated with UAE (Table 4).

Twenty (24.4%) had microalbuminuria and 62 (75.6%) had normal albumin excretion. There were no significant differences in age, duration of diabetes, HbA1c levels, eGFR decline or MOTS-c levels between patients with and without microalbuminuria (Tables 2, 3). Moreover, no significant differences were found between these two groups in terms of eGFR, lipid levels, creatinine, or cystatin-C levels.

Hyperfiltration was detected in 25 (30.9%) patients based on $eGFR_{cr}$. When the groups with and without glomerular hyperfiltration ($eGFR_{cr}$) were compared, the duration of

Table 1. The descriptive data and laboratory results of patients with T1DM

Age (years)	14.3 ± 3.4
Pre-pubertal/pubertal	9/73
Height (cm) Height SDS	157 ± 15.48 -0.04 ± 1.03
Weight (kg) Weight SDS	53.12 ± 15.71 0.02 ± 1.16
Body mass index Body mass index SDS	20.98 ± 4.03 0.04 ± 1.3
Diabetes duration (years)	6.4 ± 3.1
Insulin dose (IU/kg/day)	1.0 ± 0.3
Mean HbA1c (%)	8.53 ± 1.65
Triglyceride (mg/dL)	112.0 ± 82.7
HDL cholesterol (mg/dL)	57.5 ± 12.6
Total cholesterol (mg/dL)	167.3 ± 36.1
LDL cholesterol (mg/dL)	89.6 ± 27.4
Creatinine (mg/dL)	0.6 ± 0.1
$eGFR_{cr}$ (mL/min/1.73 m^2)	112.0 ± 20.5
Cystatin-C (mg/dL)	0.85 ± 0.11
UAE (mg/L) median (IQ) UACR (mg/g) median (IQ) MOTS-c (mg/dL)	9.9 (4.3-22.9) 9 (5-21.7) 76.3 ± 12.2

SDS: standard deviation score, UAE: urinary albumin excretion, UACR: urinary albumin creatinine ratio, $eGFR_{cr}$: creatinine based estimated glomerular filtration rate, T1DM: type 1 diabetes mellitus, HbA1c: hemoglobin A1c

Table 2. Comparison of HbA1c and markers of diabetic kidney disease in subgroups of patients with T1DM

Subgroups		Age	Diabetes duration	Mean HbA1c	UAE (mg/L)	UACR (mg/g)	$eGFR_{cr}$ decline
Metabolic control	Good (n = 12)	14.3 ± 2.7	5.0 ± 3.1	6.8 ± 0.4	10 (5.7-20.9)	11.3 (5.7-12)	9.8 (-4.4-14.0)
	Poor (n = 60)	13.3 ± 3.1	6.4 ± 3.0	8.8 ± 1.5	7.2 (3.8-18.8)	8.1 (4.8-16.4)	4.03 (-5.4-10.3)
	p	0.1	0.5	0.04	0.15	0.18	0.72
Diabetes duration	< 5 years (n = 23)	13.8 ± 2.9	3.0 ± 0.8	8.2 ± 1.0	6.3 (3-18.6)	9 (5.2-15.7)	5.2 (-7.5-12.3)
	≥5 years (n = 59)	13.2 ± 3.1	6.7 ± 1.4	8.6 ± 2.1	11.3 (5.9-29.3)	11.3 (4.8-24)	3.4 (-4.3-9.8)
	p	0.5	< 0.001	0.48	0.08	0.17	0.96
Microalbuminuria	Present (n = 20)	14.1 ± 3.1	6.9 ± 3.2	9.3 ± 2.3	48(32.1-86)	59 (37.3-142)	6.7 (-11.6-11.3)
	Absent (n = 62)	13.7 ± 2.9	6.2 ± 2.7	8.2 ± 1.3	6.6 (3.3-13.6)	7.2 (4.7-11.6)	5.1 (-5-12)
	p	0.61	0.33	0.13	< 0.001	< 0.001	0.68
Hyperfiltration ($eGFR_{cr}$)	Present (n = 25)	13.9 ± 3.0	5.2 ± 2.1	8.2 ± 1.5	13.8 (3.9-28.7)	10.9 (4.9-20.8)	10 (2.4-15.8)
	Absent (n = 57)	13.2 ± 3.1	6.7 ± 3.2	8.6 ± 1.8	7.4 (4.2-22.9)	9.9 (5.3-23.7)	0.4 (-7.4-8.6)
	p	0.33	**0.01**	0.35	0.51	0.27	**0.006**
$eGFR_{cr}$ decline	Present (n = 38)	14 ± 3.6	8.7 ± 3.5	9.0 ± 1.4	7.2 (3.2-9.7)	7.5 (4.6-14.6)	10.4 (8.2-15.6)
	Absent (n = 30)	12.4 ± 3.4	5.2 ± 2.5	8.8 ± 2.5	31 (2.7-55.6)	19.7 (4.8-99)	-6.5 [-15.1-(-)6.5]
	p	0.37	**0.03**	0.83	0.66	0.28	< 0.00

HbA1c: hemoglobin A1c, T1DM: type 1 diabetes mellitus, UAE: urinary albumin excretion, UACR: urinary albumin creatinine ratio, $eGFR_{cr}$: creatinine based estimated glomerular filtration rate

Table 3. Comparison of MOTS-c levels in subgroups of patients with T1DM

Subgroups of patients	MOTS-c (mg/dL)	p
Duration of diabetes		
> 5 year (n = 59)	75.6 ± 12.7	0.2
< 5 year (n = 23)	78.7 ± 10.5	
Obese (n = 9)	75.9 ± 9.3	0.9
Normal weight patient (n = 73)	76.3 ± 12.6	
Good metabolic control (n = 12)	74.4 ± 11.4	0.4
Poor metabolic control (n = 60)	76.2 ± 12.6	
Hyperlipidemia		
Yes (n = 16)	76.6 ± 14.1	0.9
No (n = 66)	76.2 ± 11.6	
Glomerular hyperfiltration ($eGFR_{cre}$)		
Yes (n = 25)	80.0 ± 10.2	0.07
No (n = 57)	74.7 ± 12.8	
$eGFR_{cre}$ decline		
Yes (n = 38)	74.1 ± 12.5	
No (n = 30)	77.0 ± 11.7	0.3
Microalbuminuria		
Yes (n = 20)	74.1 ± 9.7	0.4
No (n = 62)	76.7 ± 12.8	
Puberty (n = 73)	75.3 ± 12.4	0.051
Prepubertal (n = 9)	83.8 ± 6.8	

T1DM: type 1 diabetes mellitus, $eGFR_{cr}$: creatinine based estimated glomerular filtration rate, MOTS-c: mitochondrial open reading frame of 12S rRNA-c

Table 4. Correlation of MOTS-c with HbA1c and markers of diabetic kidney disease

		$eGFR_{cr}$ decline	UAE (mg/L)	UACR (mg/g)	Mean HbA1c	Diabetes duration
MOTS-c	r	-0.0	-0.15	-0.06	0.123	-0.13
	p	0.94	0.180	0.593	0.30	0.24
$eGFR_{cr}$ decline	r		0.04	-0.01	-0.26	0.08
	p		0.69	0.93	**0.03**	0.49
UAE (mg/L)	r			0.788	0.27	0.241
	p			**< 0.001**	**0.02**	**0.03**
UACR (mg/g)	r				0.11	0.18
	p				0.33	0.10
Mean HbA1c	r					0.15
	p					0.19

*Correlation was significant at the 0.05 level (2-tailed).
UAE: urinary albumin excretion, UACR: urinary albumin creatinine ratio, $eGFR_{cr}$: creatinine based estimated glomerular filtration rate, HbA1c: hemoglobin A1c, MOTS-c: mitochondrial open reading frame of 12S rRNA-c

diabetes was shorter in patients with hyperfiltration but the duration of diabetes in these patients.

GFR_{cr} monitoring was performed in 68 (82.9%) patients. Of these 38 (55.9%) experienced a decline in eGFRcr greater than 3.3%, while the remaining 30 (44.1%) did not experience any decline. Upon comparison of these two groups, there was no significant difference in terms of age, creatinine, cystatin-C, UAE, or MOTS-c levels (Tables 2, 3). The $eGFR_{cr}$ decline was greater in patients with hyperfiltration than without hyperfiltration (p = 0.006) (Table 2). Patients with GFRcr decline had a significantly longer duration of diabetes (Table 2).

Mean serum MOTS-c levels were significantly lower in the T1DM group (76.2 ± 12.2 mg/dL) than in the control group (105.2 ± 54.6 mg/dL, p < 0.01) (Figure 1). The association between serum MOTS-c levels and baseline clinical and biochemical factors was evaluated. MOTS-c levels were not correlated with baseline age, body weight, height, or BMI. Furthermore, there was no correlation between MOTS-c levels and total cholesterol, low density lipoprotein cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, HbA1c levels, serum creatinine, cystatin-C, eGFRcr, ACR, or UAE (Table 4).

No significant difference in MOTS-c levels was found among the T1DM subgroups categorized by diabetes

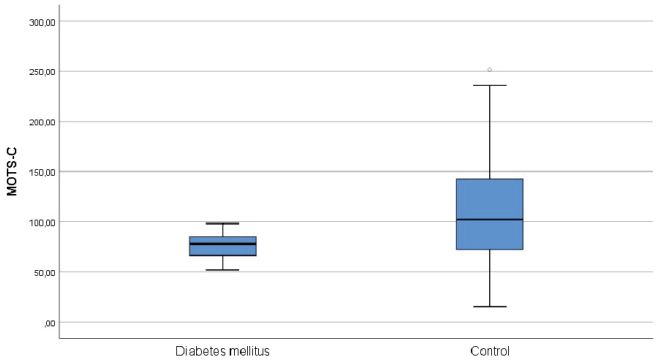

Figure 1. MOTS-c serum levels in type 1 diabetes mellitus and the healthy control group

MOTS-c: mitochondrial open reading frame of 12S rRNA-c

duration, obesity, metabolic control, hyperlipidemia, glomerular hyperfiltration, decline of eGFR, or presence of microalbuminuria (Table 3). The simple linear regression analysis results indicated that MOTS-c was not predictive for GFR decline, hyperfiltration, or microalbuminuria.

Discussion

In recent years, studies have shown the relationship between MOTS-c and adult T1DM and T2DM, childhood obesity, insulin resistance and related vascular complications (20,21,22,23). We found that MOTS-c levels were lower in the T1DM group than the control group. However, there was no correlation between MOTS-c levels and UAE or, eGFR. Although MOTS-c levels were lower in T1DM patients than in controls, there was no association between MOTS-c and indicators of diabetic nephropathy. This finding suggests that the onset of oxidative damage in T1DM is independent of diabetic nephropathy.

DKD is a significant cause of morbidity and mortality among T1DM patients, that can lead to chronic renal failure and require renal replacement therapy. Changes in the kidneys of people with diabetes generally occur in five stages (24,25). Hyperfiltration is the first stage of DKD, and the third stage is associated with the development of microalbuminuria. Hyperfiltration and microalbuminuria are believed to be strong predictors of DKD progression (4). Studies have shown that the prevalence of glomerular hyperfiltration in the pediatric population with T1DM varies between 13% and 52% (26). In the present study, 30.9% of patients had glomerular hyperfiltration, 24% had microalbuminuria, and 55% had $eGFR_{cr}$ decline. At the end of one year, the decline in $eGFR_{cr}$ was greater in patients with hyperfiltration compared to those without.

In children with T1DM, microalbuminuria is frequently detected during puberty, with a prevalence of around 10-25% after 5-10 years of diabetes duration (24,27,28,29). The development and progression of microvascular complications are influenced by puberty and duration of diabetes (24). We found that the mean diabetes duration was 7.9 ± 4.0 years and duration of diabetes was correlated with UAE and eGFR decline. Hyperfiltration was significantly more pronounced in older patients. Seventy-three patients were pubertal, and there was no difference in the frequency of microalbuminuria, GFR decline, or hyperfiltration between the pubertal and prepubertal patient groups. Poor glycemic control is well-known to be associated with the development of vascular complications. In the present study, 83% of patients had poor metabolic control and HbA1c was correlated with UAE and $eGFR_{cr}$ decline. However, there was no increase in the number of patients with glomerular hyperfiltration or microalbuminuria in the poor metabolic control group compared to the good metabolic control group.

The novel bioactive peptide, MOTS-c, has recently attracted attention as a potential prevention or therapeutic option for obesity and T2DM (20). Experimental studies have suggested that MOTS-c may serve as a new metabolic regulator and a potential therapeutic target in T2DM (8,11,30). In addition to experimental studies, studies on people with obesity and T2DM, particularly children, continue to be conducted. Du et al. (20) demonstrated that levels of circulating MOTS-c are decreased in obese male children and adolescents, and a negative correlation existed between circulating MOTS-c levels and BMI, fasting insulin levels, insulin resistance measured by homeostasis model assessment-insulin resistance (HOMA-IR) and HbA1c levels. They suggested that decreased MOTS-c concentration might be a biomarker of insulin resistance in childhood obesity. Ramanjaneya et al. (23) demonstrated that levels of circulating mitochondrial derived peptides, MOTS-c and humanin, were reduced in individuals with T2DM and significantly related to HbA1c. This study revealed that levels of MDPs were lower in people with poorly controlled T2DM compared to those with well-controlled T2DM. Luo et al. (21) showed that serum MOTS-c levels were decreased in obese children, which may be associated with impaired vascular endothelial function. Luo et al. (21) also showed that MOTS-c levels were positively correlated with HDL levels and negatively correlated with BMI, total triglycerides, and HOMA-IR. We found that there were no significant differences in MOTS-c levels between children with diabetes who had good metabolic control and those who had poor metabolic control. There was no statistically significant correlation between MOTS-c levels and BMI, HbA1c levels, or lipid levels.

Kong et al. (22) reported that adult patients with T1DM (n = 10) had significantly lower circulating MOTS-c levels than healthy controls and suggested a relationship between circulating mitochondrial-encoded peptides and the pathogenesis of autoimmune diabetes. They also demonstrated that MOTS-c treatment prevented T cell-mediated autoimmune destruction of pancreatic beta cells and autoimmune diabetes in non-obese diabetic mice. Similar to the findings of Kong et al. (22), we also found low MOTS-c levels in children with childhood T1DM. This suggests that mitochondrial damage starts in T1DM in childhood. There was no correlation between MOTS-c levels in the T1DM group and serum creatinine, cystatine-C, eGFRcr, ACR, or UAE. In addition, the absence of a significant difference in MOTS-c levels among subgroups categorized according to the presence of glomerular hyperfiltration, eGFR decrease and microalbuminuria suggests that MOTS-C is not an early indicator of DKD.

Study Limitations

The control group was younger than anticipated, which is a significant limitation. The correlation of MOTS-c with age was analysed, and no correlation was found. MOTS-c levels are known to decrease in relation to age-related illnesses (geriatric disease) and old age, but our study and control groups were children, and we do not think that the significant difference in the mean ages of the T1DM and control group affected the findings.

Conclusion

There are a limited number of published studies in patients with T2DM and a single study in adult patients with T1DM that have shown low MOTS-c levels. In the present study, MOTS-c was lower in the T1DM group than in healthy children. However, the lack of association with microalbuminuria, hyperfiltration, and eGFR decline suggested that MOTS-c is not an early marker of DKD. Moreover, the results suggest that HbA1c and duration of diabetes are significant risk factors for the development of DKD, while changes in eGFR and microalbuminuria continue to serve as indicators of DKD.

Ethics

Ethics Committee Approval: The study was approved by the Pamukkale University Local Ethics Committee (number: 10.150.1.90-106832, date: 05.01.2021).

Informed Consent: The patients along with their caregivers gave their written consent to participate in the study.

Footnotes

Authorship Contributions

Concept: İlknur Girişgen, Design: İlknur Girişgen, Tülay Becerir, Data Collection or Processing: Selda Ayça Altıncık, Murat Öcal, Gaye Malaş Öztekin, Bayram Özhan, Analysis or Interpretation: İlknur Girişgen, Esin Avcı, Murat Öcal, Gaye Malaş Öztekin, Literature Search: İlknur Girişgen, Selda Ayça Altıncık, Tülay Becerir, Bayram Özhan, Selçuk Yüksel, Writing: İlknur Girişgen, Selda Ayça Altıncık, Esin Avcı, Tülay Becerir, Selçuk Yüksel.

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ORIGINAL ARTICLE

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The Effect of Parental Collaboration on Diabetes Self-efficacy, Quality of Life and HbA1c Level in Adolescents Diagnosed with Type 1 Diabetes

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What is already known on this topic?

Adolescence is a period when the child's autonomy comes to the fore and they want to be independent. Type 1 diabetes (T1D) is a chronic disease for which the adolescent patient and parent must manage care and treatment together. Parental participation in disease management in T1D has an effect on compliance, quality of life (QoL) and metabolic control.

What this study adds?

Parental collaboration did not affect the adolescent's hemoglobin A1c level. However, the QoL increased when the adolescent performs diabetes self-management with the support of their parents.

Abstract

Objective: Type 1 diabetes mellitus (T1D) is a chronic disease that is diagnosed relatively often in childhood and adolescence. However, care and metabolic control are difficult for both adolescents and their parents. Parental participation in the care and treatment process, especially when adolescents are developing autonomy and taking responsibility for self-care, affects both the adolescent's perception of autonomy and may cause difficulties in self-management. This study was conducted to determine the effect of parental support on adolescents' self-efficacy, quality of life (QoL) and glycaemic control in adolescents with T1D.

Methods: This study was descriptive and cross-sectional. Descriptive questionnaires, The Collaborative Parental Involvement Scale for Adolescents with T1D, Diabetes Management Self-efficacy Scale for Adolescents with T1D and QoL Scale in Children with Diabetes Mellitus (PedsQL 3.0) were administered to 79 adolescents with T1D. Laboratory health records were examined about participants hemoglobin A1c (HbA1c) levels measured in the last 3 months.

Results: There was no relationship between parental collaboration and adolescent's HbA1c levels. However, there was a moderate positive relationship between parental collaboration and adolescent's QoL ($p = 0.043$) and a strong positive relationship between parental collaboration and adolescent's diabetes management self-efficacy ($p < 0.001$).

Conclusion: Adolescents who self-managed diabetes with the support of both parents, especially their fathers, who were not school absentees and had regular blood glucose measurements had better QoL. There was no relationship between HbA1c levels and parental co-operation, but there was a strong relationship between parental cooperation and adolescent self-efficacy.

Keywords: Adolescent, diabetes, parental collaboration, self-efficacy, quality of life, HbA1c level

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Introduction

Type 1 diabetes (T1D) is one of the most common chronic diseases in childhood and adolescence. Adolescence is a period when care and metabolic control of T1D is difficult for both parents and adolescents (1,2,3). Management of T1D requires multidisciplinary involvement, including endocrinologists, diabetes nurses, dieticians, psychologists and the participation of the family. This is because optimal management of T1D requires adjustments in lifestyle due to the nature of the disease, which in turn requires family committment, as well as insulin treatment, blood glucose monitoring and diet. Failure to maintain metabolic control and blood glucose levels at the desired level leads to complications, and the risk of microvascular complications accelerates throughout adolescence.

Hemoglobin A1c (HbA1c) measurement in isolation is not an ideal indicator of diabetes self-management, but it is one of the most important markers of metabolic control (4). When the HbA1c value is below 5.7%, it is classified as normal or non-diabetic, and when it is between 5.7% and 6.4%, it is classified as prediabetes. In cases where the HbA1c value is 6.5% and above, diabetes is diagnosed. The Diabetes Control and Complications Trial reported that a higher mean HbA1c level was the dominant predictor of progression of diabetic retinopathy. Achieving HbA1c levels of 7% or lower with tighter control in patients with T1D has been associated with a 35-76% reduction in microvascular complications, such as retinopathy, nephropathy, and neuropathy (5). Management of blood glucose and prevention of complications are very important for a healthy and long life in adolescents with T1D (6).

While adolescence is a period when autonomy develops and the adolescent takes responsibility for self-care, the diagnosis of T1D both affects the adolescent's perception of autonomy, due to parental involvement, and leads to additional complex difficulties in self-management (6). Adolescents with T1D have difficulty in achieving metabolic control and dietary compliance due to their efforts to be accepted by their peers, to be independent from their parents, and because of psychosocial problems (7). Besides the adolescent patient's perception of the disease and its effect on life domains, parents' emotional responses, their perspective on treatment and level of knowledge may also play a role in the behaviour of the adolescent (8).

It is known that mothers whose adolescents are diagnosed with T1D experience emotions such as shock, denial, anxiety, anger and guilt with the diagnosis, just like other mothers with adolescents with chronic diseases. Mothers may experience an intense sense of loss including the loss of normality and their healthy adolescent child (3). With the effect of anxiety and loss, parents intervene in the life of the adolescent diagnosed with diabetes and conflicts may occur between the adolescent and his/her family. Conflict may cause deterioration in the relationship between parents and adolescents. However, some adolescents with T1D and their parents manage to navigate this period with fewer problems (9).

Factors affecting the adaptation of adolescents to T1D can be explained according to the Roy Adaptation model. According to this model, age, gender, duration of diabetes diagnosis, pubertal development, family environment and treatment method are individual and familial characteristics that affect adaptation. Individual and family characteristics lead to psychological responses, such as various levels of stress, anxiety, depressive symptoms, eating disorders and behavioural disorders. Individual and family characteristics and psychological responses lead to the development of coping, self-efficacy, social competence, and self-management in the adolescent and family, and ultimately, improved quality of life (QoL) and metabolic control, which are indicators of adaptation. When harmony is achieved between the patient and the family, QoL and metabolic control are present at a good level (10). Self-efficacy, which is defined as the individual's judgement of the ability to organise and execute action plans (11), determines the disease-specific behaviours of patients with chronic diseases and is important in the realization of adaptation. Adolescents with a sense of self-efficacy are more likely to achieve the targeted metabolic control, as they are more confident and take an active role in disease management (12). As parental monitoring increases, disease management and metabolic control can be better achieved (3), and parental involvement has been reported to have a positive effect on HbA1c (13). However, conflict within the family and poor parental control may result in poor glycaemic control. It is argued that diabetes self-management will be achieved by preventing parental conflict and supporting parental involvement in the adolescent period (14).

Given all this evidence, the aim of this study was to determine the effect of parental support on self-efficacy, QoL and glycemic control in adolescents with T1D.

Methods

This descriptive and cross-sectional study was conducted in the pediatric endocrinology outpatient clinic of a university hospital in western Türkiye between March 2023 and September 2023. The study was approved by the İzmir Kâtip Çelebi University Non-interventional Clinical Studies

Institutional Review Board (protocol number: 0101, date: 23.03.2023).

Participants

According to the World Health Organization's definition, adolescence is the period between the ages of 10-19 between childhood and adulthood. Adolescents with a chronological age between 11-18 years were included in this study. The number of patients between the ages of 11-18 who were followed up at the research center between the dates of the study was 198. The study sample included adolescents with T1D who were followed up in the pediatric endocrinology outpatient clinic of the hospital and met the inclusion criteria. Adolescents who were diagnosed at least six months prior to recruitment were included in the study. Twelve adolescents with a diagnosis date of less than 6 months at the time of the study were not included in the study sample. Since having another chronic disease besides diabetes may affect self-efficacy, QoL and glycemic control, adolescents with any chronic disease in addition to diabetes were not included in the sample. The number of adolescents with a chronic disease other than diabetes (2 with Celiac disease, 1 with Down syndrome and 2 with thyroid disease) is five. These five people were not included in the sample. Adolescents who met the inclusion and exclusion criteria were invited to participate in the study, and research data were collected from 79 adolescents who agreed. According to the analysis performed with the G-Power program (3.1.9.7; Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany), based on the correlation analysis between the Collaborative parental involvement scale and the PedsQL scales, the effect size of the study was 0.47. With 95% confidence interval and 5% error for correlation analysis, the posthoc achieved power was calculated to be 0.99.

Instruments

For data collection the descriptive questionnaire, Collaborative Parental Involvement Scale for Adolescents with T1D, Diabetes Management Self-Efficacy Scale for Adolescents with T1D and QoL Scale in Children with Diabetes Mellitus (PedsQL 3.0) were introduced to the adolescents and their parents. How to fill them out was explained and the adolescents and their families completed the data collection process which was conducted online. It took an around 15-20 minutes to complete the forms.

Descriptive Questionnaire: This form consists of 17 questions developed by the researchers based on the literature (1,2,3,6,7,8,15,16,17,18). Nine of the questions included sociodemographic information, such as age, gender and education level of the adolescent and parents. Eight of the questions were related to the adolescent's diabetes, including the number of years since diagnosis, the last measured HbA1c level of the adolescent, who performs diabetes follow-up and management at home, the effect of diabetes on school attendance, and information about regularity of diabetes follow-up, insulin use and blood glucose measurements.

Collaborative Parental Involvement Scale for Youth with T1D: The parental involvement scale was developed by Nansel et al. (15), and its Turkish validity and reliability was performed by Ayar et al. (16). The scale consists of 12 items and a single sub-dimension and is scored on a 1-5 scale: 1 = almost never; 2 = sometimes; 3 = often; 4 almost always; 5 = always. The scale does not have a cut-off point and the higher the score, the closer the parental involvement.

Diabetes Management Self-efficacy Scale in Adolescents with T1D: The scale was developed by Moens et al. (17), and the Turkish validity and reliability study was conducted by Ozturk et al. (18). The scale is used to determine the educational needs of adolescents or to evaluate the effectiveness of diabetes education programmes. The scale consists of 26 items ranging from 1 (strongly agree) to 5 (strongly disagree). The scale score is calculated by dividing the total self-efficacy scores by the number of items to show the strength of perceived self-efficacy for different performance levels. The lowest total score that can be obtained from the scale is 26 and the highest is 130. A higher score indicates poorer self-efficacy. The scale has four sub-dimensions, including medical treatment and nutrition (items 1, 2, 4, 5, 7, 9, 10, 11, 14, 18, 22 and 26), assessment of glycemia, adjustment of nutrition and insulin dose (items 6, 8, 12, 13, 17, 19, 21 and 25), talking about diabetes (items 23 and 24), and honesty towards oneself and others (items 3, 15, 16 and 20). The total scores that can be obtained from the sub-dimensions of the scale are minimum 12, 8, 2, 4; maximum 60, 40, 10 and 20, respectively. The Cronbach's alpha coefficient obtained by applying the scale in Turkish children was 0.85.

Quality of Life Scale in Children with Diabetes Mellitus (PedsQL 3.0): The scale was developed by Varni et al. (19) and its Turkish validity and reliability was performed by Ayar (20). This scale measures both general domains of QoL and disease-specific domains. The scale is a comprehensive, multidimensional scale with both diabetic children and proxy (parent/caregiver) reports. The PedsQL 3.0 Diabetes Scale (28 items) includes five subscales: diabetes symptoms (11 items), treatment barriers (4 items), treatment compliance (7 items), anxiety (3 items), and communication (3 items). In the scale prepared according to the five-point Likert system, 0 = never creates a problem,

1 = almost never creates a problem, 2 = sometimes creates a problem, 3 = often creates a problem and 4 = always creates a problem. In the total score calculation of the scale, a linear conversion is applied and it is converted into 0-100 points. If the answer to the question is marked as never, it receives 100 points; if it is marked as rarely, it receives 75 points; if it is marked as sometimes, it receives 50 points; if it is marked as frequently, it receives 25 points; and if it is marked as almost always, it receives 0 points. The higher the total score, the better the health-related QoL is perceived. The reliability coefficients of the subscales in the child form were 0.81 for diabetes symptoms; 0.66 for treatment barriers; 0.66 for treatment compliance; 0.63 for anxiety; and 0.77 for communication, respectively. Similarly, the reliability coefficients of the subscales in the parent form were 0.81 (diabetes symptoms), 0.68 (treatment barriers) 0.73 (treatment compliance), 0.81 (anxiety and 0.84 (communication), respectively (19,20).

Independent variables for the present study were the adolescent's gender, age, and duration of diabetes diagnosis, together with parental education level and the score of the Collaborative Parental Involvement Scale Score for Youth with T1D.

The dependent variables were the scores obtained on the QoL Scale in Children with Diabetes Mellitus (PedsQL 3.0) and the Diabetes Management Self-efficacy Scale Score in Adolescents with T1D, together with the adolescent's HbA1c level.

Statistical Analysis

Statistical Package for the Social Sciences, version 26.0 was used for statistical analyses (IBM Inc., Armonk, NY, USA). Descriptive data of adolescents and parents are given as numbers and percentages. The Student's t-test and ANOVA test were used to analyse the variables that conformed to the normal distribution. The Kruskal-Wallis followed by pair-wise Mann-Whitney U tests were used to analyse variables that did not fit the normal distribution. Spearman's correlation analysis was used in the relationship between continuous variables.

Results

The demographic and clinical characteristics of the adolescent participants and their parents are shown in Table 1. Of the adolescents, less than a third (27.8%) followed up diabetes themselves and nearly half (45.6%) reported missing school beacuse of their diabetes. Most (88.6%) went for regular check-ups, nearly all (97.5%) used regular insulin and somewhat fewer (94.9%) had regular blood glucose measurements.

Factors that did not affect QoL included the gender of the adolescent ($p = 0.282$), education of the mother ($p = 0.521$) and father ($p = 0.481$), diabetes education received by the adolescent and family ($p = 0.926$) or regular insulin use ($p = 0.541$) (Table 2). However, the person monitoring the diabetes ($p = 0.001$), history of school absenteeism ($p < 0.001$) and regular blood glucose measurement ($p = 0.045$) were found to have an effect on QoL. Thus, the

Table 1. Descriptive characteristics of the adolescent and the family (n = 79)

Characteristic	Mean ± SD (range)	
Age (years)	14.29 ± 2.86 (11-18)	
Mother age (years)	40.75 ± 6.08 (28-62)	
Father age (years)	45.71 ± 6.79 (35-67)	
Duration of diabetes (years)	5.22 ± 3.51 (1-12)	
HbA1c level (%)	8.43 ± 1.81 (5.30-13.0)	
Gender	**n**	**%**
Female	44	55.7
Male	35	44.3
Mother education level		
Primary education	32	40.5
Secondary education	15	19
High school education	20	25.3
University education and above	12	15.2
Father education level		
Primary education	28	35.4
Secondary education	13	16.5
High school education	23	29.1
University education and above	15	19
Diabetes monitoring person		
Self monitoring	22	27.8
Only mother	21	26.6
With mother	19	24.1
With mother-father-themselves*	17	21.5
School absence due to diabetes		
Yes	36	45.6
No	43	54.4
Regular insulin use status		
Yes	77	97.5
No	2	2.5
Regular blood glucose measurement status		
Yes	75	94.9
No	4	5.1

*The group in which only the father performed diabetes follow-up and the group in which adolescents, mothers and fathers performed diabetes follow-up together were combined.
min-max: minimum-maximum, HbA1c: hemoglobin A1c, SD: standard deviation

QoL of adolescents who monitored diabetes themselves without the help of their mothers, adolescents who were not absent from school and adolescents who undertook regular blood glucose measurements had a better QoL.

In terms of self-efficacy, the gender of the adolescent (p = 0.813), mother's (p = 0.543) and father's education levels (p = 0.478), the person monitoring the diabetes (p = 0.478), school absenteeism (p = 0.148), regular insulin use (p = 0.818) or regular blood glucose measurement (p = 0.086) had no effect. The self-efficacy scores of adolescents who monitored diabetes themselves without the help of their mothers were significantly higher than the self-efficacy scores of adolescents who monitored diabetes with the help of their mothers (p = 0.036) (Table 2).

Gender of the adolescent (p = 0.322), mother's (p = 0.441) and father's education levels (p = 0.161), the person who monitores of diabetes (p = 0.457), school absenteeism (p = 0.172), regular insulin use (p = 0.644) or regular blood glucose measurement (p = 0.690) had no effect on the collaboration of parents (Table 2).

Finally, no relationship was found between parental collaboration and adolescent's HbA1c levels. However, there was a weak positive relationship between parental collaboration and adolescent's QoL (r = 0.228, p = 0.043) and a strong positive relationship between parental collaboration and adolescent's diabetes management self-efficacy (r = 0.614, p < 0.001) (Table 3).

Table 2. The relationship between scores for QoL, self-efficacy and sociodemographic variables in adolescents (n = 79)

Characteristic	QoL scale in children with diabetes mellitus (PedsQL 3.0)		Diabetes management self-efficacy scale in adolescents with T1D		Collaborative parental involvement scale	
	$\bar{x} \pm SD$	p	$\bar{x} \pm SD$	p	$\bar{x} \pm SD$	p
Gender						
Female	63.31 ± 17.18	0.282*	101.15 ± 19.68	0.813$^{\beta}$	51.77 ± 11.55	0.322$^{\beta}$
Male	58.82 ± 19.59		100.85 ± 20.59		54.14 ± 9.91	
Mother education level						
Primary education	60.99 ± 18.30	0.521$^{¥}$	77.62 ± 18.13	0.543$^{\Omega}$	51.28 ± 9.76	0.441$^{\Omega}$
Secondary education	55.89 ± 16.70		73.06 ± 20.83		49.53 ± 15.47	
High school education	65.31 ± 18.11		78.95 ± 23.32		55.52 ± 10.57	
University education and above	62.56 ± 20.78		74.66 ± 19.97		56.82 ± 5.17	
Father education level						
Primary education	57.65 ± 19.27	0.481$^{¥}$	73.53 ± 22.21	0.478$^{\Omega}$	49.75 ± 12.42	0.161$^{\Omega}$
Secondary education	65.52 ± 14.46		82.46 ± 11.13		56.46 ± 5.73	
High school education	60.86 ± 18.70		77.65 ± 21.95		51.47 ± 12.77	
University education and above	65.23 ± 19.03		75.86 ± 19.30		57.46 ± 4.10	
Person monitoring diabetes						
Self monitoring	70.04 ± 16.03	**0.001**$^{¥}$	107.54 ± 16.53	0.478$^{\Omega}$	50.95 ± 10.23	0.170$^{\Omega}$
Only mother	49.91 ± 20.52		95.95 ± 22.33		54.80 ± 10.12	
With mother	59.49 ± 13.66		96.36 ± 22.75		49.05 ± 14.18	
With mother-father-themselves	66.17 ± 16.00		104.05 ± 15.78		57.00 ± 6.10	
School absence due to diabetes						
Yes	53.47 ± 16.40	**< 0.001***	98.00 ± 19.17	0.148$^{\beta}$	50.72 ± 12.17	0.172$^{\beta}$
No	67.89 ± 17.35		103.55 ± 20.47		54.58 ± 9.40	
No	60.90 ± 18.94		102.78 ± 15.26		56.57 ± 5.16	
Regular insulin use status						
Yes	61.12 ± 18.28	0.541*	100.46 ± 19.87	0.818$^{\beta}$	52.75 ± 10.98	0.644$^{\beta}$
No	69.19 ± 24.62		122.50 ± 10.60		55.50 ± 3.53	
Regular blood glucose measurement status						
Yes	62.27 ± 17.91	**0.045***	1.56 ± 20.21	0.086$^{\beta}$	52.76 ± 11.08	0.690$^{\beta}$
No	43.52 ± 18.94		91.00 ± 11.48		54.00 ± 5.47	

*Student's t-test, $^{¥}$One-way ANOVA, $^{\beta}$Man-Whitney U test, $^{\Omega}$Kruskal Wallis test.
T1D: type 1 diabetes mellitus, QoL: quality of life, p: statistical significance value, SD: standard deviation

Table 3. The relationship between parental collaboration and QoL, diabetes management self-efficacy and HbA1c level in adolescents (n = 79)

	QoL scale in children with diabetes mellitus (PedsQL 3.0)		Diabetes management self-efficacy scale in adolescents with T1D		HbA1c level	
	r	p	r	p	r	p
Collaborative parental involvement scale	**0.228**	**0.043**	**0.614**	**< 0.001**	-0.091	0.442

T1D: type 1 diabetes mellitus, HbA1c: hemoglobin A1c, r: Spearman's correlation coefficient (rho), p: statistical significance (probability), QoL: quality of life

Discussion

Diabetes management of adolescents with T1D is monitored by measuring HbA1c levels, which are defined as glycosylated haemoglobin levels over a period of 2-3 months. Elevated HbA1c, which is considered normal below 5.7%, is associated with microvascular complications, such as retinopathy, when it exceeds 7%. Regular monitoring of blood glucose and HbA1c levels of adolescents with T1D are indicators of adolescent self-management (4,5,21,22,23). Harrington et al. (24) (2021) conducted a study in adolescents with HbA1c values of 6.5-11% in order to evaluate the relationship between diabetes self-management of adolescents aged 13-17 years with T1D, HbA1c and depression among the psychosocial outcomes. The mean final HbA1c level of adolescents participating in this study was 8.43 ± 1.81. This level suggests the presence of problems related to diabetes management in the cohort. However, the use of a single self-reported measurement of HbA1c, which is also among the limitations of this study, is not sufficient for generalizability.

Parental involvement with adolescents with T1D is considered necessary for improved glycemic control, better compliance with the T1D management regime and better self-management (6). Parental involvement in adolescents with T1D contributed to the adaptation and self-efficacy of the adolescent (8), and parental adaptation had an effect on HbA1c control (13). QoL and self-efficacy levels of adolescents who followed up their diabetes without the help of their mothers were found to be high in the present study. The group without maternal involvement included the adolescents self-managing without parental help and adolescents with paternal involvement. It is desirable for the adolescent to take responsibility in diabetes management, and the positive effect of father involvement on self-management and life capacity may be a reflection of the patriarchal family structure and the position of the father in the family. Through a cultural approach, the participation of fathers in the diabetes management of adolescents with diabetes can be further supported.

While the quality of self-care in adolescents with T1D is associated with glycemic control, reduction in complications and increase in QoL, the management of factors that inhibit self-care results in the integration of the disease into the individual's identity level in adolescents with T1D (25). The results of the present study showed that regular insulin use had no significant effect on the increase in QoL but almost all (97%) participants reported regular insulin use, while the relationship between regular blood glucose measurement and QoL was significant.

In adolescents diagnosed with T1D, there is a significant relationship between the development of disease-specific self-management skills with a decrease in complications and HbA1c level in the process of regular health care services, training and control carried out with various supportive practices (26). Regular blood glucose monitoring stands out as an important factor for individual health management in adolescents and is associated with an increase in QoL while it is considered as self-management of adolescents (27). The present study found that regular blood glucose measurement by the adolescents had an effect on QoL so that adolescents who monitored diabetes only by themselves reported significantly higher QoL than adolescents whose diabetes was monitored only by their mothers. The QoL was higher in the group in which both parents helped monitoring diabetes with the adolescent, but there was no significant difference between the other groups. Factors such as family, peer and health care team interaction, pain, and understanding care are suggested as self-management barriers and have an impact on the QoL of adolescents with T1D. QoL is significantly associated with self-management, which is considered as self-management behaviours of adolescents (28). Families of children with chronic diseases may develop overprotective and controlling behavior patterns compared to families of children without chronic diseases. This may negatively affect personality development in children and lead to the development of an externally directed, dependent personality structure, deterioration in social relations and loss of self-esteem in adolescence. Therefore, the adolescent may show angry and aggressive behavior (29,30,31,32,33). In a study examining the relationship between parental attitudes and diabetes self-management in adolescents diagnosed with T1D, it was reported that as the protective parental

attitude increased, the fasting blood glucose values of the adolescents increased (32). Increased self-management of adolescents in our study in diabetes management leads to an increase in QoL. The QoL of the adolescent decreases as family members, especially the mother, play a more active role in diabetes management. This may be attributed to the fact that families, especially mothers, have protective and controlling behaviour patterns.

In a global study conducted with young people with T1D, a decrease in HbA1c level is accepted as one of the self-management indicators and it was emphasized that the lower the HbA1c level, the higher the QoL (27). In addition, no significant relationship was found between glycemic control and QoL in studies conducted with adolescents (34,35). There was no relationship between HbA1c and QoL in the present study, in keeping with these earlier reports. However, this may be due to analysing only a single recently measured and self-reported HbA1c level. In adolescents with T1D, stigmatisation, social problems, problems experienced in school life due to factors that complicate diabetes management at school (difficulty in diabetic nutrition, inability to manage regular insulin use, inadequate physical activity) prevent diabetes management of the adolescent (36,37). Thus, QoL decreases with increased difficulty in the management of health for adolescents (34,37). Adolescents may hide the fact that they have diabetes in order to adopt the lifestyle of friends, be accepted by their peers and to avoid the prejudiced behaviours of others. For this reason, glucose monitoring and insulin injections may not be performed regularly (38). They may deliberately restrict and neglect insulin in combination with irregular eating behaviour for weight control (39). Therefore, adolescents should be questioned about whether they regularly monitor their blood glucose levels and whether they regularly administer insulin. In the present study, four adolescents reported not measuring blood glucose regularly and two adolescents reported not taking insulin regularly.

The present study found that adolescents who did not miss school reported a better QoL than those with school absenteeism. However, there was no significant relationship between school absenteeism and self-efficacy. It is a possible confounder that participants had a better QoL because they were more likely to attend school, or was it that not missing school resulted in a better QoL? It is not possible to determine causation here.

There was no significant relationship between regular blood glucose measurement and self-management. Contrary to these findings, in a qualitative study conducted with adolescents with T1D, the negative impact of not undertaking regular blood glucose measurements due to fear of injections, laziness and forgetfulness on health management was highlighted among the barriers to self-management (37). It has been shown that regular blood glucose measurement was associated with better glycemic control in adolescents with T1D (20). Similarly, the importance of regular blood glucose measurement for improved self-management has been reported in other studies (40,41).

Parents are an important factor on the QoL of adolescents with T1D (39). Parent-adolescent collaboration is important for adolescent's self-management (42) and increased adolescent self-management was associated with positive parental attitude and increased QoL of adolescents with T1D (21). Similarly, the present study found that there was a weak positive correlation between parental collaboration and adolescent's QoL.

Study Limitations

Conducting this study in a single hospital constitutes one of the limitations of the study. Since this study was conducted in a single centre and a simple sampling method was used, the representativeness and generalizability of our results may be limited. Furthermore, metabolic control was evaluated with a single, self-reported HbA1c level. This is a further limitation of our study.

Conclusion

The present study found that adolescents with T1D who followed up their diabetes themselves, adolescents who were not absent from school and those who had regular blood glucose measurements had a better QoL. Moreover, there was an inverse relationship between HbA1c levels measured at the last control and QoL. The results also showed a weak positive relationship between parental collaboration and adolescent's QoL, and a strong positive relationship between parental collaboration and adolescent's diabetes management self-efficacy. The mean HbA1c levels of the adolescents were high but ranged from normal (5.3%) to very high (13%). We believe that it would be useful to continue planning for the development of self-efficacy and self-management in adolescents and to find approaches to support more paternal participation in adolescents with T1D, as this was associated with better management and QoL in our cohort.

Ethics

Ethics Committee Approval: The study was approved by the İzmir Kâtip Çelebi University Non-interventional Clinical Studies Institutional Review Board (protocol number: 0101, date: 23.03.2023).

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

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Footnotes

Authorship Contributions

Surgical and Medical Practices: Perihan Yetim, Bumin Nuri Dündar, Concept: Beste Özgüven Öztornacı, Esra Ardahan Akgül, Nisa Yanar, Selda Akyol, Hatice Yıldırım Sarı, Design: Beste Özgüven Öztornacı, Esra Ardahan Akgül, Nisa Yanar, Selda Akyol, Hatice Yıldırım Sarı, Data Collection or Processing: Beste Özgüven Öztornacı, Nisa Yanar, Selda Akyol, Perihan Yetim, Analysis or Interpretation: Esra Ardahan Akgül, Hatice Yıldırım Sarı, Literature Search: Beste Özgüven Öztornacı, Esra Ardahan Akgül, Nisa Yanar, Selda Akyol, Gülay Baş, Hatice Yıldırım Sarı, Writing: Beste Özgüven Öztornacı, Esra Ardahan Akgül, Nisa Yanar, Selda Akyol, Gülay Baş, Hatice Yıldırım Sarı, Bumin Nuri Dündar.

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Adaptation and Validity/Reliability Evaluation of Menstrual Bleeding Questionnaire in Turkish Adolescent Girls

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What is already known on this topic?

Abnormal uterine bleeding is the most common gynecological problem in adolescence and consulting a doctor is often delayed. The Menstrual Bleeding Questionnaire (MBQ) is a scale developed to identify women with heavy menstrual bleeding.

What this study adds?

The MBQ adapted into Turkish has demonstrated good internal consistency, high reliability and acceptable validity. This study is the first to measure the severity of menstrual bleeding in adolescent girls in our Türkiye.

Abstract

Objective: The Menstrual Bleeding Questionnaire (MBQ) is a scale developed to identify women with heavy menstrual bleeding. The aim was to evaluate the validity/reliability of the Turkish version of this scale.

Methods: The MBQ was translated into Turkish and adapted to the adolescent age group. Face validity of the draft scale was tested by piloting. To ensure concurrent validity, MBQ was first applied together with the Short Form-36 (SF-36). Afterwards, both questionnaires were given to adolescent girls and the reliability of the scale was evaluated by retesting in a subgroup.

Results: The pilot study was performed with ten adolescent girls, median age 14.5 (13-16) years. The main follow-up reliability study included 251 girls medan age 16 (11-18) years, of whom 63 (25.1%) underwent retesting. There was a strong correlation between the results of the first MBQ and the second MBQ. The reliability coefficients of both the SF-36 and MBQ were above the acceptable limit of 0.70. Kaiser-Meyer-Olkin (KMO) sampling adequacy for the first application of the MBQ was above the good level (KMO = 0.831, $p < 0.001$). Eigen values of 48.73% were determined in four factors. When the pattern matrix of the first application of MBQ was examined, distribution of the items was generally regular. Receiver operator characteristics analysis of the MBQ values showed areas under the curve of the symptom effect (0.882), symptom (0.884) and severity (0.903) sub-dimension values were high. MBQ results revealed abnormal uterine bleeding in 11/251 (4.3%) cases.

Conclusion: This Turkish adaptation of the MBQ demonstrated good internal consistency, high reliability, and acceptable validity. Using it with adolescent Turkish girls will facilitate evaluation of conditions associated with abnormal uterine bleeding.

Keywords: Adolescence, abnormal uterine bleeding, questionnaire

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Introduction

Abnormal uterine bleeding (AUB) is common in adolescence. The clinical status, which was previously termed "menorrhagia", "menometrorrhagia", or "dysfunctional uterine bleeding", is now grouped under the umbrella terms "abnormal uterine bleeding" and "heavy menstrual bleeding (HMB)" (1). AUB is defined in four groups: disorders of regularity (variation >20 days over a period of one year or no bleeding in a 90-day period), disorders of frequency (one or two episodes in a 90-day period or more than four episodes in a 90-day period), disorders of amount of flow (HMB, heavy and prolonged menstrual bleeding or light menstrual bleeding) and disorders of duration of flow (menstrual periods that exceed eight days or menstrual bleeding lasting less than two days (2,3). HMB is defined as excessive blood loss that interferes with the woman's physical, emotional, social and/or material quality of life, which may be isolated or concurrent with other symptoms, and it is defined as prolonged if it exceeds eight days (3). The PALM-COEIN classification, which stands for Polyp, Adenomyosis, Leiomyoma, Malignancy, or hyperplasia (structural causes); Coagulopathy, Ovulatory dysfunction, Endometrial, Iatrogenic and Not yet classified (non-structural causes) is used to determine the etiology (4,5).

The Menstrual Bleeding Questionnaire (MBQ) is a scale developed by Matteson et al. (6) to identify women with HMB. The MBQ was originally developed to facilitate the diagnosis of HMB. However, AUB is a broader umbrella term that also includes HMB. In this study, the cases assessed using the MBQ fall under the definition of AUB. The scale primarily targets HMB based on criteria such as the amount and duration of bleeding, and its impact on quality of life. Since these parameters can also be evaluated in other types of AUB, the MBQ may also provide some information about other subtypes of AUB. The aim of the present study was to adapt the scale for Turkish adolescents and evaluate the validity/reliability of this version of this scale.

Methods

This research was conducted with adolescent girls who had started menstruating for at least six months before recruitment between June 2020 and February 2022. Girls with any chronic disease, using any treatment, doing vigorous exercise, or having uterine anomalies were not included in the study. Permission was obtained from the Ethics Committee of İstanbul Medeniyet University, Göztepe Prof. Dr. Süleyman Yalçın City Hospital (approval no: 2020/0410, date: 01.07.2020). Written informed consent was obtained from all parents of participants.

Permission from Dr. Matteson et al. (6) was received for this study. The translation from English to Turkish was performed independently, by two translators. In a meeting organized by the translators and the researcher, the texts were reviewed and a single Turkish text was agreed upon. The Turkish text was translated back into English by a professional translator. The translated texts and the original English text were compared by the researcher and senior staff and found to be compatible. To determine face validity, the questionnaire was administered to a pilot group of 10 girls and no need for alteration was found. Then, the adapted MBQ was administered to the main cohort. The Medical Outcomes Study Short Form-36 (SF-36) quality of life scale (7,8) was administered together with the MBQ to examine its concurrent validity. For the test-retest analysis, to evaluate reliability, the MBQ was administered again after two weeks to a sub-group of the main cohort.

Statistical Analysis

IBM Statistical Package for the Social Sciences, version 25.0 (IBM Corp., Armonk, NY, USA) was used for statistical analyses. The Kolmogorov-Smirnov test was used to test normality of distribution. Descriptive statistics are expressed as mean ± standard deviation for variables with a normal distribution, and as median (minimum-maximum) for non-parametric variables. The significance of difference between means was evaluated using Student's t-test, and significance of difference between median values was evaluated using the Mann-Whitney U test. The significance of difference between two pairs was assessed using the paired samples t-test and Kruskal-Wallis test, as appropriate. Pearson's correlation was used to evaluate the relationship between two variables when normal distributed, and Spearman's correlation was used in the absence of normal distribution. Chi-square tests were used for categorical variables. Statistical significance was set at $p < 0.05$.

Factor analysis was used to evaluate whether the items formed a sub-dimensional structure as in the original scale and four factors were taken. Suitability of the sample size was evaluated with the Kaiser-Meyer-Olkin (KMO) test. A KMO value of ≥0.70 was accepted. Correlation coefficient and the Cronbach's alpha coefficient were calculated to investigate internal reliability. The effect of MBQ in predicting AUB was examined by receiver operator characteristic (ROC) curve analysis. The Youden index was used to calculate cut-off values.

Results

The pilot study was performed in ten girls with a median age of 14.5 (13-16) years. Following the successful pilot

study, the adapted MBQ was tested in 251 girls with a median age 16 (11-18) years and the median age of first menstrual period was 12 (10-16) years. The test-retest subgroup consisted of 63 (25.1%) of the main cohort. The results of the MBQ identified AUB in 11/251 (4.3%). When the demographic and clinical characteristics of the girls with and without AUB were compared, no statistically significant difference was detected (Table 1).

In evaluating the adequacy of the sample size, KMO was found to be above the good level (KMO = 0.831 and chi-square for Bartlett's sphericity test = 1244.3; $p < 0.001$). The eigen value of the four factors was 48.73%.

A strong correlation was found between the responses to the first and second (test-retest) completion of the Turkish version of the MBQ in the retest subgroup ($p < 0.001$). The SF-36 general health perception score showed a significant correlation with both the MBQ and the SF-36 subscale scores ($p < 0.001$). The reliability coefficients of both SF-36 and MBQ were above the acceptable limit of 0.70 (Table 2). The average inter-item correlation coefficient for the first completion of the MBQ was 0.202 [(-0.053) -0.680]. Moreover, intra-class correlation between the first and second MBQ applications was significant and very strong (Table 3). In the ROC analyses of MBQ values, the areas under the curve of the symptom effect (0.882), symptom (0.884) and severity (0.903) sub-dimension scores were high (Figure 1). The cut-off values for these three sub-dimensions in predicting AUB were: 14.5 (sensitivity 78.9%, specificity 92.6%), 18.5 (sensitivity 84.2%, specificity 93.9%) and 10.5 (sensitivity 84.2%, specificity 93.4%), respectively.

Intra-class correlation and the correlation between MBQ applications are represented in Table 4 and Table 5, respectively.

Discussion

AUB is the most common gynecological problem in adolescent girls. Furthermore, anovulation is generally involved in the etiology of adolescent AUB. However, given the menstrual irregularities that are frequently encountered during adolescence, identifying AUB-related complications and consulting a doctor are often delayed. Therefore, patients with AUB may suffer long-term health consequences, experience decreased quality of life and have poor school attendance (6,9,10,11). The MBQ was designed to identify this problem in women and raise awareness (6). To the best of our knowledge, there is no questionnaire in the Turkish language designed to evaluate the severity of menstrual bleeding in adolescent girls. It was noteworthy that the AUB frequency was 4.3% in our adolescent group without any complaints, although it is reported that 30% of women will experience HMB at some point in their lives (6,10,11).

To assess concurrent validity, the scale undergoing validity testing should be applied at the same time as another scale with proven validity and is used in the same field, and the correlation coefficient between the two scales is examined. For example, the Pictorial Bleeding Assessment Chart and Pediatric Quality of Life module were used for the validation

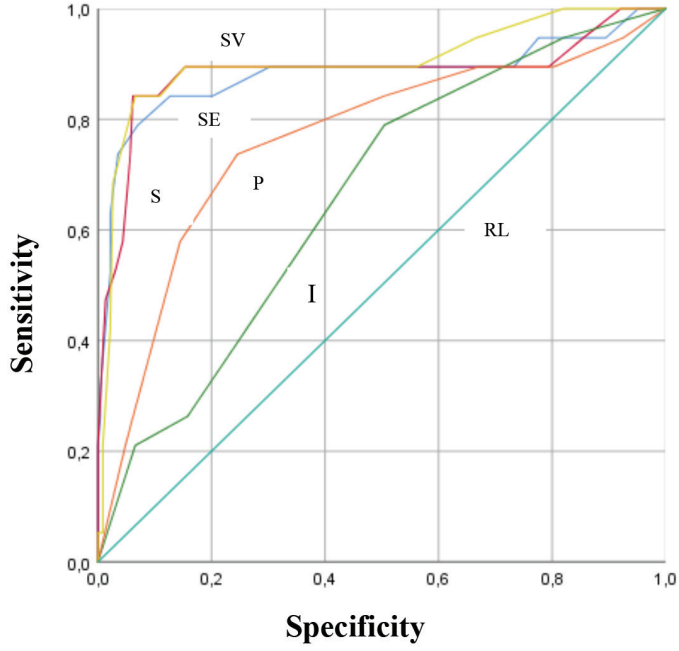

Figure 1. Receiver operator characteristic curve analysis of MBQ scores for the five sub-categories of the MBQ

MBQ: Menstrual Bleeding Questionnaire, RL: reference line, SV: severity, SE: symptom effect, I: irregularity, P: pain, S: symptom

Table 1. Comparison of demographic and clinical characteristics by the presence or absence of abnormal uterine bleeding

	Abnormal uterine bleeding		
	NO (n = 240, 95.6%)	YES (n = 11, 4.4%)	p*
Median (range) age, (years)	16 (11-18)	16 (11-18)	0.928
Median (range) age at menarche, (years)	12 (10-16)	12 (10-14)	0.457
Median (range) menstrual regularity/cycle time, (days)	30 (14-60)	30 (20-30)	0.673
Median (range) menstrual duration (days)	7 (1-10)	6 (3-10)	0.799

*Calculated using the Mann-Whitney U test

Table 2. Variance distribution according to the factor condition

Question/item	Initial eigenvalue		
	Total	Variance %	Cumulative %
1	5.115	25.574	25.574
2	1.713	8.565	34.140
3	1.627	8.135	42.275
4	1.293	6.463	48.738
5	1.095	5.477	54.215
6	0.958	4.788	59.003
7	0.905	4.523	63.526
8	0.814	4.070	67.596
9	0.787	3.935	71.531
10	0.739	3.697	75.228
11	0.708	3.542	78.770
12	0.641	3.203	81.973
13	0.587	2.936	84.909
14	0.563	2.813	87.722
15	0.508	2.541	90.263
16	0.471	2.356	92.620
17	0.453	2.267	94.886
18	0.406	2.031	96.918
19	0.350	1.752	98.670
20	0.266	1.330	100.0

Table 3. Reliability coefficients

Questionnaire	Cronbach alpha level	Number of items
SF-36	0.889	36
First response aMBQ	0.763	20
Second response aMBQ	0.835	20

aMBQ: adapted Menstrual Bleeding Questionnaire, SF-36: short form-36

Table 4. Intra-class correlation

	Intra-class correlation*	95% CI		F	p
		Minimum	Maximum		
Single measurement SE	0.954	0.924	0.972	42.118	**< 0.001**
Average measurement SE	0.976	0.961	0.986	42.118	**< 0.001**
Single measurement S	0.972	0.953	0.983	69.388	**< 0.001**
Average measurement S	0.986	0.976	0.991	69.388	**< 0.001**
Single measurement I	0.652	0.484	0.774	4.752	**< 0.001**
Average measurement I	0.790	0.652	0.873	4.752	**< 0.001**
Single measurement P	0.965	0.943	0.979		
Average measurment P	0.982	0.970	0.989		
Single measurement SV	0.960	0.934	0.975		
Average measurment SV	0.979	0.966	0.988		

*Pearson correlation coefficient.
CI: confidence interval, F: factor, SE: symptom effect, S: symptom, I: irregularity, P: pain, SV: severity

Table 5. The correlation between MBQ applications

		First MBQ SI	First MBQ Sy	First MBQ I	First MBQ P	First MBQ S	Second MBQ SI	Second MBQ Sy	Second MBQ I	Second MBQ P
aMBQF symptom	r	0.468								
	p	**< 0.001**								
aMBQF irregularity	r	0.240	0.400							
	p	**< 0.001**	**< 0.001**							
aMBQF pain	r	0.271	0.693	0.104						
	p	**< 0.001**	**< 0.001**	0.1						
aMBQF severity	r	0.473	0.864	0.179	0.398					
	p	**< 0.001**	**< 0.001**	**0.005**	**< 0.001**					
aMBQS symptom impact	r	0.944	0.669	0.499	0.400	0.643				
	p	**< 0.001**	**< 0.001**	**< 0.001**	**0.001**	**< 0.001**				
aMBQS symptom	r	0.694	0.978	0.486	0.735	0.901	0.653			
	p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**			
aMBQS irregularity	r	0.298	0.513	0.642	0.224	0.384	0.332	0.509		
	p	**0.018**	**< 0.001**	**< 0.001**	0.077	**0.002**	**0.008**	**< 0.001**		
aMBQS pain	r	0.439	0.702	0.272	0.970	0.506	0.365	0.705	0.234	
	p	**< 0.001**	**< 0.001**	**0.031**	**< 0.001**	**< 0.001**	**0.003**	**< 0.001**	0.065	
aMBQS severity	r	0.676	0.872	0.329	0.515	0.920	0.634	0.903	0.265	0.458
	p	**< 0.001**	**< 0.001**	**0.009**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.036**	**< 0.001**

r: Spearman correlation coefficient, aMBQF, adapted Menstrual Bleeding Questionnaire first application, aMBQS: adapted Menstrual Bleeding Questionnaire second (final) application, S: severity, SI: symptom impact, Sy: symptom, I: irregularity, P: pain

of another survey study that evaluated menstrual bleeding in adolescent girls (12). In the present study, the new version of the MBQ was tested against the SF-36 form in Turkish and a significant correlation was found between MBQ and SF-36 in all dimensions. The original MBQ had a moderate correlation with the SF-36 bodily pain subscale and a low correlation with the SF-36 Physical Component Score (6).

Construct validity was examined by factor analysis, that is whether the items formed a sub-dimensional structure, as in the original scale. Factor analysis is used to obtain small but independent sets of variables by combining variables that are at least moderately related to each other. The number of factors was kept constant at four in the present study, as was done in the original study. When factor analysis was performed, the four-factor model explained the data well. When the adapted MBQ pattern matrix was examined, the distribution of the items was generally regular. In this context, factor 1 measured quality of life, factor 2 measured pain, factor 3 measured menstrual irregularity, and factor 4 measured severity of bleeding. However, the question "How would you describe your menstrual period last month?" was evaluated under the severity factor in the original English language scale. The factor loading of this question (item 1) was greater in the pain domain than in the severity domain in our adapted form. Therefore, it was evaluated under the pain factor in the Turkish version.

Reliability was assessed by evaluating internal consistency and using test-retest methods. Cronbach's alpha was used for investigating internal consistency. Regarding the interpretation of the Cronbach alpha criterion value, in general, values below 0.40 are considered 'inadequate', values between 0.60-0.80 'quite reliable', and values greater than 0.80 'highly reliable' (13). When ınternal consistency of the MBQ original survey was evaluated, Cronbach's alpha was found to be between 0.87 and 0.94 (6). We obtained a Cronbach's alpha value for the 20-item MBQ Turkish version of 0.763 for the first application and 0.835 for the second application. In comparison, the Cronbach's alpha of the Turkish version of the SF-36 was 0.889. According to the consistency analysis between the items of the first and second applications of the adapted MBQ, removing the item "Rate your general concern about menstrual bleeding staining your clothes between 0-10" from the survey increased the Cronbach's alpha value (0.816-0.856). When the retests of the adapted MBQ were evaluated, the intra-class correlation between the first and second applications was significant and very strong (ICT/intraclass correlation coefficient 0.652-0.982). In the adolescent menstrual survey study of Pike et al. (12), ICT was reported to be lower than in the present study with an ICT of 0.4-0.75.

Study Limitations

Firstly, the sample was hospital-based and did not include girls attending a family doctor. Since there is no other valid and reliable Turkish language instrument that measures the severity of menstrual bleeding in adolescent girls, it was not possible to compare the adapted MBQ with another similar scale. The lack of adaptation studies conducted in other countries has made it impossible to compare the MBQ Turkish version with other language adaptations.

Conclusion

The MBQ adapted into Turkish demonstrated good internal consistency, high reliability and acceptable validity. This tool can be easily applied in a hospital outpatient setting. This study was the first to measure the severity of menstrual bleeding abnormalities in adolescent girls in Türkiye. We hope that use of this adapted MBQ will facilitate evaluation of menstrual bleeding-related conditions in adolescent Turkish girls.

Ethics

Ethics Committee Approval: Permission was obtained from the Ethics Committee of İstanbul Medeniyet University, Göztepe Prof. Dr. Süleyman Yalçın City Hospital (approval no: 2020/0410, date: 01.07.2020).

Informed Consent: Written informed consent was obtained from all parents of participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ayşe Aşık, Concept: Ayşe Aşık, Hamdi Cihan Emeksiz, Design: Ayşe Aşık, Data Collection or Processing: Ayşe Aşık, Aşan Önder Çamaş, Hamdi Cihan Emeksiz, Analysis or Interpretation: Ayşe Aşık, Aşan Önder Çamaş, Literature Search: Ayşe Aşık, Writing: Ayşe Aşık, Aşan Önder Çamaş.

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ORIGINAL ARTICLE

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Clinical Characteristics and Genotype-phenotype Correlation in Turkish Patients with a Diagnosis of Resistance to Thyroid Hormone Beta

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What is already known on this topic?

Variants in the *THRB* gene are the most common cause of resistance to thyroid hormone (RTH), termed RTH beta (RTHβ). RTHβ is a rare condition, and is mostly asymptomatic. Therefore, lack of awareness may lead to misdiagnosis, unnecessary tests or inappropriate management of the patient.

What this study adds?

In the present study, evaluating the clinical and genetic characteristics of a series of 30 Turkish patients with genetically confirmed RTHβ in comparison to variant-negative patients, the *THRB* gene variant database was expanded with three novel variants. Furthermore, the results provide evidence for prioritizing individuals for genetic analysis by comparing RTHβ patients with and without a detected variant in THRB.

Abstract

Objective: Resistance to thyroid hormone beta (RTHβ) is a rare disorder characterized by a fairly heterogeneous clinical presentation due to varying degrees of tissue response to thyroid hormone. The present study aimed to evaluate the clinical and laboratory features and genotype-phenotype relationship of Turkish patients with RTHβ.

Methods: Patients who underwent a *THRβ* gene analysis between September 2019 and September 2023 were retrospectively reviewed.

Results: Fifty patients with the clinical features of RTHβ syndrome or a family history of an index case were included. A total of eight different heterozygous pathogenic/likely pathogenic missense variants, three of which were novel, were detected in *THRB* in 30 patients from 8 unrelated families. Although most patients with RTHβ were asymptomatic, seven patients exhibited various symptoms. Moreover, seven patients had received various treatments before diagnosis. Thyroid autoantibody was positive in 23% of all cases with a variant,

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and goitre was detected in 56% of children with a variant. While thyroid nodules were detected in seven adult patients, two adults had been diagnosed with papillary thyroid cancer. One child had attention-deficit disorder, learning disability, and type 1 diabetes mellitus. Of the 20 patients without a variant, TSHoma was detected in one.

Conclusion: The present study provides an overview of clinical and genetic characteristics of patients with genetically confirmed RTHβ and expanded the *THRB* gene variant database with three novel variants. Although most patients with RTHβ are asymptomatic, molecular genetic analysis of the *THRB* gene and regular follow-up because of the apparent risk of concurrent autoimmune diseases or thyroid cancer is warranted.

Keywords: Thyroid hormones, resistance to thyroid hormone, *THRB* gene, autoimmune thyroid disease, goitre

Introduction

Defects in signaling of the thyroid hormones (TH), tetraiodothyronine (T4) and triiodothyronine (T3), TH cell membrane transport, TH metabolism, or TH action lead to reduced TH sensitivity (1,2). TH action defect is characterized by reduced response to circulating TH in target tissues, termed resistance to TH (RTH). Variants in the TH receptor gene are responsible for the etiology of the majority of RTH (3,4). There are two distinct subtypes of the TH receptor (TR); TRα and TRβ. Variants in TH receptor beta (*THRB*) gene are the most common cause of RTH, termed RTHβ (5). The prevalence of RTHβ has been reported to vary from 1 in 40,000 to 1 in 18,750 live births, with no gender predominance (6,7).

RTHβ is characterized by inappropriately normal or elevated thyroid-stimulating hormone (TSH) concentrations concurrent with extremely elevated TH levels (3). RTHβ syndrome is mainly characterized by reduced effects of T3 at the cellular and tissue level (8). Excessive TH secretion usually compensates for the impaired sensitivity in peripheral tissues. Therefore, patients with RTHβ syndrome are typically euthyroid, thereby achieving normal growth and mental development. However, elevated TH may lead to thyrotoxic symptoms, such as tachycardia in heart tissue where TRα serves as a dominant receptor or cause irritability. Although rare, patients may experience clinical features of hypothyroidism, in cases with variants that severely prevent TH receptor activity (1). These variable manifestations are in part due to variable tissue expression of the TR subtypes (9). The severity of the symptoms varies among individuals, even those from the same family with an identical *THRB* gene variant (10).

TRβ is a ligand-dependent transcription factor consisting of two functional domains: the ligand-binding domain at the carboxyl terminal, which recognizes T3, and the DNA-binding domain. The majority of the variants are located in three clusters enriched with CpG dinucleotide hot spots in the carboxy terminus of TRβ and result in mutant proteins (11). Although most cases have heterozygous variants, a few cases have harbored homozygous variants (4,12). Most variants are single nucleotide substitutions leading to an amino acid change or, less frequently, to a truncated protein. Besides, nucleotide insertions, deletions, and duplications have also been described, resulting in frame-shift and nonsense variations (8). While 75% of cases with RTHβ syndrome have a dominant inheritance, it may also occur due to a *de novo* pathogenic variant (1). Of note, the underlying molecular defect can not be detected in about 15% of individuals with the RTHβ phenotype and this condition is referred to as "TR-RTH unspecified" (13).

There are few studies evaluating the molecular genetic analysis of patients with RTHβ syndrome from Türkiye. Firstly, Poyrazoğlu et al. (14) reported a variant in the *THRB* gene in a Turkish family. Following this report, Guran et al. (15) reported the treatment outcome of their patient with the *THRB* gene variant. Işık et al. (16) highlighted the underestimation of RTHβ diagnosis in a family whose index case was misdiagnosed as thyrotoxicosis and treated with antithyroid medication. As RTHβ is rare, and most patients are asymptomatic, lack of awareness may lead to misdiagnosis, unnecessary tests, or inappropriate patient management. The aim of the present study was to evaluate the clinical and laboratory features, and genotype-phenotype relationship of a series of Turkish patients with RTHβ syndrome due to *THRB* gene variants.

Methods

Patients

A retrospective examination was conducted of all patients and their families who underwent *THRB* (NM_001354712.2) gene analysis at Ankara Bilkent City Hospital, Pediatric Endocrinology Clinic, and Endocrinology and Metabolism Clinic with a presumptive diagnosis of RTHβ between September 2019 and September 2023. The study was approved by the Clinical Research Ethics Committee of Ankara Bilkent City Hospital with decision number: 23-5676, date: 22.11.2023. The chief complaints, age of the diagnosis, sex, treatment history, body weight, height, body mass index (BMI), standard deviation scores (SDS),

pulse rate, serum TSH, free T4 (fT4), and free T3 (fT3) concentrations, anti-thyroglobulin antibody (Tg-Ab), and antithyroid peroxidase antibody (TPO-Ab) results of the patients were extracted from the patients' files. Pituitary magnetic resonance imaging (MRI) findings, thyroid ultrasound, echocardiography, and genetic analysis results were evaluated. The thyroid function tests of all patients undergoing molecular genetic analysis were double-checked, as lab and assay-specific variations could potentially affect correct phenotyping and thus pick-up rate for a variant. Besides, none of the patients from our cohort were using biotin or any medication that could interfere with the TH measurement.

All patients were evaluated for goitre. In children, the volume of each lobe was calculated using the formula of length × width × depth × 0.52. The thyroid volume was determined as the sum of both lobes and then SDS were calculated using age- and sex-specific references (17). Values above 2 SDS were considered to be goitre in children. In adults, the thyroid volume of each lobe was calculated using the formula of V (mL) = 0.479 × width (cm) × depth (cm) × length (cm) (18). A thyroid volume above 10 mL in women and 15 mL in men was considered to represent goitre (19). The hormonal profile of patients with a pathogenic or likely pathogenic variant was compared with age and sex-matched healthy controls. The fT4/fT3 ratio was calculated after unifying fT4 and fT3 units as pmol/L.

Molecular Genetic Analyses

Genomic DNA was extracted from peripheral blood leukocytes. For the index patients, all of the coding exons and exon-intron boundaries of the *THRB* gene were amplified by specific primers via polymerase chain reaction (PCR). After cycle sequencing, all PCR products were purified and sequenced on an ABI 3100 Genetic Analyzer (Applied Biosystems®, California, USA). All the sequences were aligned to the reference genome and analyzed using SeqScape® Software (Applied Biosystems®, California, USA). For the relatives of the probands, only variant-associated exonic primers were used and were analyzed with Sanger sequencing. All variants were interpreted according to the American College of Medical Genetics and Genomics Guidelines (20). Written informed consent was obtained from all patients/their legal guardians.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences, version 24.0 (IBM Corporation, Armonk, NY, USA). The mean and standard deviation (SD), median and quartile values of numerical variables were calculated. Categorical variables were expressed as frequency and percentages (%). Shapiro-Wilk test was used to evaluate the normal distribution of data. Normally distributed numerical variables were evaluated using the Student's t-test, and the Mann-Whitney U test was used if the parametric test assumptions were not met. Chi-square analysis and Fisher's exact tests were used to compare categorical variables. A $p < 0.05$ was considered to indicate statistical significance.

Results

The study included 50 patients with the clinical features of RTHβ syndrome or a family history of an index case. All participants underwent *THRB* gene analysis. Thirty patients from eight unrelated families were found to have pathogenic/likely pathogenic variants in the *THRB* gene. Clinical characteristics and hormonal features of patients with a pathogenic variant are shown in Table 1. Variant characteristics and classifications detected in our cohort are displayed in Table 2.

The number of children and adults with a pathogenic or likely pathogenic variant was 16 [female/male (F/M): 11/5] and 14 (F/M: 7/7), respectively. While 12 of these adults were family members of our pediatric patients, two families (family 7 and 8) of two adult patients could not be evaluated because they were either deceased or unwilling to participate in the study. Furthermore, the thyroid function test results and molecular genetic analysis of the parents of children with a likely pathogenic variant from Family 6 were normal, suggesting a *"de novo"* variant. The pedigrees of families 1, 2, 3, 4, 5, and 6 are presented in Figure 1. The median (1st and 3rd quartile) age in children with a pathogenic or likely pathogenic variant was 9.7 (5.2-11.4) years, and the median (1st and 3rd quartile) age in adults with a pathogenic or likely pathogenic variant was 36 (33.8-46) years. All children had a height SDS above -2 SD. BMI-SDS was within the normal range in all children except for two cases with malnutrition (case 3-6 from family 2 and 3-1 from family 5) (Table 1). One of the children (3-1 from family 5) had a diagnosis of type 1 diabetes mellitus (T1DM). While 23 of the 30 patients were asymptomatic, seven (23.3%) had various symptoms [two patients had palpitations, two had goitre, one had sweating, one had anxiety, and one had attention-deficit disorder (ADD), and learning disability (LD)]. Seven (23.3%) had received various forms of treatment at other centres before the diagnosis of RTHβ (Table 1).

Table 1. Clinical and laboratory findings of the patients with *THRB* gene pathogenic or likely pathogenic variants

Family no	Individual	Sex	Age at dx years	Initial presentation	Previous treatment	Tachycardia	Height cm (SDS) BMI (SDS)	FT3 ng/L (adult: 2.3-4.2) (children: 3-4.7)
1	1-1	M	55	Asymptomatic	None	No	NA	NA
	2-1	F	36	Asymptomatic	None	No	153 25.6	4
	2-3	F	35	Palpitation	Triiodothyronine sodium	Yes	157 28.3	4.7
	3-1	F	10.5	Asymptomatic	None	No	142.7 (0.14) 15.9 (-0.71)	7.5
	3-2	F	6.9	Asymptomatic	None	No	115 (-1) 13.6 (-1.3)	8.3
	3-3	F	9.9	Asymptomatic	None	No	127.7 (-1.5) 13.9 (-1.62)	6
	3-4	F	4.9	Asymptomatic	None	No	104.5 (-0.66) 14.8 (-0.43)	7.3
	3-5	M	13.5	Asymptomatic	None	No	153 (-0.97) 15.4 (-1.86)	7.3
2	1-2	F	56	Goitre	NA	No	153 38.4	6.2
	2-1	M	33	Asympotomatic	None	No	178 24.9	7.2
	2-3	M	40	Asympotomatic	None	No	NA	NA
	2-5	M	33	Asympotomatic	None	No	175 24.4	6.9
	3-1	F	11.5	Asymptomatic	Propranolol, mmz	No	152 (0.36) 21.9 (1)	9
	3-2	M	17.1	Asymptomatic	None	No	178 (0.57) 27.1 (1.38)	8.9
	3-3	F	10.1	Sweating	Propranolol	No	144.7 (0.95) 17.1 (0.01)	7.6
	3-4	F	1.5	Asymptomatic	None	No	80 (0.49) 16.1 (-0.08)	9.4
	3-5	M	1.1	Asymptomatic	None	No	75.8 (-0.67) 16.3 (-0.51)	10.9
	3-6	F	7.8	Asymptomatic	None	No	119 (0.89) 16.4 (-2.46)	7.8
3	2-1	M	36	Goitre	None	No	174 27	5.77
	2-3	F	19.5	Asymptomatic	L-T4	No	157 20.6	6.8
	3-1	F	1.1	Asymptomatic	None	No	73 (-1.09) 19.1 (1.58)	8.57
4	2-2	F	40	Asymptomatic	None	No	155 36.6	7.9
	3-1	F	16.9	Asymptomatic	None	No	152 (-1.82) 25.3 (1.4)	5.5
5	2-1	M	36	Asymptomatic	None	No	170 24.2	7.8
	2-3	M	43	Asymptomatic	Propranolol	No	170 20.7	5.51
	3-1	M	6	ADD, LD	Propranolol	No	108.5 (-1.45) 12.4 (-2.64)	6.6
	3-2	M	11	Asymptomatic	None	No	143 (-0.15) 25.4 (1.8)	9.47
6	2-1	F	9.5	Anxiety disorder	None	No	137.7 (0.47) 20.4 (1.27)	7.43
7		F	20.5	Palpitation	Propranolol	Yes	160 23.4	8.6
8		F	53	Asymptomatic	NA	No	NA	5.62

Table 1. Continued

Family no	FT4 ng/dL (adult: 0.89-1.76) (children: 0.83-1.43)	TSH mU/L (0.5-4.78)	Autoimmunity markers	Thyroid volume (SDS or mL)	Thyroid nodule	Pituitary MRI	Other clinical features
1	NA	NA	NA	NA	NA	No	NA
	1.74	0.72	NA	NA	NA	No	(-)
	1.6	5.4	Anti-TPO + Anti Tg-	13.1 mL	Multinodular	No	(-)
	1.98	1.4	Anti-TPO + Anti-Tg +	2.3 SDS	No	No	(-)
	2.7	1.3	Negative	2.2 SDS	No	No	(-)
	1.9	1.8	Negative	-0.15 SDS	No	No	(-)
	1.9	4.1	Negative	1.1 SDS	No	No	(-)
	1.7	1.6	Negative	2.1 SDS	No	No	(-)
2	2.45	2.81	Negative	NA	Total thyroidectomy papiller thyroid cancer	No	Type 2 DM
	1.95	5.52	Negative	NA	Total thyroidectomy papiller thyroid cancer	No	(-)
	NA	NA	NA	NA	NA	No	NA
	1.98	3.1	Negative	NA	Total thyroidectomy benign multinodular goitre	No	(-)
	2.2	3.8	Negative	9.8 SDS	No	No	(-)
	2.1	3.3	Negative	2.64 SDS	No	No	(-)
	2.5	1	Anti-TPO + Anti-Tg +	9 SDS	No	No	(-)
	2.2	2.5	Negative	1.1 SDS	No	No	(-)
	2.6	3.9	Negative	2 SDS	No	No	(-)
	2.4	3.3	Negative	2.5 SDS	No	No	(-)
3	2.68	3.2	Negative	NA	Multinodular, FNAB recommended	No	(-)
	2.7	5.8	Negative	NA	No	Yes, normal	(-)
	2.54	3.6	Negative	1 SDS	No	No	Premature
4	2.7	7.9	Anti-TPO + Anti-Tg +	18.7 mL	Multinodular FNAB: Benign	Yes, microadenom	(-)
	2.6	2.4	Anti-TPO + Anti-Tg +	5.8 SDS	No	No	(-)
5	2.8	2.6	NA	NA	NA	No	(-)
	2.57	1.75	Negative	NA	No	Yes, normal	(-)
	2.7	2	Negative	1.8 SDS	No	No	Type 1 DM
	2.79	3.9	Negative	1.68 SDS	No	No	(-)
6	2.13	2.96	Negative	1.17 SDS	No	No	(-)
7	4	3	Negative	NA	No	No	NA
8	2.26	7.9	Anti TPO + Anti Tg-	NA	Multinodular	Yes, normal	NA

Age at dx: age at diagnosis, SDS: standard deviation score, NA: not available, BMI: body mass index, FNAB: fine-needle aspiration biopsy, FT3: free triiodothyronine, FT4: free tetraiodothyronine, TSH: thyroid-stimulating hormone, Mmz: methimazole, LT4: levothyroxine sodium, Anti-TPO: antithyroid peroxidase, Anti-Tg: anti-thyroglobulin, DM: diabetes mellitus, MRI: magnetic resonance imaging, ADD: attention-deficit disorder, LD: learning disability, F: female, M: male

Twenty-six patients with a pathogenic or likely pathogenic variant could be evaluated for autoimmune thyroiditis. Six (from four different families) (23.1%) of 26 patients had autoimmune thyroiditis. Of these, all were female. There was no significant difference between TSH (p = 0.466), fT4 (p = 0.420), and fT3 (p = 0.168) levels of patients with negative or positive thyroid autoantibodies.

Table 2. *THRB* gene variants detected in the families and variant classification according to the guidelines

Family	Mutation (cDNA/protein)	Cluster region/ domain	Status	ACMG classification	Inheritance
1	c.959G > A (p.R320H)	2	Clinvar-RCV000760097	Pathogenic; PS3, PM5, PM1, PM2	Familial
2	c.701C > A (p.A234D)	3	Reported by literature (43)	Likely pathogenic; PM5, PM1, PM2, PP3	Familial
3	c.794A > T (p.D265V)	3	Novel	Likely pathogenic; PM1, PM2, PP3-S	Familial
4	c.1291A > C (p.I431L)	1	Novel	Likely pathogenic; PM5, PM1, PM2, PP3	Familial
5	c.939G > A (p.M313I)	2	Novel	Likely pathogenic; PM5, PM1, PM2, PP3	Familial
6	c.749T > C (p.I250T)	3	Clinvar-RCV000760094	Likely pathogenic; PM1, PM2, PP3, PP5-M	*de novo*
7	c.1012C > T (p.R338W)	2	Clinvar-RCV000013385	Pathogenic; PS3, PM1, PM2, PP3-S	Unknown
8	c.980C > A (p.T327N)	2	Clinvar-RCV000582153	Likely pathogenic, PM1, PM2, PP3-S, PP5	Unknown

ACMG: American College of Medical Genetics and Genomics

Table 3. Comparison of the anthropometric and laboratory findings of the patients with a variant and without a variant but having clinical/laboratory findings similar to RTHβ

	Children *THRB* +	Children *THRB*-	p value	Adult *THRB* +	Adult *THRB*-	p value	All group variant +	All group variant-	p value
Female/male	11/5	3/4	0.363$^{\phi}$	7/7	2/8	0.210$^{\phi}$	18/12	5/12	0.044*
Age (year)	9.7 (5.2-11.4)	9.5 (8.3-11.9)	0.664$^{\Psi}$	36 (33.8-46)	27 (22.6-45.5)	0.253$^{\Psi}$	17 (9.1-36)	18.4 (10.4-33.5)	0.690$^{\Psi}$
Height SDS	-0.34 ± 0.91	0.32 ± 1.28	0.245$^{\mu}$	164.50 ± 9.85	NA	NA	NA	NA	NA
BMI SDS	-0.19 ± 1.49	0.11 ± 1.00	0.564$^{\mu}$	26.96 ± 6.15	NA	NA	NA	NA	NA
FT3	7.99 ± 1.38	6.17 ± 1.25	**0.009$^{\mu}$**	6.40 ± 1.39	5.40 ± 1.22	0.099$^{\mu}$	7.31 ± 1.58	5.74 ± 1.25	**0.001$^{\mu}$**
FT4	2.32 ± 0.34	1.87 ± 0.15	**0.004$^{\mu}$**	2.46 ± 0.64	2.07 ± 0.26	0.088$^{\Psi}$	2.38 ± 0.48	1.98 ± 0.25	**0.002$^{\Psi}$**
TSH	2.68 ± 1.04	2.23 ± 0.50	0.287$^{\mu}$	4.06 ± 2.28	1.95 ± 0.80	**0.016$^{\mu}$**	3.27 ± 1.79	2.07 ± 0.68	**0.014$^{\Psi}$**
Autoimmunity	3	0	NA	3 (33.3%)	1 (12.5%)	0.576$^{\phi}$	6 (24%)	1 (6.7%)	0.224$^{\phi}$
Thyroid SDS	2.87 ± 2.83	0.15 ± 0.58	**0.02$^{\Psi}$**	NA	NA	NA	NA	NA	NA
fT4/TSH	1.03 ± 0.54	0.87 ± 0.20	0.894$^{\Psi}$	0.87 ± 0.63	1.26 ± 0.68	0.136$^{\Psi}$	0.96 ± 0.57	1.09 ± 0.55	0.242$^{\Psi}$
fT4/fT3	2.96 ± 0.67	3.16 ± 0.91	0.662$^{\Psi}$	3.88 ± 0.67	4.20 ± 1.21	0.859$^{\Psi}$	3.35 ± 0.80	3.74 ± 1.18	0.479$^{\Psi}$
fT3/fT4	0.35 ± 0.06	0.33 ± 0.07	0.635$^{\mu}$	0.27 ± 0.05	0.26 ± 0.05	0.905$^{\mu}$	0.31 ± 0.07	0.29 ± 0.07	0.386$^{\mu}$

*Chi-square test, $^{\phi}$Fisher's exact test, $^{\mu}$Student's t-test, $^{\Psi}$Mann-Whitney U test. Data are presented as mean ± SD, or median (Q1-Q3).
BMI: body mass index, SDS: standard deviation (SD) score, NA: not available, fT3: free triiodothyronine, fT4: free tetraiodothyronine, TSH: thyroid-stimulating hormone, RTHβ: resistance to thyroid hormone β

Table 4. Comparison of the anthropometric and laboratory findings of the patients with a detected variant of RTHβ and healthy controls

	Children *THRB* +	Children control	p value	Adult *THRB* +	Adult control	p value
Female/male	11/5	14/10	0.505*	7/7	9/14	0.517*
Age (year)	9.7 (5.2-1.4)	9.4 (5.6-11.1)	0.945$^{\Psi}$	36 (33.8-46)	35 (25-43)	0.316$^{\Psi}$
fT3	8 ± 1.38	3.94 ± 0.22	**< 0.001$^{\mu}$**	6.41 ± 1.40	3.51 ± 0.38	**< 0.001$^{\mu}$**
fT4	2.32 ± 0.35	1.13 ± 1.12	**< 0.001$^{\mu}$**	2.46 ± 0.64	1.18 ± 0.15	**< 0.001$^{\mu}$**
TSH	2.69 ± 1.05	2.88 ± 1.2	0.605$^{\mu}$	4.07 ± 2.29	1.89 ± 1.00	**0.002$^{\Psi}$**
fT4/TSH	1.03 ± 0.54	0.45 ± 0.16	**< 0.001$^{\Psi}$**	0.88 ± 0.63	0.78 ± 0.35	0.972$^{\Psi}$
fT4/fT3	2.96 ± 0.67	2.89 ± 0.34	0.868$^{\Psi}$	3.88 ± 0.67	4.36 ± 0.63	0.053$^{\mu}$
fT3/fT4	0.35 ± 0.06	0.35 ± 0.04	0.877$^{\mu}$	0.27 ± 0.05	0.30 ± 0.04	0.054$^{\mu}$

*Chi-square test, $^{\mu}$Student's t-test, $^{\Psi}$Mann-Whitney U test. Data are presented as mean ± SD and (median (Q1-Q3).
fT3: free triiodothyronine, fT4: free tetraiodothyronine, TSH: thyroid-stimulating hormone, RTHβ: resistance to thyroid hormone β, SD: standard deviation

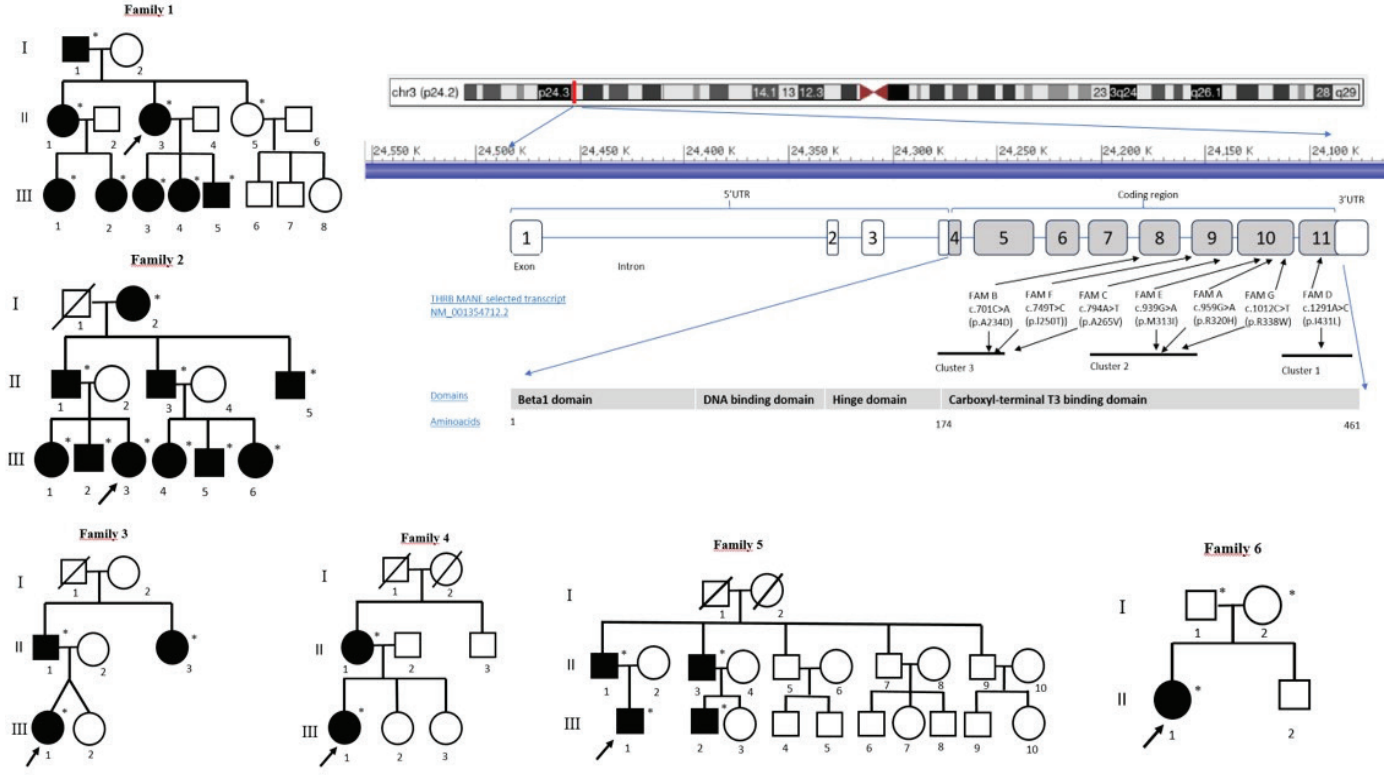

Figure 1. Schematic presentation of the chromosomal location, exon-intron organization, and protein domain content of the *THRB* gene. The detected variants have been aligned on the exonic and cluster levels. On the pedigrees of familial cases; black-filled squares and circles indicate affected individuals, and those marked with an asterisk indicate individuals with *THRB* gene analysis

In the RTHβ group, thyroid volume was above 2 SDS in 9/16 (56.25%) children. Furthermore, thyroid nodules were found in 7/14 (50.0%) adult patients. In one patient (2-2 from family 4), a fine-needle aspiration biopsy revealed benign cytology. In family 2, three individuals (1-2, 2-1, and 2-5) underwent total thyroidectomy. Of those two, (1-2 and 2-1) had papillary thyroid carcinoma (PTC) while patient 2-5 had a benign cytology result (21). A pituitary MRI, performed in four adult patients with a pathogenic or likely pathogenic variant, revealed pituitary incidentaloma in one and normal MRI in three patients.

A total of eight different heterozygous variants were detected in eight families. Three of these variants were novel. All variants were missense type resulting from a single nucleotide change. Three variants were in Cluster 3, four in Cluster 2, and only one in Cluster 1.

No variant was detected in the *THRB* gene in 20 patients. While 17 of the 20 patients were examined because of clinical and/or laboratory findings suggesting RTHβ, the remaining three euthyroid individuals were examined as part of family screening. Clinical characteristics and hormonal features of 17 patients (8 children, 9 adults) who underwent genetic analysis but no variant was detected are shown in Supplementary Table 1. Of the 17 patients without a variant, 16 were assessed for autoimmune thyroiditis. Out of these 16 patients, only one (7%) adult female had evidence of autoimmunity. The thyroid volume could be calculated in all children but in only seven/nine or 7/9 adult patients with no variant. In the pediatric group, thyroid volume was above 2 SDS in three children (37%), whereas 4/7 adults (57%) had goitre. Out of nine adult patients, seven underwent an cranial MRI scan. In one patient, a TSH-producing pituitary adenoma (TSHoma) was detected, while in another patient, a nonfunctional adenoma (incidentaloma) was detected. Since there was no Multiplex Ligation-dependent Probe Amplification kit available for *THRB*, two patients without detected variants underwent high-resolution array comparative genomic hybridization (CytoScan HTCMA_96r3.1, Thermo Fisher Scientific, Waltham, MA, USA). No pathology was detected.

Comparison of the anthropometric and laboratory findings of patients with a variant (n = 30) and without a variant, but having clinical/laboratory features of RTHβ (n = 17), are summarized in Table 3. In the pediatric group, patients

with a variant had significantly higher fT4 (p = 0.004) and fT3 (p = 0.009) levels than the variant-negative group, while no difference was observed in TSH levels (p = 0.287). Remarkably, in the adult group, while there was no significant difference between fT3 (p = 0.099) and fT4 (p = 0.088) levels of patients with and without variants, TSH levels were higher in patients with variants compared to the patients without variants [(4.06 ± 2.28 mIU/mL vs. 1.95 ± 0.80 mIU/mL), p = 0.016]. In addition, there was no difference between the fT4/TSH, fT4 (pmol/L) / fT3 (pmol/L), and fT3 (pmol/L) / fT4 (pmol/L) ratios of patients with or without a variant (Table 3).

Comparison of the laboratory findings of patients with a variant and a healthy control group are summarized in Table 4. There was no difference between TSH values in children. However, TSH levels were higher in adults with a variant. In addition, while the fT4/TSH ratio was higher in children with a variant, no difference was detected between the fT4 (pmol/L) / fT3 (pmol/L) (children, p = 0.868; adult, p = 0.053) and fT3 (pmol/L) / fT4 (pmol/L) ratios (children, p = 0.877; adult, p = 0.054) between the patients with a variant and healthy controls.

A comprehensive cardiological evaluation was performed for all pediatric patients regardless of variant status. No abnormalities were found on either echocardiography or electrocardiography of the patients, including holter monitoring performed in nine children.

Discussion

In the present study, evaluating *THRB* gene analysis in a series of 50 patients with signs and symptoms of RTHβ syndrome or a history of the index case in their families, eight *THRB* variants, of which three were novel, were detected in 30 out of 50 individuals.

Clinical manifestations of RTHβ syndrome vary widely. Although euthyroidism may be present in most patients with high TH values which are sufficient to stimulate the mutated receptors in most tissues, the phenotypes of the patients vary depending on the location of the hormonal resistance (9). The most common symptoms reported in the literature are goitre (65-85%), tachycardia (33-75%), attention-deficit/hyperactivity disorder and LD (33-68%), respectively (8,22,23). Less commonly reported symptoms were increased incidence of speech disorder, short stature, increased frequency of ear, nose, and throat infections, underweight in children, hearing loss, and cardiac abnormalities (23). In the present series, most of patients with a pathogenic or likely pathogenic variant were asymptomatic, consistent with the literature. RTHβ syndrome due to single amino acid changes in the *THRB* gene is reported to be milder than those due to insertion, deletion, or truncation variants (8,24). In our series, all patients with a pathogenic or likely pathogenic variant had missense heterozygous variants and we therefore attributed the high rate of asymptomatic cases to the universal presence of missense variants as the underlying molecular genetic etiology.

In a study evaluating RTHβ patients, 41.7% of the patients were reported to have received inappropriate treatments, including antithyroid therapy, thyroidectomy and radio-iodine ablation (25). While treatment is recommended for symptomatic cases, except for limited experiences with TH analogues (triiodothyroacetic acid, TRIAC), there is no specific treatment option for patients with RTHβ syndrome (26,27). Nevertheless, the rate of inappropriate treatment was decreased in the present study compared to previous publications, which may be attributed to the increased awareness of RTHβ and the opportunity to access molecular genetics analysis.

The increased prevalence of goitre despite mostly normal TSH has been reported to be due to alterations in terminal sialic acid residues, which enhance the biological potency of TSH (28). In our series, diffuse goitre was more prevalent in pediatric patients with a variant (56%) than in patients without a variant (37%).

TSHoma was detected in one of 17 patients with clinical/laboratory findings of RTHβ whilst no variants were detected in the *THRB* gene. There was no identified cause in the remaining 16 patients. The inability to explore the underlying etiology in these patients might be due to several factors, such as lack of facility to conduct further investigations and genetic analyses, errors in laboratory tests, the possible presence of somatic mosaicism, the existence of variants not covered by coding region sequencing, such as deep intronic variants, variants in inter- or intra-genic regions that regulate gene expression, or may be due to new modifier genes that has not yet been identified (1,4). Indeed, mosaicism in RTHβ was first reported by Mamanasiri et al. (29) who did not detect a variant in the *THRB* gene in 15% of individuals who had the RTHβ phenotype. Lack of measurement of serum biomarkers of TH effects on peripheral tissues, such as cholesterol, creatine kinase, alkaline phosphatase, osteocalcin and sex hormone binding globulin may be considered a limitation of our study. However, Refetoff et al. (3) reported that these values are less reliable unless measured before and after administration of T3.

The number of studies comparing patients with and without RTHβ is scarce. In the study of Brucker-Davis et al. (23), individuals with RTHβ were younger, exhibited a higher rate

of palpable goitre, had shorter stature, lower body weight, lower IQ scores, higher fT3 and fT4 levels, and higher T4/TSH and T4/T3 ratios. In the present series, in children with a variant, fT3 and fT4 values were higher than those without variants, while in the adult group, no differences were observed in these values. Furthermore, fT4/TSH, fT4/fT3, and fT3/fT4 ratios of patients with and without a variant did not differ. Compared to healthy controls, TSH levels were not different in children but were higher in adults with a variant. Moreover, the fT4/TSH ratio was higher in children with a variant whilst no difference was detected between the fT4/fT3 and fT3/fT4 ratios. This finding was consistent with the results of Refetoff et al. (3) indicating the total T3/total T4 ratio of patients with generalized RTHβ was only slightly above the mean value found in euthyroid-healthy individuals.

Individuals with RTHβ have been reported to have a higher likelihood of developing autoimmune thyroid disease (AITD) (30). In the present study, the rate of AITD was 23% in RTHβ and all cases were female which was consistent with the previously reported female predominance (25). While Gavin et al. (31) suggest that high TSH in RTHβ might activate intrathyroidal lymphocytes and increase proinflammatory cytokines and thyroid cell destruction, Barkoff et al. (30) reported that this hypothesis does not explain the increased autoimmunity in RTHβ. Moreover, the role of TH on the immune system is still poorly understood, and TH is reported to activate the immune system by acting directly on thymic epithelial cells, neutrophils, natural killer cells, macrophages, and dendritic cells (9,32,33). Besides, while there is a well-known female predominance in thyroid autoimmunity, there is no sex difference in RTHβ. However, all patients with thyroid autoimmunity were female in our series and some of the other studies suggest a need for further investigation of the mechanism behind the association between AITD and RTHβ which remains unclear.

RTHβ has been reported in patients with renal failure, ichthyosis-eczema, psychotic attacks, oesophagal atresia, reflux, celiac disease, congenital heart disease, and T1DM and T2DM (23,26,34,35). One patient in our series was also diagnosed with T1DM. TRs such as TRα1 and TRβ1 have been shown to be expressed in pancreatic beta cells (36). In addition, it has been reported that T3 induces the proliferation of pancreatic β-cells by activating phosphoinositide 3-kinase/Akt kinase pathways. Therefore, T3 could be considered a survival factor for islet cells, by protecting them from apoptosis (37). Except for the single case in our series, T1DM has only been reported in two other cases. There is insufficient evidence to consider whether this association was coincidental or not. Although the results of studies evaluating the effects of TH on insulin secretion are controversial, the effect of mutant TH receptors on islet cell function is not fully understood, and assessment of glucose metabolism in these patients is warranted (38,39).

Studies investigating the role of TH receptors in cancer have argued that decreased TR gene expression in cancer tissues due to hypermethylation or TR gene deletions can be explained by the potential tumour-suppressive function of TRs. Furthermore, these studies have highlighted the association of somatic variants in TRs with human cancers, suggesting that the loss of normal TR function might lead to uncontrolled cell growth and poor differentiation (40). In 2001, Taniyama et al. (41) reported the first case of RTHβ associated with PTC. In 2022, Fang et al. (42) published a literature review of 17 cases including their case. Two patients in Family 2 investigated in the present study were also reported in this series (21).

Study Limitations

Our study has some limitations. First, the sample size was relatively small. Due to the low frequency of RTHβ, further multicenter or nationwide studies with larger sample sizes are needed to elucidate the clinical characteristics and genotype-phenotype association of RTHβ. Second, in some patients, in whom we could not detect a *THRB* gene variant, further investigations using advanced genetic and laboratory analysis methods were not performed.

Conclusion

In conclusion, in the present study evaluating the clinical and genetic characteristics of a series of 30 Turkish patients with genetically confirmed RTHβ, the *THRB* gene variant database was expanded by the addition of three novel variants. Moreover, our results provide insights into prioritizing individuals for genetic analysis by comparing RTHβ patients with and without a variant. Although most patients with RTHβ are asymptomatic, prompt molecular genetic analysis for *THRB* gene variants and regular follow-up for potential concurrent autoimmune diseases and thyroid cancer is warranted.

Ethics

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Ankara Bilkent City Hospital with decision number: 23-5676, date: 22.11.2023.

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Gönül Büyükyılmaz, Keziban Toksoy Adıgüzel, Oya Topaloğlu, Büşra Erozan Çavdarlı, Cevdet Aydın, Sema Hepşen, Erman Çakal, Nur Semerci Gündüz, Fatih Gürbüz, Mehmet Boyraz, Serkan Bilge Koca, Hüseyin Demirbilek, Concept: Gönül Büyükyılmaz, Büşranur Çavdarlı, Hüseyin Demirbilek, Design: Gönül Büyükyılmaz, Büşranur Çavdarlı, Hüseyin Demirbilek, Data Collection or Processing: Gönül Büyükyılmaz, Keziban Toksoy Adıgüzel, Oya Topaloğlu, Büşra Erozan Çavdarlı, Cevdet Aydın, Sema Hepşen, Erman Çakal, Nur Semerci Gündüz, Fatih Gürbüz, Mehmet Boyraz, Serkan Bilge Koca, Hüseyin Demirbilek, Analysis or Interpretation: Gönül Büyükyılmaz, Serkan Bilge Koca, Büşra Erozan Çavdarlı, Nur Semerci Gündüz, Erman Çakal, Hüseyin Demirbilek, Literature Search: Gönül Büyükyılmaz, Serkan Bilge Koca, Nur Semerci Gündüz, Oya Topaloğlu, Cevdet Aydın, Hüseyin Demirbilek, Writing: Gönül Büyükyılmaz, Serkan Bilge Koca, Büşranur Çavdarlı, Hüseyin Demirbilek.

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ORIGINAL ARTICLE

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Assessment of Quadriceps Muscle Strength and Thickness in Adolescents with Polycystic Ovary Syndrome: A Case-control and Longitudinal Follow-up Study

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What is already known on this topic?

To date, no studies have investigated muscle strength and thickness in adolescents with polycystic ovary syndrome.

What this study adds?

Quadriceps muscle thickness and strength were comparable between adolescents with polycystic ovary syndrome and healthy controls. Girls using levonorgestrel showed significantly greater gains in quadriceps muscle strengths than those using cyproterone acetate.

Abstract

Objective: To date, muscle strength and thickness have not been investigated in adolescents with polycystic ovary syndrome (PCOS). This study aimed to investigate whether differences exist in these parameters between adolescents with PCOS and healthy controls. Additionally, we evaluated the effects of six months combined oral contraceptive (COC) treatment on quadriceps muscle characteristics.
Methods: The study included adolescents diagnosed with PCOS and healthy peers. Dynamometers were used to measure knee muscle and hand grip strengths, and ultrasound was used to measure quadriceps muscle thickness. In the PCOS group, all measurements were repeated after six months of COCs treatment.
Results: There were 20 participants in each of the PCOS and control groups. There were no significant differences between the groups in terms of age, weight, height, pubertal stage, Physical Activity Questionnaire scores, quadriceps muscle thickness, grip strength and isokinetic knee strengths at baseline. Within the PCOS group, significant increase were observed in weight, height, quadriceps strength and lipid levels after six months of treatment (all $p < 0.05$). Subgroup analysis of COC treatments revealed significantly greater gains in quadriceps muscle strength among levonorgestrel users (n = 6) compared to those using cyproterone acetate users (n = 13).
Conclusion: Quadriceps muscle thickness and strength were comparable between adolescent with PCOS and controls, indicating no intrinsic muscular deficit. However, significantly greater improvements in quadriceps muscle strength were observed in those using levonorgestrel-containing COCs users compared to cyproterone acetate users. These findings suggest a potential role of progestin androgenicity in muscle strength. Further longitudinal studies with larger cohorts are warranted to validate these preliminary findings and to explore the impact of COCs with varying androgenic properties.
Keywords: PCOS, isokinetic, ultrasound, levonorgestrel, oral contraceptive

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Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous condition that occurs most commonly in women of reproductive age. It is characterized by hyperandrogenism and chronic anovulation. Symptoms may appear in adolescence, and the condition is associated with various comorbidities, such as insulin resistance/hyperinsulinemia, impaired glucose tolerance, hypertension, non-alcoholic fatty liver disease, dyslipidemia and sleep apnea (1). The criteria for diagnosing PCOS in adults are not considered valid in adolescents, and it is often difficult to diagnose PCOS in adolescents. The diagnosis is typically made when irregular menstrual bleeding persists for at least two years post-menarche, accompanied by clinical and/or biochemical hyperandrogenism (1,2).

Hormonal and metabolic changes such as hyperandrogenism, hyperinsulinism, and hyperlipidemia may lead to alterations in muscle morphology and strength in with PCOS patients. However, there is a paucity of publications on this subject, especially in adolescents with PCOS, and there are conflicting results (3,4,5,6). As androgens and insulin have anabolic effects on skeletal muscle, they may influence muscle mass and/or strength (7,8,9,10,11,12,13). Furthermore, progesterone in combined oral contraceptives (COCs), commonly prescribed to treat PCOS, may have androgenic or antiandrogenic effects (14), potentially causing muscle changes.

Therefore, the aim of this study was to investigate whether there are differences in quadriceps muscle thickness and strength between adolescents with PCOS and controls of similar age, anthropometric characteristics, and pubertal stage. The quadriceps muscle was selected for the study due to its functional importance and substantial muscle mass (15,16). Additionally, we aimed to evaluate the effects of different COCs treatments on this muscle. We found no published studies investigating quadriceps muscle thickness by using musculoskeletal ultrasound (US) in adolescents diagnosed with PCOS.

Methods

This study was conducted at the Departments of Adolescent Health and Physical Medicine and Rehabilitation, Hacettepe University Faculty of Medicine, Türkiye. Approval was obtained from the Hacettepe University Faculty of Medicine Local Ethics Committee (decision number: KA-20083, date: 20.10.2020). The study met the standards of the Declaration of Helsinki for human experimentation. The study group consisted of adolescents with PCOS and healthy controls of similar age, weight, height and pubertal stage who were admitted to the adolescent health unit between September 2020 and December 2022. Informed consent was obtained from all participants and their families. PCOS diagnosis was made using the 2015 Pediatric Endocrine Society criteria (17). Biochemical hyperandrogenism was defined as a total testosterone level >42 ng/dL, and clinical hyperandrogenism was considered by a modified Ferriman-Gallwey score was ≥8 (1,2).

Considering that the mean age of menarche in Turkish females is 12.2 ± 0.9 years (18) participants were selected from adolescents aged 14 to 18 years whose menarche had already occurred, and pubertal development was Tanner stage ≥4. Adolescents engaged in regular training or sports, having chronic systemic diseases, on prescription or non-prescription drugs (including those affecting testosterone metabolism), or with mentally disabilities were excluded.

Study Protocol

Each participant's height and weight were measured using a stadiometer and a digital scale. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2) and expressed as BMI standard deviation (SD) score (BMI Z-scores) based on national data for Türkiye. Adolescents with a BMI at or above the 95th percentile were classified as obese, and those with BMI between the 85th and 94.9th percentiles as overweight, and those with BMI between the 5th and 84.9th percentiles as normal weight (19). Pubertal staging was done according to the Marshall-Tanner system (20). Hair growth in the patient group was assessed using the modified Ferriman-Gallwey scale (FGS) (21).

Lifestyle changes, healthy nutrition, and walking for 30-45 minutes at least 3 days a week, 15 minutes of weight training, and 15 minutes of muscle-strengthening exercise were recommended for all participants. In addition, a weight-loss diet was also recommended for both overweight and obese patients. Patients were monitored monthly to compliance with these suggestions. All patients were prescribed either COCs or with progestin-only drugs if COCs were contraindicated. COC regimens included 0.15 mg levonorgestrel and 0.03 mg ethinyl estradiol (Microgynon®) or a combination of 2 mg cyproterone acetate and 0.035 mg ethinyl estradiol (Diane 35®), while norethisterone (Primolut®) was used for progestin-only drug. In our routine clinical practice, Microgynon® and Diane 35® were the preferred COCs.

Laboratory Measurements

At the start of the study, serum samples were collected to diagnose PCOS, evaluate metabolic and hormonal changes, and exclude other differential diagnoses. To evaluate the

metabolic/hormonal changes, biochemical and hormonal tests were also performed after sixth month of treatment. Serum samples for glucose, insulin, triglyceride, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), total cholesterol, and luteinizing hormone (LH), follicle-stimulating hormone (FSH), and total were obtained after an 8-hour fast at 8:00 AM. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the standard formula: HOMA-IR = [(fasting insulin (uU/mL) x fasting plasma glucose (mg/dL)) / 405].

Isokinetic Muscle Strength Assessment

Prior to treatment, all participants underwent isokinetic measurement of knee extension and flexion strength on the dominant side using the Biodex System 3 Pro device (Biodex Medical Systems, Shirley, NY, USA) (22). After a 5-minute warm-up, participants were seated with the hip/knee joints in 90° flexion, the lateral femoral condyle of the femur positioned through the center of motion of the dynamometer and secured to the distal end of the dynamometer with a strap two cm above the malleoli. The thighs/trunk were secured to the seat with straps to prevent force distribution and ensure stabilization. The concentric strengths of the quadriceps and hamstrings muscles were measured for five repetitions at 60°/sec and 15 repetitions at 180°/sec, with 60-second rest intervals, and verbal motivation was provided during the test (23). Peak torque values were recorded for analyses.

Grip Strength Assessment

Dominant hand grip strength was measured using a hydraulic dynamometer (JAMAR Baseline Hydraulic Hand Dynamometer, Irvington, NY, USA). Participants were positioned in a standing position with the shoulder in full adduction, the elbow in 90° flexion and the hand in neutral. They were asked to perform three maximal grip strength trials, and the highest value was recorded.

Ultrasonographic Measurements

Prior to treatment, all participants underwent muscle US to assess quadriceps muscle thickness using a 7-12 MHz linear probe (Logiq P5, GE Medical Systems, AD). All US measurements were performed on the dominant hand side with minimal compression. Muscle thickness was measured in axial view at the 50% level between the anterior, superior iliac spine and the patella, with the participants in the supine position. Measurements were performed by a physiatrist (MK) experienced in musculoskeletal US.

Physical Activity Evaluation

The Physical Activity Questionnaire in Adolescents (PAQ-A) was used to assess the physical activity level over the last seven days of the school term (24). It consists of eight questions, scored from 1 (low activity) to 5 (high activity), and nine questions about barriers to physical activity in the past week, and was adapted for the Turkish population (25).

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (version 23, IBM Inc., Armonk, NY, USA). Descriptive statistics are presented as mean ± SD or median (interquartiles) for numerical variables. Normality was tested using the Shapiro-Wilk test. Student's t-test or Mann-Whitney U test was used to compare the variables between the groups, and paired t-test or Wilcoxon test was used to compare within-group/subgroup comparisons. Fisher's exact test was used for categorical variables. Correlations between continuous variables were assessed using Pearson or Spearman's correlation analysis. A $p < 0.05$ was considered statistically significant.

Results

A total of 20 adolescents with PCOS and 20 control adolescents were included in the study. Weight distribution in comparable between the groups, with the majority of participants classified as having normal weight (13 individual; 65% in each group). Table 1 shows the comparison of the clinical parameters between the groups. There were no significant differences between the groups in terms of o age, weight, height, pubertal stage, PAQ-A scores, quadriceps muscle thickness, grip strength, or isokinetic knee muscle strength parameters.

Among the PCOS group, 10 participants (50%) had clinical hyperandrogenism only, two (10%) had biochemical hyperandrogenism only, and eight (40%) presented with both. Of the 13 normal-weight patients, only four (30.7%) adhered to dietary recommendations and seven (53.8%) followed the walking recommendations of the exercise program. None of the overweight (n = 2) or obese (n = 5) patients adhered to either dietary or exercise program. Regarding pharmacologic treatment, six patients (30%) received Microgynon® (levonorgestrel 0.15 mg and ethinyl estradiol 0.03 mg), 13 patients (65%) received Diane 35® (cyproterone acetate 2 mg and ethinyl estradiol 0.035 mg), and one patient (5%) received Primolut® (norethisterone). All patients reported regular use of medication for six months.

In the PCOS group, weight was positively correlated with quadriceps muscle thickness ($r = 0.462$), knee extensor strength at 60° ($r = 0.496$), knee flexor strengths at 60° ($r = 0.514$) and 180° ($r = 0.545$), and handgrip strength ($r = 0.475$) (all $p < 0.05$). Similarly, BMI Z-score demonstrated

positive correlations with quadriceps muscle thickness (r = 0.672), knee extensor strength at 60° (r = 0.633), and knee flexor strengths at 60° flexion (r = 0.786), and 180° (r = 0.591) (all $p < 0.01$). In addition, serum insulin levels were positively correlated with knee extensor strength at 60° (r = 0.498), and knee flexor strengths at 60° (r = 0.457), and 180° (r = 0.565) (all $p < 0.05$). No significant correlations were found between quadriceps muscle thickness or strength values with height, modified FGS scores, disease duration, fasting blood glucose (FBG), or testosterone levels.

The PAQ-A scores indicated that both patients and controls were predominantly in the very low or low physical activity categories. Only three patients and two controls were categorized as moderately active. However, no correlations were found between PAQ-A scores and muscle thickness or strength in the PCOS group.

Following six months of treatment (Table 2), patients demonstrated significant increases in weight, height, quadriceps muscle strengths, HDL, LDL, total cholesterol, and triglyceride levels, while LH, FSH and total testosterone levels significantly decreased (all $p < 0.05$). No significant differences were observed in PAQ-A scores, quadriceps muscle thickness, or handgrip strength (all $p > 0.05$). Additionally, changes in weight and height did not correlate with changes in knee muscle strength after treatment (all $p > 0.05$).

Subgroup analyses based on type of COC use (Table 3) revealed that all knee muscle strength parameters increased significantly among users of levonorgestrel containing COCs, however, in the cyproterone acetate group, a significant increase was observed only in knee extension strength at 180° (all $p < 0.05$). Both subgroups demonstrated significant increases in LDL, total cholesterol, and triglyceride levels (all $p < 0.05$). However, HDL levels increased significantly only in the cyproterone acetate group ($p = 0.001$). Significant decreases in LH, FSH, and total testosterone levels were found in the levonorgestrel group, while only LH levels decreased in the cyproterone acetate group (all $p < 0.05$). Comparison of the post-treatment changes between the groups showed significant differences in knee extensor strength at 60° ($p = 0.009$) and HDL levels ($p = 0.001$).

Discussion

To our best notice, this is the first study investigating quadriceps muscle thickness and strength using objective US and isokinetic assessments in adolescents with PCOS, with additional analyses following six months of different COCs treatment. Our preliminary findings suggest that while muscle strength improves with treatment, this can be more attributable to COCs type than to intrinsic effects of PCOS itself.

At baseline, quadriceps muscle thickness and strength values did not differ significantly between adolescents with PCOS and healthy controls. Following six months of COC treatment, increases were observed in weight, height, quadriceps muscle strength and lipid profiles. The increases in quadriceps muscle strengths appeared to be associated with levonorgestrel, while elevations in lipid profile and hormone suppression were observed in both groups of COC users.

Although there is strong evidence that anabolic androgens increase muscle mass, their effects on muscle strength

Table 1. Comparison of clinical parameters of groups (n = 40)

Characteristics	PCOS (n = 20)	Control (n = 20)	p	Effect size
Age (years)	15.7 ± 0.6	15.8 ± 1.3	0.684	0.130
Weight (kg)	67.6 ± 20.3	58.0 ± 9.3	0.892	0.021
Height (cm)	163.5 ± 6.6	163.0 ± 6.5	0.821	0.072
BMI (kg/m²)	21.6 (19.5-25.3)	21.3 (19.0-23.5)	0.808	0.075
BMI Z-score	0.74 (0-1.68)	0.80 (-0.30-1.61)	0.760	0.071
Tanner stage (4 vs. 5)	5/15	5/15	0.347	
PAQ-A	2.2 ± 0.8	2.0 ± 0.8	0.461	0.291
Quadriceps MT (mm)	42.4 ± 7.4	40.8 ± 8.2	0.525	0.026
Grip strength (kg)	27.0 ± 4.1	26.5 ± 4.5	0.746	0.024
Knee muscle strength (Nm)				
60° extension	93.2 ± 29.1	94.5 ± 25.5	0.889	0.002
60° flexion	44.3 ± 17.1	43.0 ± 11.2	0.774	0.006
180° extension	61.8 ± 20.5	59.7 ± 18.8	0.402	0.133
180° flexion	37.7 ± 14.5	38.8 ± 10.7	0.791	0.007

Data are shown as mean ± SD, median (interquartiles) or number (n).
PAQ-A: Physical Activity Questionnaire in Adolescents, MT: muscle thickness

Table 2. Clinical parameters of the patients at baseline and after treatment (n = 20)

Characteristic	Baseline	After treatment	p	Effect size
Weight (kg)	67.6 ± 20.3	69.1 ± 17.2	**0.035**	0.473
Height (cm)	163.5 ± 6.6	163.9 ± 6.6	**0.010**	0.640
BMI (kg/m²)	21.6 (19.5-25.3)	23.2 (21.6-28.8)	**0.030**	0.486
BMI Z-score	0.74 (0-1.68)	0.70 (0.28-1.64)	0.093	0.376
PAQ-A	2.2 ± 0.8	1.9 ± 0.7	0.105	0.291
Quadriceps MT (mm)	42.4 ± 7.4	43.8 ± 8.1	0.117	0.026
Grip strength (kg)	27.0 ± 4.1	27.8 ± 3.9	0.096	0.024
Knee muscle strength (Nm)				
60° extension	93.2 ± 29.1	103.5 ± 21.5	**0.008**	0.002
60° flexion	44.3 ± 17.1	48.0 ± 14.1	**0.018**	0.006
180° extension	50.2 ± 17.0	60.7 ± 20.8	**0.001**	0.133
180° flexion	37.7 ± 14.4	42.4 ± 13.1	0.075	0.007
Laboratory test				
LDL (mg/dL)	106.0 ± 24.5	132.4 ± 27.6	**0.001**	0.891
Total cholesterol (mg/dL)	171.0 ± 32.7	209.2 ± 39.0	**< 0.001**	1.028
Triglyceride (mg/dL)	91.3 ± 40.1	129.6 ± 49.8	**0.003**	0.668
HDL (mg/dL)	52.0 ± 11.8	58.9 ± 11.8	**0.030**	0.486
LH (mIU/mL)	9.5 ± 5.2	2.9 ± 3.8	**< 0.001**	0.938
FSH (mIU/mL)	5.3 ± 1.9	3.4 ± 3.0	**0.017**	0.606
Total testosterone (ng/dL)	45.7 ± 21.7	29.1 ± 13.3	0.001	0.890
FBG (mg/dL)	85.4 ± 7.8	83.8 ± 8.6	0.466	0.163
Insulin (µIU/mL)	11.1 ± 5.0	14.0 ± 7.9	0.126	0.342
HOMA-IR	2.9 ± 2.2	3.0 ± 1.5	0.204	0.284
ALT (U/L)	17.6 ± 10.7	23.0 ± 15.5	0.099	0.369
AST (U/L)	18.1 ± 5.6	20.8 ± 10.9	0.222	0.273

Data are shown as mean ± SD or median (interquartiles).
PAQ-A: Physical Activity Questionnaire in Adolescents, MT: muscle thickness, LDL: low density lipoprotein cholesterol, HDL: high density lipoprotein cholesterol, LH: luteinizing hormone, FSH: follicle stimulating hormone, FBG: fasting blood glucose, ALT: alanine aminotransferase, AST: aspartate aminotransferase, SD: standard deviation, BMI: body mass index, NB: statistically significant variables are shown as bold

remain unclear (9,10,11). Studies investigating the effects of hyperandrogenism on skeletal muscle in PCOS patients are limited in adults and lacking in adolescents (3,4,5,6). Thomson et al. (4) compared 10 adult patients with PCOS to 16 healthy controls using isokinetic dynamometer measurements and found no difference in quadriceps muscle strength. Our results are consistent with these findings. Additionally, the same study also found no correlation between testosterone levels quadriceps muscle strength, suggesting that hyperandrogenism may not play a significant role in determining muscle strength in this population. Nonetheless, this relationship remains a topic of debate in the literature (26).

A recent meta-analysis (26) investigating the relationship between muscle strength and mass in adults reported that PCOS patients had greater total muscle mass compared to controls. While no association was found between total muscle mass and total testosterone levels, a significant correlation was observed between BMI and total muscle mass. The meta-analysis also emphasized the inconsistent findings in the literature regarding muscle strength in PCOS-some studies reported an increase, while others observed a decrease. The authors concluded that further research is necessary to clarify these discrepancies (26).

In the present study, quadriceps muscle thickness, recognized as a valid and reliable parameter in current sarcopenia guidelines (39), was measured and compared to the muscle mass assessment methods cited in the referenced meta-analysis. No significant differences were observed in quadriceps muscle mass or strength between adolescents with PCOS and controls. Consistent with the meta-analysis findings, quadriceps muscle thickness was positively correlated with BMI-Z scores, but not with testosterone or insulin levels. However, a significant correlation was observed between insulin levels and certain knee muscle strength parameters.

Table 3. Clinical parameters of the patients at baseline and after treatment (n = 19)

Characteristic		Levonorgestrel-EE (n = 6)	Cyproterone acetate-EE (n = 13)	Effect size
Knee strength (Nm)				
60° extension	Baseline	81.5 ± 9.0	98.7 ± 34.8	
	After	105.1 ± 12.2	103.1 ± 25.8	
	p	**0.002**	0.288	0.336
60° flexion	Baseline	41.7 ± 16.9	45.6 ± 18.4	
	After	55.5 ± 12.8	45.1 ± 14.5	
	p	**0.046**	0.919	0.132
180° extension	Baseline	54.5 ± 12.9	47.4 ± 18.9	
	After	68.5 ± 11.1	56.9 ± 24.2	
	p	**0.010**	**0.015**	0.066
180° flexion	Baseline	35.5 ± 12.3	39.3 ± 16.1	
	After	44.5 ± 17.0	41.4 ± 12.2	
	p	**0.017**	0.549	0.084
Laboratory tests				
LDL (mg/dL)	Baseline	92.3 ± 34.2	112.2 ± 18.0	
	After	116.3 ± 15.9	141.1 ± 29.4	
	p	**0.039**	**0.010**	0.006
Total cholesterol (mg/dL)	Baseline	145.7 ± 50.0	175.6 ± 25.0	
	After	182.7 ± 28.7	223.3 ± 36.2	
	p	**0.034**	**0.006**	0.021
Triglyceride (mg/dL)	Baseline	80.5 ± 29.0	99.7 ± 44.7	
	After	109.7 ± 20.5	144.8 ± 52.6	
	p	**0.039**	**0.015**	0.024
HDL (mg/dL)	Baseline	54.8 ± 8.4	47.8 ± 7.7	
	After	52.5 ± 10.9	62.5 ± 11.5	
	p	**0.602**	**0.001**	0.531
LH (mIU/mL)	Baseline	8.0 ± 3.0	10.1 ± 6.1	
	After	2.2 ± 2.9	3.4 ± 4.3	
	p	**0.028**	**0.023**	0.003
FSH (mIU/mL)	Baseline	4.6 ± 1.4	5.7 ± 2.0	
	After	2.1 ± 2.2	3.9 ± 3.2	
	p	**0.029**	0.118	0.012
Total testosterone (ng/dL)	Baseline	42.6 ± 9.1	47.2 ± 26.4	
	After	21.6 ± 12.4	33.3 ± 12.8	
	p	0.022	0.056	0.025

Data are shown as mean ± SD. Statistically significant variables are shown in bold.
Baseline: at start of treatment period, After: following six months of treatment with the specified COC, EE: ethinyl estradiol, LDL: low density lipoprotein cholesterol, HDL: high density lipoprotein cholesterol, LH: luteinizing hormone, FSH: follicle stimulating hormone, COC: combined oral contraceptive, SD: standard deviation

Aksun et al. (27) reported that short-term use of COCs (121 days) did not affect muscle composition or strength in their study of 20 adult patients with PCOS, in which muscle strength and body composition were evaluated. They also identified a correlation between total testosterone levels and mean lower extremity power. In contrast, our study did not observe any association between total testosterone and muscle strength. The discrepancy may be attributed to differences in the characteristics of the study populations. Additionally, the shorter duration of COC use in the study of Aksun et al. (27) compared to the present study may have influenced the divergent muscle strength outcomes observed following treatment.

In a study by Kogure et al. (28), which evaluated physical performance in adults with PCOS and control groups using a hand dynamometer, 70 women in the PCOS group were compared with 93 participants in the control group. The study reported that handgrip strength was significantly higher in the PCOS group and was positively correlated with

lean body mass. However, in our study, we observed similar handgrip strength values between the patient and control groups. Furthermore, no correlation was found between BMI-Z scores and handgrip strength.

A systematic review of limited studies investigating the effects of COCs on muscle hypertrophy, power and strength adaptations to resistance exercise concluded that there is no evidence-based rationale either supporting or against the use of COCs (29). However, the presence of estrogen and progesterone receptors in skeletal muscle suggests potential direct effects of both endogenous and exogenous estrogen and progesterone hormones on muscle tissue (30). Although androgens are known to have anabolic effects on skeletal muscle (7,8), the androgenic or antiandrogenic properties of COCs vary depending on the specific progestin content. A molecular study has shown that second-generation COC use in young, untrained women led to an increase in satellite cell numbers and skeletal muscle molecular markers after 10 weeks of resistance training, compared with non-users (30). Additionally, a COC containing progestin (Dienogest) at physiologic concentrations was shown to positively influence muscle cell proliferation and myogenic potential *in vitro*, suggesting a possible anabolic effect of certain COCs *in vivo* (31).

A meta-analysis including nine studies in adults reported no significant effect of COC use on muscle strength (32). However, the limitations of this analysis included the lack of consideration for differences in progestin content across studies, small sample sizes, and methodological inconsistencies in measuring muscle mass and strength. In the present study, we examined the impact of progestin content on muscle thickness and strength in adolescents with PCOS over a 6-month treatment period. Among progestin-containing COCs, Levonorgestrel exhibits the strongest androgenic activity, whereas cyproterone acetate demonstrates anti-androgenic properties (14). Our findings revealed significantly greater improvements in all measures of knee muscle strength among girls who used levonorgestrel-containing COCs compared to those who received cyproterone acetate. These results suggest that the differing androgenic or anti-androgenic effects of progestins, despite similar ethinyl estradiol content, may account for the observed differences in muscle strength outcomes.

Although previous studies have investigated the effect of COCs on exercise adaptation, their impact on exercise performance remains poorly understood. Dalgaard et al. evaluated the effects of third-generation COCs on muscular adaptations to resistance training in young, untrained women (33). Following a 10-week resistance training program, COC users (n = 14) exhibited a trend towards a greater increase in quadriceps cross-sectional area (p = 0.06) and a significantly greater increase in type 1 muscle fiber area (p = 0.04) compared with non-users (n = 14). Similarly, Ruiæ et al. (34) examined the (anti)androgenic effects of COCs on muscle strength and fat-free mass over a 16-week of exercise program in young women. Their findings indicated that the use of COC containing levonorgestrel (n = 24) led to significantly greater gains in muscle strength and fat-free mass compared to COC containing cyproterone acetate (n = 26). The authors attributed the relatively lower increase in the cyproterone acetate group to its antiandrogen properties. In contrast, our findings suggest that the more pronounced gains in muscle strength observed in levonorgestrel users may be attributable to the higher androgenic potential of levonorgestrel (14).

The safety and metabolic effects of COCs in individuals with PCOS remain to be fully elucidated. In our study, patients received progestins in the form of cyproterone acetate (n = 13), levonorgestrel (n = 6), or norethisterone (n = 1). Intrauterine contraceptive devices containing levonorgestrel have been associated with clinically meaningful changes in clinical and metabolic parameters (35). It has been reported that COCs containing cyproterone acetate or third generation progestin can lead to elevations in the lipid parameters, without affecting body weight or FBG (36). In a study including 14 to 19 years with PCOS, treatment with either desogestrel and ethinyl estradiol or cyproterone acetate and ethinyl estradiol resulted in significant increases in lipid profile in both drug groups (37). Consistently, we observed increases in the lipid levels following six months of treatment; however FBG, insulin levels, and HOMA-IR values remained unchanged. These changes in lipids may be attributed to the ethinyl estradiol and/or progestin components of the COCs.

Study Limitations

This study has several limitations. First, the sample size was relatively small, and only a single follow-up measurement was conducted. However, it is known that the effect of exercise or hormonal interventions on muscles typically progress over time, often beginning with improvements in power, followed by strength, and eventually increases in muscle mass (38,39). Although our findings indicated greater increases in quadriceps muscle strengths with levonorgestrel containing COC treatment compared to cyproterone acetate, we hypothesize that significant increases in quadriceps muscle thickness may have been observed with a larger sample size and/or a longer duration of treatment.

Conclusion

In summary, adolescents with PCOS showed similar quadriceps muscle thickness and strengths compared to healthy controls, suggesting no intrinsic deficit in muscle thickness and function. Knee muscle strength improvements observed after six months of COC treatment seem to be more closely related to weight gain and the COC formulation (containing levonorgestrel) than changes in physical activity or muscle hypertrophy. Larger, long-term studies are needed to clarify our preliminary but novel findings.

Ethics

Ethics Committee Approval: Approval was obtained from the Hacettepe University Faculty of Medicine Local Ethics Committee (decision number: KA-20083, date: 20.10.2020).

Informed Consent: Informed consent was obtained from all participants and their families.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ayşe Gül Güven, Murat Kara, Sinem Güneri, Demet Aygün Arı, Erdem Karabulut, Hüseyin Demirbilek, Gürkan Bozdağ, Orhan Derman, Concept: Ayşe Gül Güven, Murat Kara, Hüseyin Demirbilek, Gürkan Bozdağ, Orhan Derman, Design: Ayşe Gül Güven, Murat Kara, Hüseyin Demirbilek, Gürkan Bozdağ, Orhan Derman, Data Collection or Processing: Ayşe Gül Güven, Murat Kara, Sinem Güneri, Demet Aygün Arı, Orhan Derman, Analysis or Interpretation: Ayşe Gül Güven, Murat Kara, Erdem Karabulut, Orhan Derman, Literature Search: Ayşe Gül Güven, Murat Kara, Writing: Ayşe Gül Güven, Murat Kara, Sinem Güneri, Demet Aygün Arı, Hüseyin Demirbilek, Gürkan Bozdağ, Orhan Derman.

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CASE REPORT

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Clinical and Genetic Analyses of Two Unrelated 46,XX Girls with Combined 17α-hydroxylase/17,20-lyase Deficiency from China

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What is already known on this topic?

17α-hydroxylase/17,20-lyase deficiency (17OHD) is a rare autosomal recessive disease caused by homozygous or compound heterozygous mutations in *CYP17A1* gene. 17OHD can be classified into complete and partial forms based on the phenotypes resulting from cytochrome P450 17α-hydroxylase (P450c17) enzyme defects of different severities.

What this study adds?

For the first time, we describe a 46,XX case of complete 17OHD accompanied by nocturnal enuresis. We identified a new compound heterozygote (p.R347C and p.R362H) of *CYP17A1* gene in a 46,XX case with partial 17OHD.

Abstract

Cytochrome P450 17α-hydroxylase (P450c17) enzyme, encoded by the *CYP17A1* gene, catalyzes 17a-hydroxylation and 17,20-lyase reactions essential for cortisol and sex steroid synthesis. 17α-hydroxylase/17,20-lyase deficiency (17OHD) is a rare autosomal recessive disease caused by CYP17A1 mutations, classified into complete and partial forms based on P450c17 defect severity. We report two unrelated girls diagnosed with 17OHD at the age of 15 and 16. Both presented with primary amenorrhea, infantile genitalia, absent axillary and pubic hair, and hypergonadotropic hypogonadism. Case 1 exhibited undeveloped breasts, nocturnal enuresis, hypertension, hypokalemia, and reduced cortisol and 17α-hydroxyprogesterone. Case 2 showed a growth spurt, spontaneous breast development, elevated corticosterone, and decreased aldosterone. Both had a 46,XX karyotype. Genetic analysis revealed a homozygous p.S106P mutation in Case 1 and compound heterozygous p.R347C/p.R362H mutations in Case 2, with the latter representing a novel combination. Based on the clinical, laboratory and genetic findings, Case 1 and Case 2 were definitively diagnosed as complete and partial forms of 17OHD, respectively. Both received estrogen and glucocorticoid replacement therapy, leading to gradual development of the uterus and breasts, and the onset of first menstruation. In Case 1, hypertension, hypokalemia, and nocturnal enuresis were significantly alleviated. In conclusion, we report the first case of complete 17OHD accompanied by nocturnal enuresis and identify a novel compound heterozygote (p.R347C / p.R362H) of *CYP17A1* gene in a case of partial 17OHD.

Keywords: Congenital adrenal hyperplasia, 17α-hydroxylase/17,20-lyase deficiency, *CYP17A1* gene, mutation, nocturnal enuresis

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Introduction

Congenital adrenal hyperplasia (CAH) is a group of seven autosomal recessive diseases caused by mutations in genes encoding key enzymes involved in cortisol biosynthesis (1). The cytochrome P450 17α-hydroxylase (P450c17) enzyme, encoded by cytochrome P450 family 17 subfamily A member 1 (*CYP17A1*) gene, catalyzes both the 17a-hydroxylation and 17,20-lyase reactions required for the production of cortisol and sex steroids (2,3). 17a-hydroxylase/17,20-lyase deficiency (17OHD) is a rare type of CAH with an estimated incidence of 1:50,000 worldwide, accounting for about 1% of all CAH cases (1,4).

Various mutations in the *CYP17A1* gene cause the complete or partial loss of either or both 17a-hydroxylase and 17,20-lyase activities, which has been recognized as the molecular basis of 17OHD (3). Most mutations are associated with the classic phenotype of combined 17OHD, which results in the substantial reduction of cortisol and sex hormones, and the accumulation of mineralocorticoid precursors, such as deoxycorticosterone (DOC) and corticosterone (1). Deficiency of sex hormones causes 46,XY disorders of sex development and sexual infantilism in females. Both 46,XX and 46,XY patients have female external genitalia and usually present with absence of secondary sexual characteristics and hypergonadotropic hypogonadism during puberty (5). Elevated levels of DOC lead to hypertension and hypokalemia with suppression of aldosterone production, while excess corticosterone exhibiting glucocorticoid activity prevents patients from an adrenal crisis although cortisol production is low or absent (5). The clinical and laboratory features has been reported to be milder in patients with partial combined 17OHD due to a degree of sex hormone production (6).

Several major structural domains are necessary for the normal function of P450c17 enzyme based on the molecular modeling of enzyme structure, including the membrane attachment domain, the heme-binding site, the substrate-binding pocket, and the redox-partner binding site (2,3). *CYP17A1* mutations affecting the steroid-binding pocket [e.g., p.S106P (7), and D487_F489 deletion (8)] or heme-binding site [e.g., p.H373L (9), and p.R440H (10)] have been found to result in combined 17OHD either completely or partially, whereas mutations in the redox-partner binding site [e.g., p.R347H (11), and p.R358Q (11)] may preferentially impair 17,20-lyase activity. Thus, genetic mutation analysis is critical for making definite diagnosis and understanding the molecular mechanism of 17OHD.

Here, we report two unrelated 46,XX cases, one with complete and one with partial 17OHD. The preliminary diagnoses were made by the estimation of clinical and laboratory features, and were confirmed by the identification of *CYP17A1* mutations.

Case Reports

Clinical and Laboratory Features at Baseline

Two unrelated, phenotypic girls with combined 17OHD are described. Both were referred to the gynecological endocrinology clinic during July to October in 2021.

Case 1 was a 15-year-old girl who presented because of primary amenorrhea and absence of secondary sexual characteristics. Her parents were first-degree cousins. They reported that she has been weak and sickly since childhood, and often felt tired. She had nocturnal enuresis since childhood, once or twice a night. She denied chronic constipation, fecal incontinence, and other urinary symptoms, including frequent micturition, urgent micturition, painful micturition, dysuria, or daytime incontinence. No significant psychological or behavioral problems were observed on clinical screening. She was 153.0 cm tall and weighed 33.0 kg, both significantly below the 50th percentile reference values for 15-year-old girls (159.8 cm and 49.8 kg) in China. Physical examination revealed a blood pressure of 144/101 mmHg, Tanner stage 1 breast development, infantile external genitalia, and absence of axillary and pubic hair. The patient also showed delayed bone age of approximately 11 years, and decreased bone mineral density with a T-score between -1 and -2.5. Ultrasound imaging exhibited a low-echo strip of 4.8 cm × 0.3 cm behind the bladder without ultrasonographically evident intrauterine space, a 2.6 cm × 1.3 cm echo-free area on the left ovary and a 2.8 cm × 1.3 cm echo-free area on the right ovary with 10-12 follicles in bilateral ovaries. Spina bifida occulta was excluded by X-ray examination. No urinary tract abnormalities were detected by ultrasound examination.

Case 2 was a 16-year-old girl who sought medical advice because of primary amenorrhea. The patient was the only daughter and her parents were not consanguineous. The patient began breast development and growth spurt about two years before presentation. Physical examination showed a weight of 57.0 kg, height of 164.0 cm, body mass index of 21.2 kg/m^2, blood pressure 131/89 mmHg, Tanner stage 2 breast development, infantile female external genitalia and no axillary or pubic hair. Ultrasound imaging revealed a small uterus (1.7 × 1.2 × 1.7 cm^3) as well as 3.0 cm × 2.3 cm and 3.8 cm × 2.2 cm echo-free areas on the left and right ovaries respectively. Bilateral adrenal glands were normal in size, as assessed by color ultrasonography.

The serum hormone and electrolyte concentrations of both patients were retrieved from the electronic medical records and are shown in Table 1. Hypokalemia was detected in Case 1. With regard to the sex hormone profile, reduced levels of estradiol, testosterone and dehydroepiandrosterone sulphate and elevated levels of progesterone and luteinizing hormone (LH) were detected in both cases, indicating hypergonadotropic hypogonadism. Low cortisol and 17α-hydroxyprogesterone (17OHP) were found in Case 1. DOC, corticosterone and aldosterone were measured in Case 2, and the results showed elevated corticosterone and decreased aldosterone.

Based on the clinical presentations and laboratory findings, Case 1 was initially diagnosed as complete 17OHD while Case 2 was suspected of suffering from partial 17OHD. More laboratory tests were conducted for Case 1 to investigate the potential cause of nocturnal enuresis. Routine blood test revealed normal counts of red blood cells, white blood cells and platelets. Renal function indicators, including creatinine 55.0 umol/L, uric acid 269.00 umol/L, and urea nitrogen 4.40 mmol/L, were within normal range. Normal fasting blood glucose (4.82 mmol/L) was observed. The results of routine urine examination were normal. The urine specific gravity was 1.01, within normal limits. Through the structured history and systematic physical examination, these secondary causes of nocturnal enuresis were excluded, including urinary system diseases, spina bifida occulta, diabetes mellitus, and diabetes insipidus.

Genetic Analyses

Molecular genetic testing was conducted for definitive diagnosis of 17OHD. The chromosome karyotype for both patients was 46,XX. Clinical exome sequencing, which included coding exons for about 5000 clinically relevant disease-causing genes (12), was performed for both patients at the AmCare Genomics Lab, Guangzhou, China. The enriched DNA samples were sequenced on the Illumina HiSeq2000 (Illumina, San Diego, CA, USA) with 150 bp, single-end read length. Gene variants were annotated using population and literature databases, including GnomAD, ClinVar, OMIM, and HGMD. The pathogenicity of gene variants was classified according to the American College of Medical Genetics guidelines (13). Suspected mutations were validated by Sanger sequencing of both the patients and their parents. Case 1 harbored a homozygous mutation

Table 1. Laboratory tests for two cases with 17α-hydroxylase/17,20-lyase deficiency at presentation

Parameters	Case 1	Case 2	Normal range
FSH, IU/L	29.33	7.90	3.5-12.5
LH, IU/L	41.52	20.30	2.4-12.6
Estradiol, pmol/L	18.35	18.35	45.4-854
Testosterone, nmol/L	0.087	0.094	0.29-1.67
Progesterone, nmol/L	28.30	34.29	0.18-2.84
Prolactin, mIU/L	390.10	181.50	102-496
SHBG, nmol/L	126.10	42.60	26.1-110
DHEAS, ug/dL	12.33	12.75	15-1000
ACTH, pg/mL	49.67	-	7.0-65.0
Cortisol, nmol/L	7.80	172.60	101.2-535.7
17OHP, nmol/L	0.150	6.850	1.32-7.07
DOC, ng/mL	-	0.257	≤0.30
Corticosterone, ng/mL	-	43.644	0.18-19.70
Aldosterone, ng/mL	-	0.055	0.07-0.35 (standing)
K, mmol/L	2.75	3.84	3.5-5.3
Na, mmol/L	143.00	141.30	137-147
Cl, mmol/L	105.20	105.70	99-110
Ca, mmol/L	2.48	2.41	2.15-2.55

FSH: follicle-stimulating hormone, LH: luteinizing hormone, SHBG: sex hormone binding globulin, DHEAS: dehydroepiandrosterone sulphate, ACTH: adrenocorticotropic hormone, 17OHP: 17α-hydroxyprogesterone, DOC: deoxycorticosterone

(c.316T > C, p.S106P) in exon 2 of *CYP17A1*, and both parents were heterozygous for the p.S106P mutation (Figure 1, Table 2). Case 2 was a compound heterozygote for p.R347C and p.R362H mutations in exon 6 of *CYP17A1*, which were inherited from her mother and father, respectively (Table 2, Figure 2).

Follow-up Data During Treatment

Upon definite diagnosis of 17OHD, Case 1 was treated with dexamethasone (0.5 mg per day) and estrogen (1 mg per day), and was also given vitamin D and calcium supplementation. Case 2 was treated with estrogen (1 mg per day), followed by the addition of prednisone (5 mg per day). The follow-up data of the two cases were collected at 3 months and 9 months after treatment, as shown in Table 3. For Case 1, there was significant weight gain, blood pressure approached the normal range, and serum potassium increased to normal level. The immature uterus and breasts developed gradually. Ultrasonography showed the uterus was $2.6 \times 1.9 \times 2.7$ cm^3, the left ovary 3.0 cm × 1.4 cm, and the right ovary 2.8 cm × 1.4 cm in size. First menstruation occurred after nine months of treatment. The nocturnal enuresis improved greatly, and had almost resolved after six months. Case 2 had her first menstruation after 4 months of treatment. Thereafter, menstrual flow gradually became regular, which occurred every 25-30 days and lasted 3 to 5 days. Her breasts and uterus continued to develop. The latest ultrasonography showed the uterus was $2.6 \times 1.7 \times 2.2$ cm^3, the left ovary 2.9 cm × 1.5 cm, and the right ovary 3.2 cm × 1.8 cm in size.

Written informed consents were obtained from the two patients and their parents to publish this study.

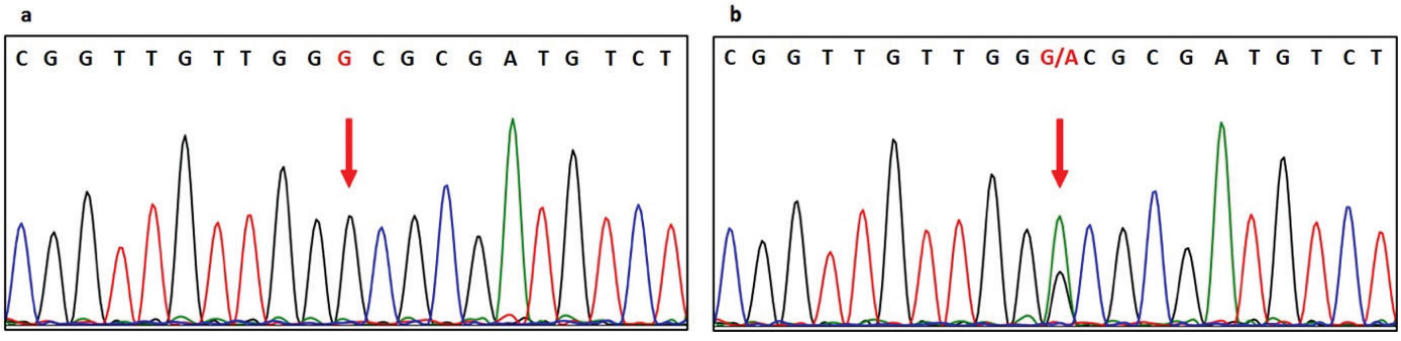

Figure 1. The *CYP17A1* gene mutation analysis for Case 1 and her parents. A) A homozygous missense mutation (A > G) at position 316 in exon 2 was detected in the patient. B) A heterozygous mutation (A > G) at position 316 in exon 2 was detected in both her mother and father

Table 2. Genetic analyses for two cases with 17α-hydroxylase/17,20-lyase deficiency (17OHD)

Parameters	Case 1	Case 2
Karyotype	46,XX	46,XX
Mutant gene	*CYP17A1* gene	*CYP17A1* gene
Mutation	c.316T > C (p.S106P)	c.1039C > T (p.R347C)/ c.1085G > A (p.R362H)
Zygosity	Homozygote	Compound heterozygote
MAF[a]	0.000008	0.000008/0.000032
Location	Exon 2	Exon 6
ACMG classification	Pathogenic	Pathogenic
Disease (OMIM)	17OHD	17OHD
Mutation inherited from mother	c.316T > C (p.S106P)	c.1039C > T (p.R347C)
Mutation inherited from father	c.316T > C (p.S106P)	c.1085G > A (p.R362H)
Loss of P450c17 enzyme acitivity[b]	Complete (7)	Partial (26)/complete (4)

[a]From gnomAD-Exomes, [b]From references.
MAF: Minor Allele Frequency, ACMG: the American College of Medical Genetics and Genomics

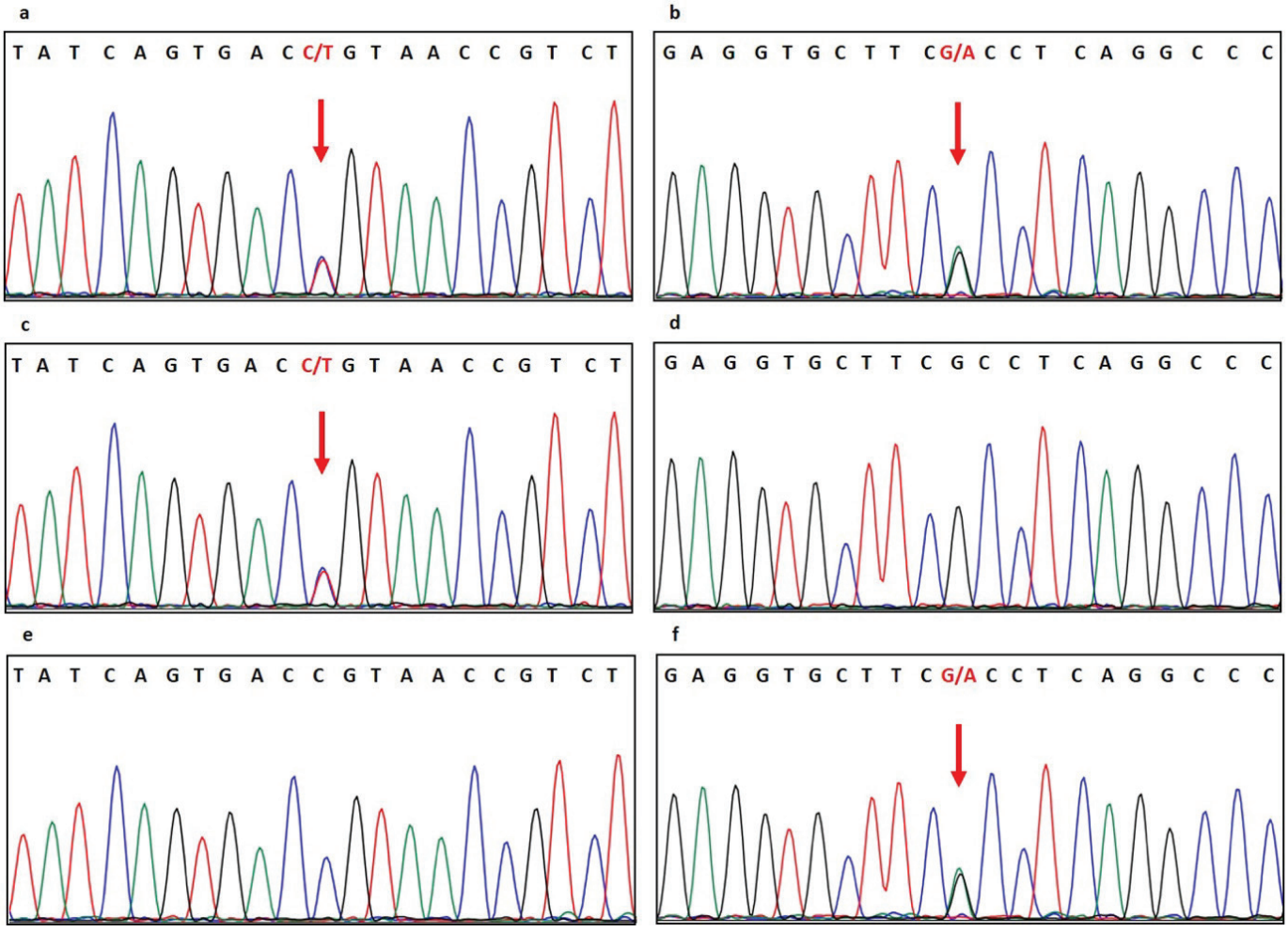

Figure 2. The *CYP17A1* gene mutation analysis for Case 2 and her parents. A) A heterozygous mutation (C > T) at position 1039 in exon 6 was detected in Case 2. B) A heterozygous mutation. (G > A) at position 1085 in exon 6 was detected in Case 2. C) A heterozygous mutation (C > T) at position 1039 in exon 6 was detected in her mother. D) A wild type at position 1085 in exon 6 was detected in her mother. E) A wild type at position 1039 in exon 6 was detected in her father. F) A heterozygous mutation (G > A) at position 1085 in exon 6 was detected in her father

Discussion

CAH due to 17OHD was firstly described in 1966 by Biglieri et al (14). To date there have been at least two hundred cases published. Notably, 46,XX cases are much rarer than 46,XY cases (15). The diagnosis of combined 17OHD in genetic females is generally made at puberty, when patients exhibit absent or delayed puberty development, and hypergonadotropic hypogonadism (16). This phenomenon was also observed in the two 46,XX cases in the current presentation.

Case 1 presented with the typical clinical and laboratory manifestations of complete deficiency of P450c17 enzyme, including primary amenorrhea, infantile external genitalia, no axillary or pubic hair, absent breast development, hypertension, hypokalemia, extremely low levels of cortisol and sex hormones but elevated progesterone, FSH and LH. It was not difficult to make a clinical diagnosis of complete 17OHD. However, Case 2 had a growth spurt and spontaneous breast development as well as normal levels of cortisol, 17OHP and DOC in spite of reduced serum estradiol and testosterone, indicating a less severe estrogenic and androgenic deficit caused by partial loss of both 17a-hydroxylase and 17,20-lyase activities.

In addition to the classic manifestations, Case 1 also showed delayed bone age, decreased bone density and long-lasting nocturnal enuresis. Research has found that bone age retardation and osteoporosis were relatively frequent in 17OHD patients and closely related to the reduced production of sex steroids (17). It is worth noting that this is the first report of nocturnal enuresis in 17OHD patients.

Table 3. The follow-up data of two cases with 17α-hydroxylase/17,20-lyase deficiency during treatment

Parameters	Case 1		Case 2	
	At 3 months	At 9 months	At 3 months	At 9 months
Height, cm	153.0	153.0	166.0	166.0
Weight, kg	38.0	41.5	59.0	61.0
BMI, kg/m^2	16.2	17.7	21.4	22.1
BP, mmHg	110/67	98/79	120/70	112/75
Tanner stage	B2P1	B3P1	B3P1	B4P1
FSH, IU/L	22.25	19.82	10.39	7.69
LH, IU/L	27.67	37.16	13.78	25.59
Estradiol, pmol/L	77.61	31.26	18.35	22.42
Testosterone, nmol/L	-	-	0.09	0.09
Progesterone, nmol/L	17.91	18.17	22.30	20.52
Prolactin, mIU/L	-	-	319.50	303.60
ACTH, pg/mL	36.29	5.90	-	122.71
Cortisol, nmol/L	0.10	38.30	-	133.50
K, mmol/L	3.52	5.17	4.18	-
Na, mmol/L	145	138.50	143	-
Cl, mmol/L	107.10	103.80	105.5	-
Ca, mmol/L	2.40	2.51	2.58	-

BMI: body mass index, BP: blood pressure, FSH: follicle-stimulating hormone, LH: luteinizing hormone, ACTH: adrenocorticotropic hormone

Nocturnal enuresis is intermittent involuntary voiding during sleep in the absence of physical disease in a child aged 5 years or more, which is the most common type of urinary incontinence in children (18). The prevalence of nocturnal enuresis decreases with age, affecting only about 1% of adolescents by age 15 years (19). The etiology of primary enuresis is not completely understood. It is presumed that long-lasting nocturnal enuresis in Case 1 may be linked to her elevated blood pressures, which was thought to cause suppression of vasopressin and sodium regulating hormones secretion resulting in increased renal excretion of solutes and water (20). Another possible explanation is that estrogen deficiency may affect the normal function of the female lower urinary tract, affecting urine storage and elimination. The bladder and urethra, which originate from the urogenital sinus, are under the influence of estrogen, just like the vagina (21). Evidence suggested that estrogen treatment can improve or even cure urinary incontinence, especially urge incontinence (22). Interestingly, the symptom of nocturnal enuresis in Case 1 improved greatly after six months of estrogen and dexamethasone treatment. Of course, coincidence cannot be ruled out because primary enuresis has a spontaneous disappearance rate of around 15% per year (18).

Using molecular diagnostics, a homozygous p.S106P and a compound heterozygous p.R347C/p.R362H mutation in *CYP17A1* were identified in Case 1 and Case 2, respectively. The findings confirmed the diagnosis of 17OHD, which has been recognized as an autosomal recessive disease caused by the homozygous or compound heterozygous mutations of *CYP17A1* gene. The *CYP17A1* gene, located on chromosome 10q24.3, consists of 8 exons and 7 introns encoding a 508 amino acid protein P450c17 (17). More than 100 mutations have been reported since the *CYP17A1* gene was first cloned in 1987 (23). The majority appear to be random, while several mutations reoccur in certain ethnic groups, suggesting a founder effect, such as p.W406R and p.R362C mutations in Brazilians (4), and D487_F489 deletion and p.Y329fs in Chinese (24,25). Here, though, the three variants identified in the present study are not prevalent in Chinese but they all have been identified in 17OHD cases previously. In addition, the enzymatic activities of these mutants have been reported and explained in the literature (4,7,26,27).

Homozygous S106P mutation was first reported in two unrelated Guamanian genetic males with the complete form of 17OHD in 1991 (7). Afterwards, two Chinese 17OHD patients were found to be compound heterozygotes for S106P and other mutations in *CYP17A1* gene (28,29). Site-

directed mutagenesis experiment showed that the mutant S106P had neither 17α-hydroxylase nor 17,20-lyase activity (7). According to molecular modeling of the human P450c17 sequence, the mutant S106P destroys all P450c17 enzyme activity by altering the positioning of I112 which is a highly conserved residue that forms one edge of the substrate-binding pocket (3).

There have been several previously reported patients carrying the c.1039C>T (p.R347C) mutation (25,26,30,31,32). The majority were compound heterozygotes (25,26,30), while only two cases were homozygote, a 67-year-old Japanese woman with partial combined 17OHD (31) and a 46,XY case with isolated 17,20-lyase deficiency (32). The arginine of codon 347 lies in the redox-partner binding site and contributes positive charges to the proximal surface of P450c17, at which cytochrome b5 interacts with the P450c17-oxidoreductase complex to promote electron transfer (2,3). Though it was found that normal functioning of the redox-partner binding site is essential for the 17α-hydroxylase/17,20-lyase activities of P450c17, the mutations affecting a cluster of basic residues usually lead to subtle defect in electron transfer and selectively disrupt 17,20-lyase activity without substantial reductions in 17α-hydroxylase activity, such as p.R347H and p.R358Q (11). Remarkably, R347C disrupts the function of the whole protein more seriously than p.R347H probably because of the formation of abnormal cysteine dimers (26). An *in vitro* study found that the R347C mutation had 13.6% and <1% of 17α-hydroxylase and 17,20-lyase activities, respectively (26). Nevertheless, some 17,20-lyase activity may be retained due to the accumulation of cytochrome b5 and oxidoreductase, resulting in the development of secondary sexual characteristics (11).

With regard to the other *CYP17A1* mutation in Case 2, p.R362H has been identified in a Mexican mestizo, a Turkish, and a Chinese previously, all with complete 17OHD (25,27,33). The R362 residue comprises part of the highly conserved ExxR motif at the C-terminus of the K helix, a motif present in all known cytochrome P450 enzymes (3,23). The hydrogen bonding between the adjacent E and R residues in this motif stabilizes the structure of the K helix, and helps to form the redox-partner binding site (3). Studies suggested that Arg362His replacement weakens hydrogen bonding within the ExxR motif and completely impaired the enzymatic activities (4,27).

Although the p.R347C and p.R362H mutations of *CYP17A1* gene have been reported separately before, their compound heterozygote was firstly described in Case 2 in the present study. This new compound heterogenous mutation leads to partial 17OHD, which may result from the affected function of the redox-partner binding site. Further cases or functional analyses are needed to draw a conclusion on genotype-phenotype correlations of the compound heterogenous mutation.

Conclusion

17OHD is a rare cause of CAH, and arises from the homozygous or compound heterozygous mutations of *CYP17A1*. The present study identified two unrelated 46,XX cases with complete and partial 17OHD respectively. The homozygous p.S106P mutation detected in the case with complete 17OHD has been reported previously. Although the p.R347C and p.R362H mutations of *CYP17A1* gene have been reported separately before, their compound heterozygote was firstly identified in Case 2 with partial 17OHD. Moreover, we describe a case of complete 17OHD accompanied by nocturnal enuresis for the first time.

Ethics

Informed Consent: Written informed consents were obtained from the two patients and their parents to publish this study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ting Han, Yingxia Wang, Yinglan Wu, Concept: Yamei Li, Yinglan Wu, Design: Yamei Li, Yinglan Wu, Data Collection or Processing: Yamei Li, Ting Han, Yingxia Wang, Analysis or Interpretation: Yamei Li, Jie Gao, Jianglin Zhang, Yinglan Wu, Literature Search: Yamei Li, Ting Han, Writing: Yamei Li, Jie Gao.

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CASE REPORT

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Sotos Syndrome and Nephrocalcinosis a Rare But Possible Association Due to Impact on Contiguous Genes

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What is already known on this topic?

Up to 15% of patients with Sotos syndrome present with renal disorders, the most common of which is vesicoureteral reflux. However, nephrocalcinosis is a less common clinical disorder reported in cases of Sotos syndrome due only to deletion that includes *SLC34A* and other genes, associated with phosphate wasting and hypercalciuria.

What this study adds?

In this patient group, delayed growth and impaired kidney function are possible and long-term follow-up is recommended. The exceptional circumstance presented in this brief report are the presentation and long-term follow-up of a patient with genetically confirmed Sotos Syndrome in association with a less common clinical disorder, nephrocalcinosis. This association can be explained by the alteration of contiguous genes included in the deletion found in 5q35 and the unusual fibroblast growth factor 23 values.

Abstract

One-month old, breastfeeding infant, born at term, with normal anthropometric measurements at birth was referred to Pediatric Nephrology due to a nephrocalcinosis. The patient presented with dysmorphic features and heart disease. A metabolic study was conducted on blood and urine yielding results within normal parameters, except for the renal concentration test and acidification test. At six months of age, the patient presented with overgrowth, which along with other clinical signs aroused the suspicion of Sotos syndrome. Molecular genetic testing identified a heterozygous deletion in 5q35 between bands q35.2 and q35.3, affecting the genes *NSD1, SLC34A1* and *FGFR4*, which was compatible with Sotos syndrome and with nephrocalcinosis as a rare association.

Keywords: Sotos syndrome, nephrocalcinosis, *NSD1, SCL34A1, FGFR4*, case report

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Introduction

Sotos syndrome is a dominant, autosomal, hereditary disease with a prevalence of approximately 1 in 14,000 newborns (1,2). It is characterized by overgrowth, a distinctive facial phenotype and learning disabilities (3). Sotos syndrome is a multisystemic disorder and up to 15% of patients present with renal disorders, the most common of which is vesicoureteral reflux. Most cases of Sotos syndrome are found to harbor point mutations in *NSD1* gene and these are *de novo* in over 95% of cases. Clinical findings in European patients show 10-15% microdeletion of 5q35 affecting this gene, as found in the presented case discussed herein (2). The exceptional circumstance in this patient is the association of genetically confirmed Sotos syndrome in association with a less common clinical disorder, nephrocalcinosis. This association may be explained by the alteration of contiguous genes included in the deletion found.

Case Report

A 37-day old, male, breast-feeding infant was referred to pediatric nephrology due to a finding of bilateral, medullary nephrocalcinosis on an ultrasound examination conducted at one month of life. The patient was born from a well-mamaged pregnancy without incident, with normal prenatal echographic findings, from non-consanguineous parents and with no relevant family history. Labor at 42 weeks of pregnancy, concluded by C-section due to failed induction, with adequate weight at birth of 3900 g [+0.93 standard deviation (SD) score (SDS)]. The infant was admitted to the neonatal unit at 3 hours of life due to hypoglycemia, developed histologically-confirmed eosinophilic colitis symptoms at eight days of life and exhibited good progress after 10 days of parenteral feeding. During his hospital stay, the patient received aminoglycoside treatment.

The infant was monitored in pediatric cardiology due to *ostium secundum* atrial septal defect without hemodynamic compromise, patent ductus arteriosum (PDA) and mild pulmonary valvular stenosis, and in pediatric neurology due to mild hypotonia and subtle dysmorphic features (broad forehead, lack of frontotemporal hair, long and narrow face, single transverse palmar crease, scaphocephaly and anteverted nares). There was also a front left paraventericular ependymal cyst and FLAIR hyperintensity in the corona radiata on brain magnetic resonance imaging. Stimulation was begun in early care due to mild neurodevelopmental retardation. Neonatal metabolic screening was compatible with normal characteristics and otoacoustic emissions yielded negative results. He received a daily supplement of vitamin D_3 (400 IU) and omeprazole. Normal ophthalmological examination was reported.

Given the presence of bilateral nephrocalcinosis and the patient's history, he was investigated for an initial differential diagnosis of a probable syndrome, a renal condition and/or a multi-factor etiology due to associated neonatal comorbidity. A metabolic study was conducted on blood and urine with normal results, including thyroid hormones, lactate, pyruvate and organic acids, although a gradual decrease of blood phosphate levels was observed, reaching values even lower than the reference values for the patient's age, with phosphate reabsorption and normal fibroblast growth factor 23 (FGF23) values which were inappropriate for phosphatemia. Of note, he also exhibited an increase in 1,25 $(OH)_2$ vitamin D_3 and parathyroid hormone (PTH) was in the low-normal range, appropriate for his serum calcium levels which were at the upper end of the normal range (Table 1). He had normal proteinuria results, including tubular proteinuria. Initially, renal function showed an increase in cystatin C and N-acetylglucosaminidase (NAG) values, as well as abnormal renal concentration test results after stimulation with desmopresin, reaching maximum urinary osmolality of 307 mOsm/Kg (normal value >562 mOsm/Kg), as well as altered renal acidification capacity, reaching a maximum pCO_2 of 54 mmHg after stimulation with bicarbonate and acetazolamide (normal value >70 mmHg).

At four months of age, he presented again with *Escherichia coli* urinary tract infection with fever, with normal cystourethrogram, while the renal gammagraphy showed right kidney hypodysplasia with no associated cortical lesions. At six months, overgrowth was detected with weight +2.50 SDS, height +2.63 SDS and head circumference +2.38 SDS. However, the anthropometric values gradually return to normal. Furthermore, bone age corresponded to patient's age and no associated skeletal disorders were observed.

Sotos syndrome was suspected due to clinical observations of dysmorphic syndrome, overgrowth and heart disease. Given the association with nephrocalcinosis, an expanded genetic study was requested to search for microdeletions that affect genes other than *NSD1* due to contiguity. The fluorescence in situ hybridization analysis revelaed an absence of hybridization signal in one of the loci for Sotos syndrome on chromosome 5, with a normal pattern in the parents (Figure 1). In addition, oligo-comparative genomic hybridization (CGH) array confirmed a *de novo* deletion of approximately 2 Mb in 5q35.2 to q35.3 (from 175,580,042 to 177,386,153 bp) in the patient, as oligo-CGH array using maternal versus paternal DNA yielded a

Table 1. Analytical data of the patient over time

Age	1 m	2 y	4 y	6 y	7 y
Plasma (reference values in parentheses)					
Cr (mg/dL) (0.32-0.59)	0.34	0.34	0.46	0.59	0.55
Cystatin C (mg/L) (0.62-1.11)	2	0.62	1.02	0.96	-
Urate (mg/dL) (2.2-4.5)	2.4	2.7	-	3.1	2.6
Ion Ca (mg/dL) (4.6-5.3)	5.8	5.5	5	5.3	-
Phosphate (mg/dL) (4.1-5.9)	5.3	3.9	3.9	3.2	4
Magnesium (mg/dL) (1.6-2.6)	2.1	2.0	1.8	1.8	1.8
AP (U/L) (142-335)	500	335	302	379	319
PTH (pg/mL) (11-60)	14	24	14	15	14
25 (OH) Vit D3 (ng/mL) (17-49)	-	42	39	33	39
1,25 $(OH)_2$ Vit D3 (pg/mL) (45-102)	-	249	-	99	-
Intact FGF23 (pg/mL) (36 ± 18)	-	51.3	-	-	-
pH_p (7.35-7.45)	-	7.37	7.39	7.37	7.38
Bicarbonate (mEq/L) (20-26)	-	25	23	22	23
Urine (reference values in parentheses)					
pH_u	7.5	7	7	7	7.5
Ca/Cr (mg/mg) (< 0.20-0.28)	0.52	0.35	0.28	0.26	0.25
Citrate/Cr (mg/g) (> 250-420)	706	687	307	172	314
Ca/citrate (mg/mg) (< 0.33)	0.74	0.51	0.92	1.49	0.58
Oxalate/Cr (mg/g) (< 110)	130	60	50	-	41
Prot/Cr (mg/mg) (< 0.2)	0.68	0.18	0.18	0.11	0.11
NAG/Cr (U/g) (< 6-11)	150	15	-	3	3
TRP (mg/dL GFR) (91.05 ± 4.71)	91	90	79	80	88
TP/GFR (mg/dL) (4.6 ± 0.6)	4.8	3.62	3.1	2.3	3.5
V/GFR (mL/dL GFR) (0.59 ± 0.22)	1.44	1.10	1.20	0.91	0.98

m: month, y: years, Cr: creatinine, Ca: calcium, AP: alkaline phosphatase, PTH: parathyroid hormone, 25(OH) vit D_3: 25-hydroxyvitamin D_3, 1,25$(OH)_2$ vit D_3: 1,25-dihydroxyvitamin D_3, pHp: plasma pH, pHu: urine pH, Prot/Cr: protein/creatinine ratio, TRP: tubular reabsorption of phosphate, TP/GFR: tubular reabsorption of phosphate per dL of glomerular filtrate, V/GFR: urinary volume per dL of glomerular filtrate

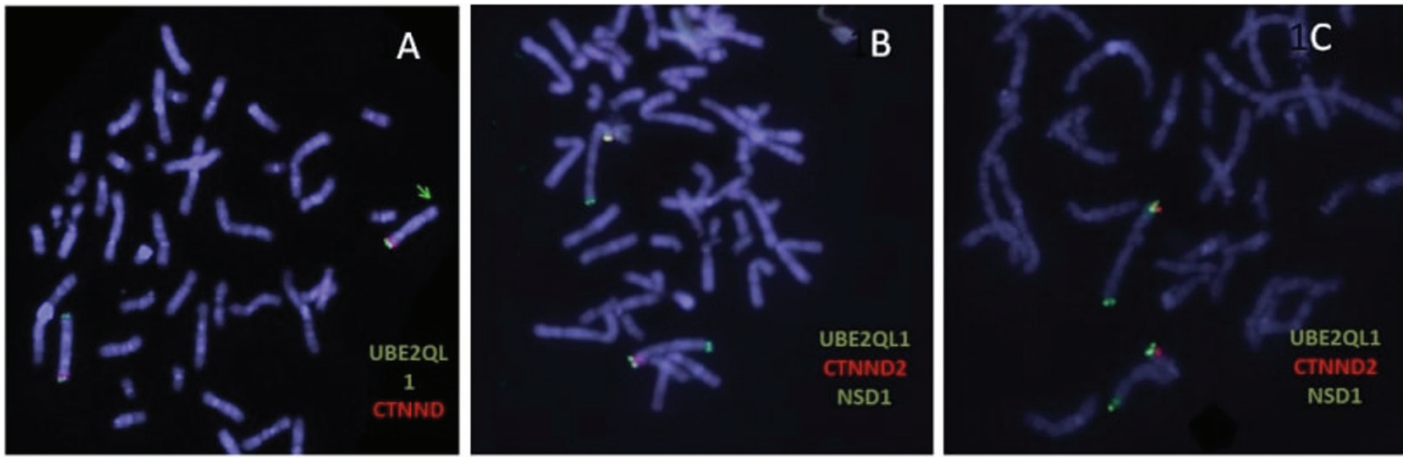

Figure 1. (A) FISH of the patient with probe combined for the loci of the Cri-du-chat (*UBE2QL1*, 5p15.31; *CTNND2*, 5p15.2) and Sotos (*NSD1*, 5q35) syndromes. The signals in 5p15 are present in both chromosome 5 pairs. The arrow shows lack of hybridization of the *NSD1* clone (green) in one of the chromosome 5 pairs. B, C) FISH of the father and mother, respectively, with the same probe. Both chromosome 5 pairs show signals both in 5p15 and 5q35

FISH: fluorescence in situ hybridization

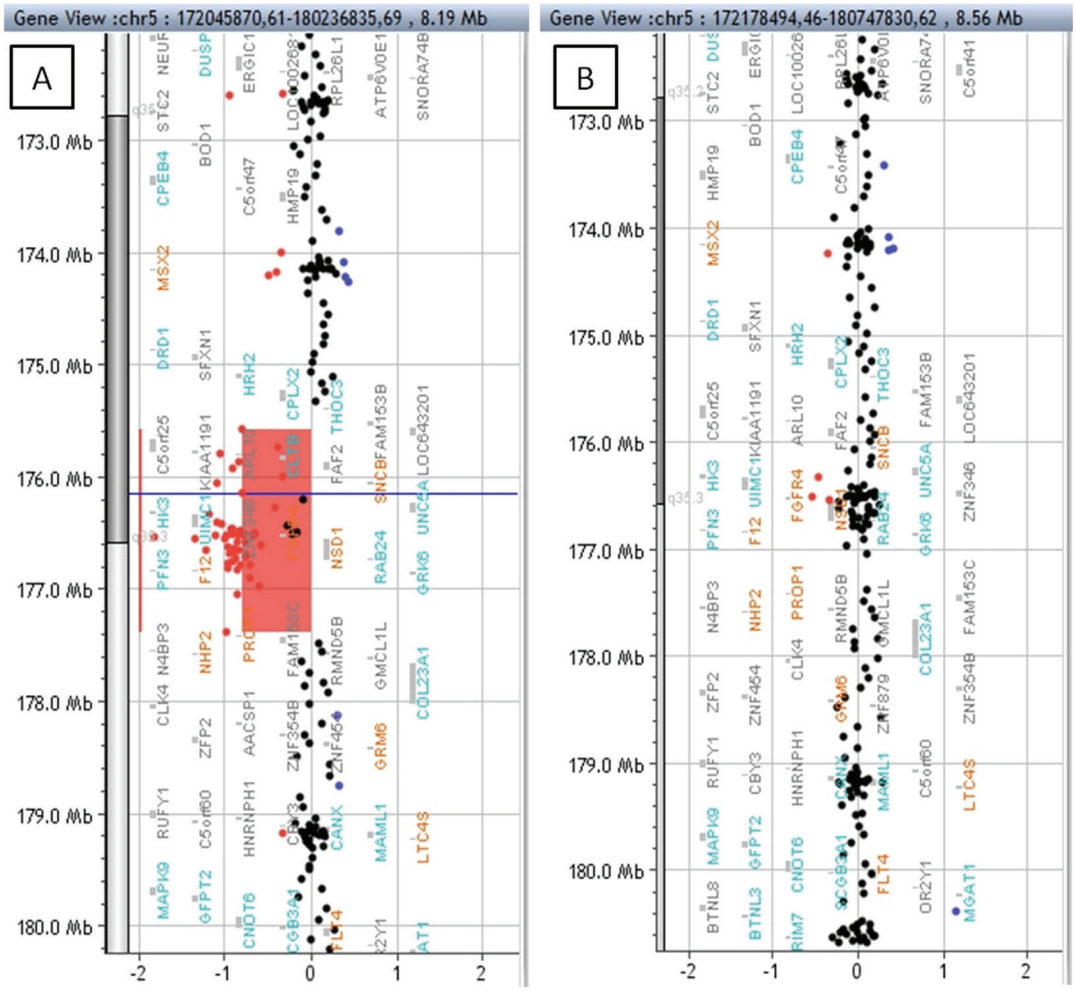

Figure 2. (A) Oligo-array CGH of the patient, showing an enlargement of the deleted region (red bar) in chromosoma 5. B) Oligo-array CGH of the same region in chromosome 5 after comparing the DNA of the parents, illustrating a normal result

CGH: comparative genomic hybridization

normal hybridization profile, including the genes *SLC34A1* and *FGFR4* in this region, among others (Figure 2).

At four years of age, oral phosphate was prescribed, with poor tolerance, so he finally began thiazides due to improvement in serum calcium levels. In the last assessment at seven years of age, weight and height were normal at -0.07 SDS and +0.44 SDS, respectively, with normal growth rate (-0.7 SD) and insulin-like growth factor-1 (IGF-1) and IGF binding protein 3 values within the normal range for the patient's age. Bilateral medullary nephrocalcinosis persisted, with renal asymmetry (right kidney, 50-75th percentile and left kidney >95th percentile), and the abnormal test results shown in Table 1, along with mild hypercalciuria. However, there was an improvement in phosphate levels and normalization of renal function tests. Cardiological evaluation showed continuation of mild PDA without other findings. In terms of neurological development, the patient continues to receive cognitive and communication stimulation and his psychomotor retardation was improving. Cow's milk protein challenge was undertaken and tolerance was good.

Discussion

The *NSD1* gene is the only gene currently known to cause Sotos syndrome. Among European patients with typical findings of this syndrome, up to 15% present 5q35 microdeletion that affects NSD1, and associated disorders may appear when these deletions affect other genes (2,4). There is no genotype-phenotype correlation in this syndrome, but in cases due to 5q35 microdeletion, overgrowth is less obvious and usually has more neurological impact, as is the case in our patient, while nephrocalcinosis is a phenotypic characteristic reported only in cases of Sotos syndrome due to microdeletion (4).

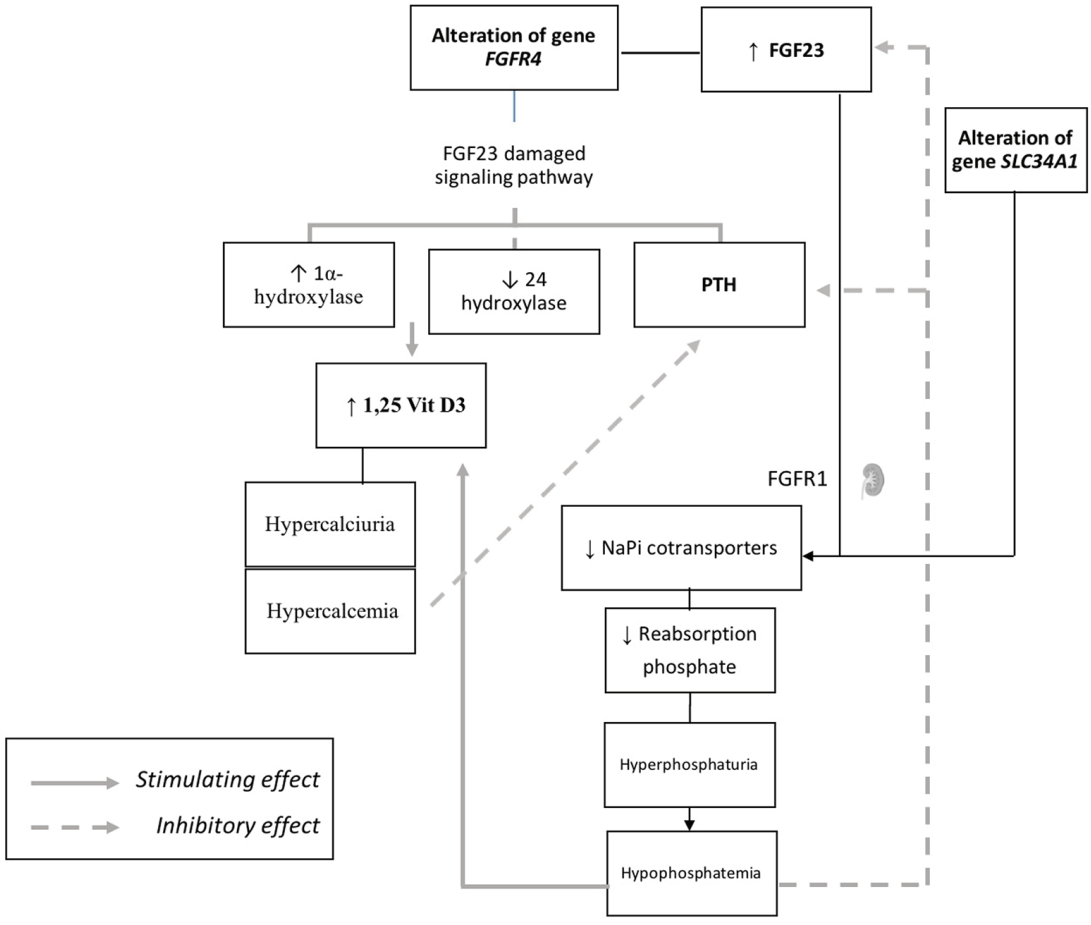

Figure 3. The damaged signaling pathway due to an alteration of gene *FGFR4* may cause an activation of PTH and 1,25 $(OH)_2$ vitamin D3, due to stimulus of the enzyme 1α hydroxylase and the lowered expression of 24 hydroxylase, thus favoring hypercalcemia and hypercalciuria. In addition, this alteration of *FGFR4* may also contribute to the inappropriately normal FGF23 level which, through binding to FGFR1, would decrease tubular reabsorption of phosphorus into the proximal tubule, exacerbating the hypophosphatemia associated with renal loss due to the inactivation of the NaPi-IIa carrier. Hypophosphatemia would be another major stimulus for increased 1,25 $(OH)_2$ vitamin D_3, although with a negative impact on the production of FGF23 and PTH

PTH: parathyroid hormone, 1,25$(OH)_2$ vit D_3: 1,25-dihydroxyvitamin D_3, FGFR: fibroblast growth factor receptor

The literature contains very few cases of nephrocalcinosis in Sotos syndrome patients. Saugier-Veber et al. (5), 2007, published three cases due to deletion with associated nephrocalcinosis, suggesting a genetic predisposition in the deletion area. Kenny et al. (6), 2011, presented two pediatric cases of 5q35 chromosome microdeletion that included *NSD1* and *SLC34A1*, explaining the wider phenotypic spectrum. This last gene encodes an important renal phosphate carrier (NaPi-IIa) and mutations thereof have been associated with nephrolithiasis/osteoporosis, hypophosphatemia 1 (OMIM #612286), Fanconi reno-tubular syndrome 2 (OMIM #613388) and Hypercalcemia infantile 2 (OMIM #616963). Recessive mutations of this gene have been associated with Fanconi syndrome and hypophosphatemic rickets, as well as familial cases of hypophosphatemia and nephrocalcinosis (7,8). However, heterozygous mutations of *SLC34A1* in patients with nephrolithiasis and osteoporosis have been published (9,10). Moreover, Schlingmann et al. (11) described a homozygous mutation in the same gene as a cause of idiopathic hypercalcemia in familial and sporadic cases, explaining the hypercalcemia with the suppression of FGF23 caused by hypophosphatemia, caused in turn by inactivation of the renal phosphate carrier NaPi-IIa. These authors suggested that supplementation with oral phosphate could help correct calcium metabolism in patients with *SLC34A1* mutation, although this treatment was not tolerated in our patient.

More recently, overlapping phenotypes associated with *SLC34A1*, *SLC34A3* and *CYP24A1* mutations have been described, and that not all the patients showed improvements in hypercalciuria and nephrocalcinosis, despite improvement in hypercalcemia and 1,25 $(OH)_2$

vitamin D_3 levels, as happened in the current case report (12,13). Moreover, an attenuation of renal phosphate wasting with advancing age has been observed, which may reflect the decreasing importance of NaPi-IIa for phosphate homeostasis over time, and other studies found impaired kidney function at a mean age of 23.8 years, even in subjects with a heterozygous mutation, suggesting the need for long-term follow-up of these patients (14,15).

Mutsaers et al. (16) described a case of Sotos syndrome due to 5q35 microdeletion that affected *NSD1* and the FGF receptor gene 4 (*FGFR4*), presenting with transient hypercalcemia but without nephrocalcinosis, in contrast to the present case report. It was suggested that the case reported by Mutsaers et al. (16) implied the existence of a change in the expression of FGF receptors (FGFR) during human renal development and that the expression of FGFR4 decreased with age. The authors proposed that the heterozygous microdeletion detected caused inactivation of this FGF23 receptor, leading to damaged signaling at an early stage of development, thus affecting calcium homeostasis, mainly mediated by FGF23 binding to FGFR3 and FGFR4. Under normal conditions, osteocytes increase FGF23 release in response to elevated calcemia, This mechanism is aimed at achieving a negative calcium balance through decreasing 1,25 $(OH)_2$ vitamin D_3 and levels of PTH, which cannot be achieved when this signaling pathway is damaged.

Considering the complex regulation mechanisms of mineral metabolism and taking into account the studies published and the findings in our case, which showed normal intact *FGF23* values, we suggest that the damaged signaling pathway caused by the alteration to *FGFR4* may have activated 1,25 $(OH)_2$ vitamin D_3, thus favoring the development of hypercalcemia and hypercalciuria. In addition, this alteration of *FGFR4* may also contribute to the inappropriately normal FGF23 level, which may in turn inhibit the expression of renal carriers NaPi of the proximal tubule through binding mainly to FGFR1. This would constitute an additional stimulus for the renal loss of phosphate and hypophosphatemia in the presented patient, already boosted by the inactivation of carrier NaPi-IIa due to the mutation of *SLC34A1*. Although hypophosphatemia was not very significant, due to the heterozygous mutation of gene, and that this defect could be partially compensated by the NaPi-IIc contransporter, it may have been another major stimulus for the increase of 1,25 $(OH)_2$ vitamin D_3 (17,18). On the other hand, the normalization of cystatin C, NAG values and renal water handling suggested an association with neonatal kidney injury and treatment with aminoglycoside (Figure 3).

Lastly, the patient's growth decreased in the most recent period after an initial period of overgrowth. This may be explained by the alteration of several genes, as delayed growth in hypophosphatemic syndromes is common, although not constant and variable. Furthermore, the regulatory action of FGF23 on bone mineralization widely recognized (19,20).

Conclusion

In conclusion, nephrocalcinosis is a phenotypic characteristic that has been reported in cases of Sotos syndrome due to deletions that include *SLC34A* and other genes associated with phosphate wasting, hypercalcemia, hypercalciuria and elevated 1,25 $(OH)_2$ vitamin D_3 levels. In these patients, long-term follow-up is recommended due to the risk of impaired kidney function, although the optimal treatment for affected patients is unknown and would require much more evidence, including genotype-phenotype relationships for different combinations of variant genes.

Ethics

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

Footnotes

Authorship Contributions

Surgical and Medical Practices: Juan D. González-Rodríguez, Esther Q. Inglés-Torres, José E. Cabrera-Sevilla, Salvador Ibáñez-Micó, Francisca Bermejo-Costa, Ascensión Vera-Carbonell, Juan A. Bafalliu-Vidal, Pedro Cortés-Mora, Ana Lorente-Nicolás, José María Donate-Legaz, Concept: Juan D. González-Rodríguez, Esther Q. Inglés-Torres, Design: Juan D. González-Rodríguez, Esther Q. Inglés-Torres, José E. Cabrera-Sevilla, Salvador Ibáñez-Micó, Francisca Bermejo-Costa, Ascensión Vera-Carbonell, Juan A. Bafalliu-Vidal, Pedro Cortés-Mora, Ana Lorente-Nicolás, José María Donate-Legaz, Data Collection or Processing: Juan D. González-Rodríguez, Esther Q. Inglés-Torres, Analysis or Interpretation: Juan D. González-Rodríguez, Esther Q. Inglés-Torres, José E. Cabrera-Sevilla, Salvador Ibáñez-Micó, Francisca Bermejo-Costa, Ascensión Vera-Carbonell, Juan A. Bafalliu-Vidal, Pedro Cortés-Mora, Ana Lorente-Nicolás, José María Donate-Legaz, Literature Search: Juan D. González-Rodríguez, Esther Q. Inglés-Torres, Writing: Juan D. González-Rodríguez, Esther Q. Inglés-Torres, José E. Cabrera-Sevilla, Salvador Ibáñez-Micó, Francisca Bermejo-Costa, Ascensión Vera-Carbonell, Juan A. Bafalliu-Vidal, Pedro Cortés-Mora, Ana Lorente-Nicolás, José María Donate-Legaz.

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CASE REPORT

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Insulinoma Associated with MEN1 Syndrome: A Case of Persistent Hypoglycemia in a School-aged Child

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What is already known on this topic?

Insulinoma is a rare cause of non-ketotic hypoglycemia in pediatric patients, mostly due to isolated benign lesions, but can also be part of multiple endocrine neoplasia type 1 (MEN1) syndrome.

What this study adds?

This case illustrates that insulinoma may be the first manifestation of MEN1 syndrome, as in our patient, in whom a likely pathogenic variant in the *MEN1* gene was found, which had not been previously reported.

Abstract

Insulinoma is a rare cause of non-ketotic hypoglycemia, both in adults and in children. Pediatric patients account for approximately 5% of all cases, mostly due to isolated benign lesions, but insulinoma may also be part of a multiple endocrine neoplasia type 1 (*MEN1*) syndrome. We report the case of a patient with multiple hospitalizations related to hypoglycemia and neuroglycopenia symptoms. Multiple studies demonstrated the presence of an insulinoma. Subsequently, an unreported likely pathogenic variant in the *MEN1* gene was identified, suggesting that the clinical presentation of this patient should be part of the spectrum of MEN1 syndrome. The primary significance of this report is to underscore that insulinoma may present as the initial manifestation of MEN1 syndrome, reported to account for around 10% of pediatric insulinomas which are associated with MEN1 syndrome. Furthermore, we describe a previously unreported, likely pathogenic variant in the *MEN1* gene. This report highlights the importance of the convergence of clinical, biochemical and molecular investigations in establishing a precise diagnosis, prognosis, and appropriate follow-up for pediatric patients with insulinoma.

Keywords: Hypoglycemia, insulinoma, pediatrics, MEN1 syndrome

Introduction

Hypoglycemia is the most common metabolic disorder in childhood (1). It is universally defined as a plasma blood glucose concentration low enough to cause signs and symptoms of impaired brain function (2) and cannot be defined as a specific concentration because brain response thresholds are individual, and the injury extent is influenced by the duration and degree of hypoglycemia (3). However, it has been shown that neurogenic symptoms are perceived at a plasma glucose concentration < 55 mg/dL (< 3.0 mmol/L), and at < 50 mg/dL (< 2.8 mmol/L) it disrupts cognitive

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function (neuroglycopenia) (3). The serum glucose level that defines hypoglycemia remains controversial (1), with the American Association of Pediatrics defining hypoglycemia as a blood glucose level of < 47 mg/dL (2.61 mmol/L) and the Pediatric Endocrine Society suggesting a blood glucose level of < 50 mg/dL (2.77 mmol/L) (4). Its incidence is inversely proportional to age, given the higher glycemic demands in the first two years of life (3).

The etiology of hypoglycemia is broad and varies with age. It includes low food intake, metabolic disorders of endocrine origin, medications, inborn errors of metabolism, iatrogenesis, and tumors, including insulinoma (3).

With an incidence of three cases per million adults, which peaks between the third and sixth decades (5), insulinoma is a rare tumor in children and adolescents (6,7). In the pediatric age group, 90% are single benign tumors; the remaining 10% are usually multiple, malignant, and generally associated with the multiple endocrine neoplasia type 1 (MEN1) syndrome. Early diagnosis is essential for the prevention of irreversible neurological lesions. In the case of MEN1, it is also important to monitor and follow the development of other associated endocrinopathies for timely treatment.

Here we present the case of a schoolboy with multiple hospitalizations related to hypoglycemia and neuroglycopenic symptoms, in whom an intrapancreatic insulinoma was diagnosed. Given the patient's age, a molecular study was performed, which revealed a likely pathogenic variant in the *MEN1* gene not previously reported, thus likely confirming the diagnosis of MEN1 syndrome.

Case Report

An eight-year-old boy presented with a two-year history of episodes described by his mother as "increased sleepiness" mainly in the morning; later associated with irritability, loss of tone and episodes of disconnection with apparent abnormal movements and, occasionally, relaxation of the urinary sphincter, and likely postictal phase. The etiological study documented an echocardiogram, Holter electrocardiogram, tilt table test, simple cranial computed tomography (CT), and an electroencephalogram, all unremarkable.

He was hospitalized when hypoglycemia was documented during an episode of abnormal movements. At the referral center, he had two critical samples, reporting non-acidotic, non-ketotic hypoglycemia and detectable insulin levels, with an insulin/glucose ratio of less than 0.3, in addition to normal lactate, ammonium, cortisol, and growth hormone levels. In addition, reports of a contrast-enhanced abdominal CT scan and a contrast-enhanced abdominal magnetic resonance imaging (MRI) were both unremarkable. He was transferred for further evaluation.

At admission to our center, besides these clinical findings, his personal and family medical history and physical exam were all unremarkable. His weight was 1.3 standard deviation score (SDS), height 1.5 SDS and body mass index 0.7 SDS.

Fatty acid profile testing was not performed due to restrictions on the shipment and processing of international referral tests during Coronavirus disease-2019 lockdown. Due to a possible beta-oxidation of fatty acids defect, specifically medium-chain triglycerides, dietary management was initiated, without a complete weaning from intravenous glucose infusion rate.

After fasting for five hours and without intravenous dextrose infusion, a new critical sample was obtained, with evidence of hyperinsulinemic, non-ketotic hypoglycemia [plasma glucose 33.5 mg/dL, negative serum ketone bodies, insulin 13.3 µUI/mL (2.6-24.9)], and glucose elevation after glucagon administration (response at 10 minutes, 71 mg/dL and at 20 minutes, 98 mg/dL). A thorough review of medication use suggestive of Hirata syndrome was negative. There was no evidence of surreptitious use of insulin-type medications or sulfonylureas. There were no findings suggestive of overgrowth syndromes associated with hypoglycemia (8).

A C-peptide level of 2.9 ng/mL (1.1-4.4 ng/mL) and the elevated C-peptide/glucose ratio suggested endogenous hyperinsulinemia, so a new contrasted abdominal MRI was performed, which showed an image consistent with an insulinoma in the uncinate process of the pancreas (Figure 1). Enucleation by laparotomy was performed. Pathology revealed a well-differentiated, neuroendocrine pancreatic tumor measuring 2.7 x 2.2 x 1.6 cm and weighing 4.6 grams, which was negative for vascular or perineural invasion. Immunohistochemical staining showed positivity for chromogranin, and weak homogeneous positivity for an insulin immunolabel in the tumor cells (Figure 2). This result confirmed the diagnosis of insulinoma.

The patient had a satisfactory course with remission of symptoms, and normalization of glycemia. Calcium, phosphorus, parathyroid hormone, prolactin, and insulin-like growth factor-1 levels did not suggest associated endocrinopathies.

Given the low frequency of insulinomas in children, his case was evaluated by the medical genetics service. A single clinical exome was performed, with evidence of a variant in the *MEN1* gene (NM_001370259.2) c.1121delA

p.(Asn374Thrfs*3) in heterozygosis, classified as likely pathogenic according to the American College of Medical Genetics and Genomics criteria (9), and not previously reported. Family history for MEN1 syndrome was negative. The patient continues endocrine and genetic follow-up close to his home. Two years after the insulinoma resection, he remains normoglycemic and under surveillance for related endocrinopathies.

Discussion

Insulinoma is a neuroendocrine tumor (NET) arising from the insulin-producing pancreatic beta cells, causing hyperinsulinemia (8). It is suspected when documenting hypoglycemia using the Whipple triad (neuroglycopenic symptoms, biochemical hypoglycemia, and reversal of symptoms after carbohydrate ingestion) (3), and fulfilling criteria for hyperinsulinemic hypoglycemia, which include: plasma glucose <50 mg/dL, detectable insulin levels, C-peptide ≥0.5 ng/mL, beta-hydroxybutyrate <2 mmol/L, glycemic response to glucagon ≥30 mg/dL, low free fatty acids, and negative blood sulfonylurea levels (2).

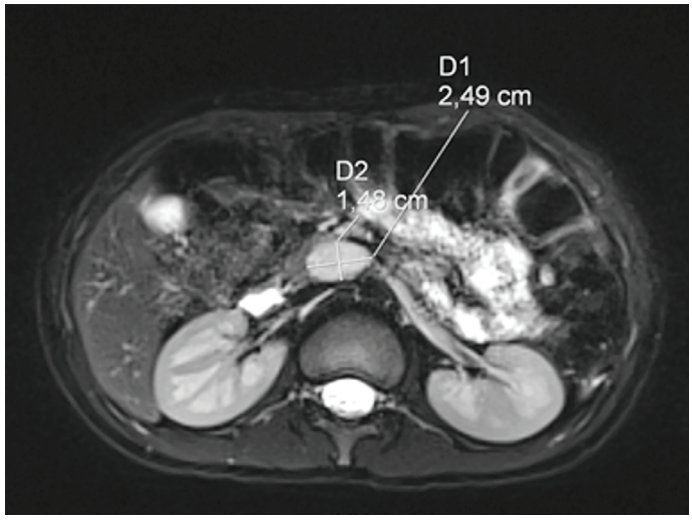

Figure 1. Abdominal magnetic resonance imaging. Oval solid mass with well-defined borders, 2.5 x 1.5 cm, hypervascular in the uncinate process of the pancreas, compatible with insulinoma by imaging features and clinical presentation

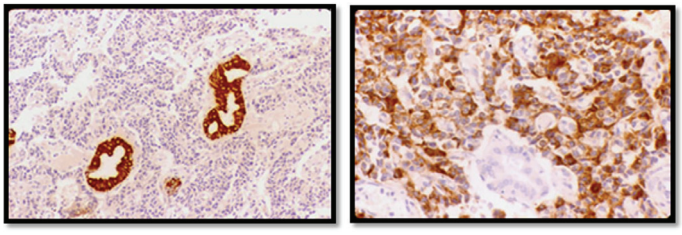

Figure 2. Histology of insulinoma. Immunohistochemical staining with antibodies against insulin, glucagon, chromogranin A and B, and keratins; positive to keratin and weak to insulin in ductal cells. Cell proliferation marker Ki-67 value of 2%, compatible with insulinoma

Insulinoma is a rare pediatric tumor (6). A study that analyzed insulinoma cases over 60 years showed that pediatric patients accounted for only 5.8% of all cases, with no gender predilection (10). Most insulinomas originate in the pancreas (98%). Approximately 90% are benign, unifocal lesions, smaller than 2 cm, uniformly distributed throughout the pancreatic structure. The remaining 10% are usually multifocal and malignant (10).

Preoperative localization includes imaging studies, such as endoscopic ultrasound, MRI, and CT; functional studies such as DOTATATE PET/CT, glucagon-like peptide 1 receptor scintigraphy; or more invasive techniques, such as celiac trunk angiography, selective intra-arterial stimulation, and intraoperative pancreatic ultrasound (2). Abdominal MRI is a reasonable starting study. Insulinomas can be characterized by immunohistochemistry, but no clear histopathological parameters or histochemical markers predict tumor behavior (6,11). Surgery is the mainstay of insulinoma treatment, with a remission rate ranging from 77% to 100% (5).

Insulinoma can be sporadic or associated with MEN1 syndrome, which represents about 4% of insulinomas (12). MEN1 syndrome (OMIM 131100) is an autosomal dominant disorder caused by mutations in the *MEN1* gene. It is characterized by endocrine and non-endocrine tumors. The most common endocrine tumor in MEN1 is parathyroid tumor, present in 90% of patients by age 50 years, followed by pituitary tumor in 30-40% of patients (prolactinoma being the most common), and gastroenteropancreatic NETs (GEP-NETs), including gastrinoma (40%), insulinoma (10%), and glucagonoma (<3%). Associated tumors include adrenal cortex tumors (40%) and pheochromocytoma (<1%), among others (13).

The *MEN1* gene is located on chromosome 11q13, (14) and consists of 10 exons; it encodes for a 610 amino acid protein called menin. Although, its specific function is yet to be established, it has been shown to interact with proteins related to transcriptional regulation, genomic stability, proliferation, and cell division (15). Approximately 1,133 germline mutations in the *MEN1* gene have been reported (13), the most frequent being frameshift mutations (40%), as in our patient, followed by nonsense mutations (23%), missense mutations (20%), splice site mutations (9%), deletions/insertions without reading frame alteration (6%) and large deletions in 1% and loss-of-function variants represent 75% of these mutations (15). MEN1 is a tumor suppressor gene with increased risk of developing neoplasms

when mutated. MEN1 syndrome has a prevalence of approximately 1 in 10,000 to 100,000 individuals, varying by geographical region. It is more prevalent in Finland due to a founder effect (16). The penetrance reaches 95% by 40 years of age (17). Currently, no genotype-phenotype correlation has been identified (18).

In 10% of patients, insulinoma is the first manifestation of MEN1 syndrome, typically before age 20 years, and presenting with symptomatic fasting hypoglycemia (13). MEN1-associated insulinomas tend to be larger than 1 cm and can be multiple in about 30% of MEN1 patients (19). MEN1-associated insulinomas in children have a higher risk of metastasis in approximately 50% of cases; whereas in patients without MEN1, insulinomas occur after the age of 40 years and metastasize in only 10% of cases (13).

Padidela et al. (20) described a series of nine pediatric patients, including two with MEN1 syndrome. Both cases exhibited insulinoma as the first endocrinopathy, with one later developing a parathyroid adenoma. Bhatti et al. (21) reported 12 patients aged between 4 and 16 years, with five displaying clinical and molecular features of MEN1 syndrome, one patient presenting with insulinoma as the first endocrine manifestation, and none having parathyroid adenoma. These series indicate a higher frequency of MEN1-associated insulinoma in children compared to adults and emphasize the importance of performing molecular studies to clarify the diagnosis, due to its implications for continuous follow-up and surveillance. None of the previously reported cases had the variant presented in the current case report. The report of new variants is beneficial to establishing a genotype correlation for future cases.

Conclusion

The patient presented with an insulinoma as part of MEN1 syndrome spectrum, with a previously unreported, likely pathogenic variant in the *MEN1* gene. Insulinoma is a rare cause of non-ketotic hypoglycemia in pediatric patients. Due to the higher frequency of MEN1-associated insulinoma in children compared to adults, it is advisable to perform genetic testing in all pediatric patients with insulinoma, as insulinoma can be the first manifestation of MEN1 syndrome. These patients will require clinical follow-up and continuous monitoring due to the risk of developing other tumors and endocrinopathies associated with MEN1 syndrome.

Ethics

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

Footnotes

Authorship Contributions

Concept: Rodrigo Lemus-Zepeda, Aura María Salazar-Solarte, Diana Marcela Vasquez-Forero, Design: Rodrigo Lemus-Zepeda, Mario Jr. Angulo-Mosquera, Liliana Mejía-Zapata, Aura María Salazar-Solarte, Diana Marcela Vasquez-Forero, Data Collection or Processing: Rodrigo Lemus-Zepeda, Aura María Salazar-Solarte, Diana Marcela Vasquez-Forero, Analysis or Interpretation: Rodrigo Lemus-Zepeda, Aura María Salazar-Solarte, Diana Marcela Vasquez-Forero, Literature Search: Rodrigo Lemus-Zepeda, Aura María Salazar-Solarte, Diana Marcela Vasquez-Forero, Writing: Rodrigo Lemus-Zepeda, Mario Jr. Angulo-Mosquera, Liliana Mejía-Zapata, Aura María Salazar-Solarte, Diana Marcela Vasquez-Forero

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CASE REPORT

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A 15-year-old Girl with a Lateral Neck Mass Turning Out to Be Papillary Thyroid Carcinoma - Lateral Ectopic Papillary Thyroid Carcinoma or Lymph Node Metastasis?

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What is already known on this topic?

Lateral neck lesions in children are common and involve various etiologies. A rather unusual cause of a lateral neck mass is the presence of ectopic thyroid tissue. Malignant degeneration of ectopic thyroid tissue located laterally in the neck is very rare and has been sparsely reported. It can be challenging to distinguish a lymph node metastasis from primary thyroid carcinoma located in ectopic thyroid tissue, especially when no primary tumor is located within the thyroid gland.

What this study adds?

We report on a 15-year-old girl in whom papillary thyroid carcinoma was found in a lateral neck lesion without a primary thyroid tumor. We discuss the diagnostic and therapeutic challenges in this case, and compare our experience with the existing literature, thereby adding knowledge to the very scarce available evidence in pediatric cases.

Abstract

Lateral neck lesions in children are common and involve various infectious or inflammatory etiologies, as well as embryological remnants such as branchial cleft cysts. Although unusual, ectopic thyroid tissue may also present as a lateral neck mass. Here, we present an unusual case of a 15-year-old girl treated for an asymptomatic lateral neck mass that, after surgical removal, was found to be papillary thyroid carcinoma (PTC). However, after removal of the thyroid gland, no primary thyroid tumor was found. The question arose whether

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the lateral neck lesion was a lymph node metastasis without identifiable primary tumor by histological evaluation, or rather malignant degeneration of ectopic thyroid tissue. Total thyroidectomy was performed with postoperative adjuvant radioactive iodine ablation. Even though PTC in a lateral neck mass without a primary thyroid tumor has been described previously, pediatric cases have not been reported. In this report we share our experience on diagnosis, treatment and follow-up, and review the existing literature.
Keywords: Thyroid dysgenesis, thyroid papillary carcinoma, cervical neck mass

Introduction

Lateral neck lesions in children are common and involve a variety of etiologies, including infectious or inflammatory disease, as well as embryological remnants, such as branchial cleft cysts. A rather unusual cause of a lateral neck mass may be an abnormally formed thyroid gland or a remnant of its embryological development. Ectopic thyroid tissue occurs in approximately seven percent of the adult population, mostly women (75%), based on previous cadaver studies (1,2,3). Ectopic thyroid tissue may be the only thyroid tissue with simultaneous absence of the thyroid gland itself (4). Ectopic thyroid tissue is most likely situated in the midline and is unusual in the lateral neck compartment with an incidence of one in 100,000 (2,5,6). Midline ectopic thyroid tissue is the consequence of an incomplete or aberrant descent of the thyroid anlage in the fetus, that normally migrates from what later becomes the base of the tongue through the thyroglossal duct to its final position at the anterior tracheal wall (7). Failure of duct obliteration may give rise to thyroglossal duct cysts of which up to 45% contain normal-appearing ectopic thyroid tissue (8). Lateral ectopic thyroid tissue seems to be the consequence of inadequate fusion of the medial thyroid anlage, which gives rise to the thyroid parenchyma, and the lateral thyroid anlage, that is part of the fourth branchial cleft, trapping normal thyroid tissue (7,9). Others have reported benign thyroid follicular inclusions in cervical lymph nodes as an underlying etiology of lateral cervical ectopic thyroid tissue (10). Degeneration of ectopic thyroid tissue in any location forms less than one percent of all thyroid carcinomas, with the majority of cases showing degeneration to papillary thyroid carcinoma (PTC) (2,11,12).

Herein, we report on our experience of a 15-year-old girl in whom a PTC was found in a lateral neck lesion without a primary thyroid tumor.

Case Report

A 15-year-old girl with an unremarkable medical history presented at the outpatient pediatric department with a non-painful, palpable, lateral neck swelling, noticed first one year earlier. Over time, slight enlargement was recognized without any additional symptoms, in particular there were no B-symptoms, as a sign of a possible underlying malignancy. Her family history was unremarkable concerning thyroid disorders and/or malignancies. An infectious cause was ruled out by serological laboratory testing. No lymphadenopathy was found during physical examination.

Cervical ultrasound was performed showing a clearly distinguishable, atypical lesion of 1.3 x 2 x 2 cm positioned ventrally of the sternocleidomastoid muscle with several calcifications, a cystic component and slightly enhanced vascular flow (Figure 1). Apart from the calcifications, there were no ultrasound findings associated with malignancy. The thyroid itself was normal-sized and no cervical lymphadenopathy was recognized. As there was no definite radiological diagnosis, surgical excision of the lesion was performed under the presumption of an embryological remnant. During surgery the lesion was located medially from the sternocleidomastoid muscle without any connection to surrounding structures or the skin. On gross examination the appearance of the lesion most closely resembled a lymph node, but during surgery it opened and fluid was exuded.

Histological examination revealed a cystic lesion consisting of papillae lined by follicular epithelium with papillary nuclear features, consistent with PTC (Figure 2, 3). There was lymphoid tissue surrounding the lesion. No other epithelial structures, such as ciliated epithelium or squamous epithelium were seen. Immunohistochemistry (IHC) of the lesion showed positivity for thyroglobulin, paired-box gene-

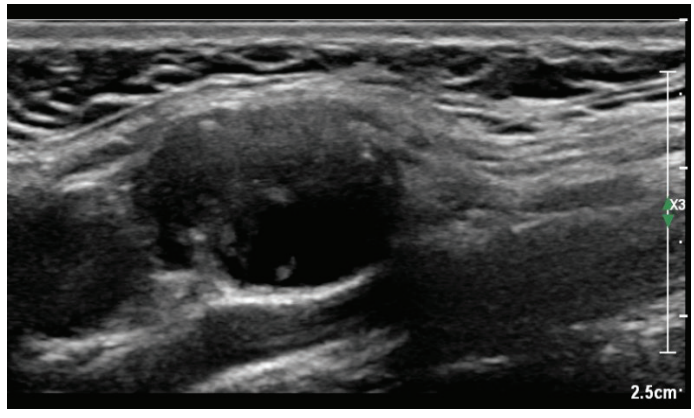

Figure 1. Sonography of the lateral neck mass showing calcifications

8 and thyroid transcription factor-1 proteins. *BRAF* mutation was ruled out. Results of IHC confirmed the diagnosis of PTC. However, it was challenging to distinguish if the lesion was a lymphatic metastasis of a primary thyroid carcinoma or if the lesion was primary ectopic PTC, as only a small rim of lymphoid tissue was found at the edge of the histological specimen (Figure 4).

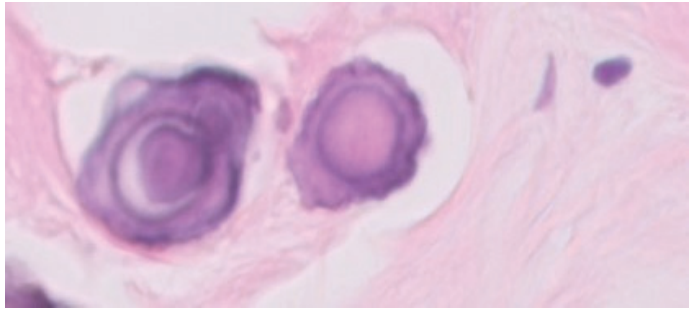

Figure 2. Psammoma bodies found in the resected lateral neck mass characteristic for papillary thyroid carcinoma

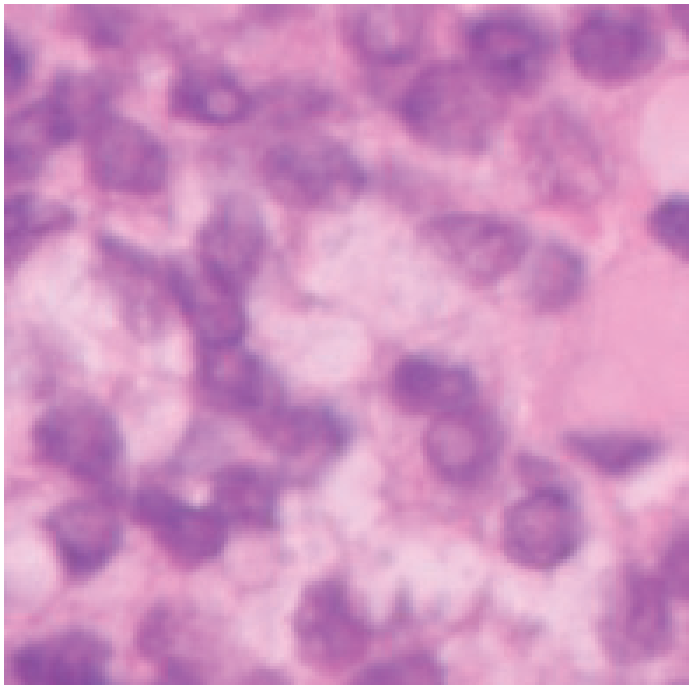

Figure 3. Nuclear pseudo-inclusion confirming papillary thyroid carcinoma

After the unexpected histological diagnosis of PTC, the pediatric endocrinologist was consulted. The patient did not report any symptoms of thyroid dysfunction and biochemical evaluation showed euthyroid status (Table 1). In a search for the primary thyroid tumor ultrasound was repeated and there was a suspicion of a four-millimeter node in the left thyroid lobe with benign appearance. There were no findings suspicious of metastatic disease. A cervical magnetic resonance imaging scan was also unable to identify a primary thyroid tumor or lymphatic metastases, nor a connection between the thyroid and the area of the previously resected PTC. Although additional diagnostic work-up showed no primary thyroid tumor, the presence of a primary thyroid microcarcinoma could not be ruled out. Therefore, the multidisciplinary team advised a total thyroidectomy as treatment for pediatric PTC. During surgery close to the thyroid, a single lymph node overlying the thyroid gland was resected. Histological examination of the complete thyroid showed no evidence of a primary tumor and examination of the resected lymph node showed no PTC.

Based on the pathology findings of the lateral lesion and the thyroid gland it was difficult to distinguish between a primary PTC in ectopic lateral thyroid tissue, or a lymphatic

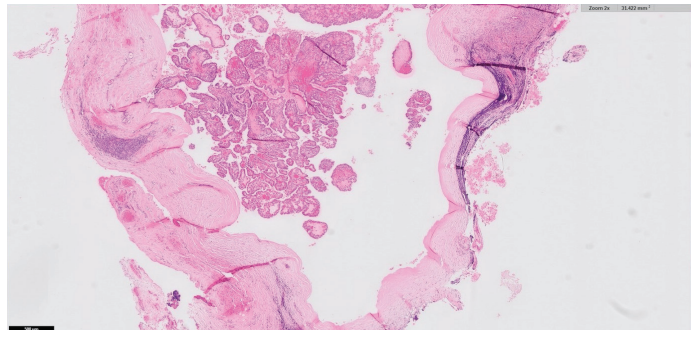

Figure 4. Histological presentation of a part of the resected lesion showing the papillary architecture characteristic for papillary thyroid carcinoma with some lymphoid tissue at the outer edge

Table 1. Laboratory findings and anthropometric data preoperative to thyroidectomy

	Patient values	Local reference values and units
Anti-thyroglobulin	< 4.1 IU/mL	< 10 IU/mL
Free T4 (thyroxine)	14.8 pmol/	12.0-22.0 pmol/L
Tg	61.7 pmol/L	0-60 pmol/L
TSH	1.6 mU/L	0.5-5 mU/L
Height	172.5 cm (+ 0.66 SDS)	
Weight	81.55 kg (+ 2.10 SDS for height)	

IU/mL: international units per milliliter, pmol/L: picomoles per liter, mU/L: milliunits per liter, SDS: standard deviation score, Tg: thyroglobulin, TSH: thyroid stimulating hormone

neck metastasis of a primary thyroid tumor, since no primary tumor was found and the lesion showed only a small edge of lymphoid tissue. To specify further treatment, it was decided to classify the lateral neck lesion as an extra-thyroidal localization of the thyroid tumor (T3bN0Mx) instead of classifying it as a nodular lesion (pT0N1bMx), based on both the absence of any signs of a primary tumor within the normally located thyroid gland on histological evaluation, and the absence of additional signs of other lymphatic metastases.

Subsequently to the surgical resection, postoperative adjuvant ablative radioactive iodine treatment (3667 MBq) was given according to the current national pediatric guideline on thyroid cancer (13). After finishing this course, thyroxine hormone supplementation was started. Scintigraphy post radioactive iodine therapy showed no signs of radioactive iodine avid metastases. Further follow-up was uneventful.

Discussion

PTC is the most common subtype of thyroid cancer in children, even though its incidence is low with approximately three cases per million people below the age of 14 years increasing to eleven cases in the 15-17 years old age group (14).

Several cases of PTC arising in a thyroglossal cyst or in lateral branchial cleft cysts have been reported, but only a few cases report the finding of PTC in a lateral neck mass, and to the best of our knowledge, none have been reported in pediatric patients.

In a case comparable to ours, but in a 53-year-old man, an ectopic PTC was found in the clavicular head of the sternocleidomastoid muscle, without evidence of a primary thyroid tumor. No evidence of branchial cleft tissue or lymphatic tissue could be found. Clinically there were no signs of lymph node involvement (15). In this patient a total thyroidectomy was performed along with central and selective neck dissection. No malignancy was found in the thyroid gland nor in the lymph nodes, histologically. The patient received ablative radioactive iodine treatment postoperatively (15).

Another case report of a 30-year-old woman, diagnosed with Hashimoto's thyroiditis three years before and who subsequently presented with a lateral neck mass that turned out to be PTC has been published (16). After the initial removal of the lateral neck mass she was treated with total thyroidectomy and partial neck dissection. The histopathological examination reported thyroid gland with no tumor, but with tissue around the thyroid showing carcinomatous infiltration. The resected neck mass contained neoplastic PTC cells within lymph node parenchyma with extra capsular invasion. In two of the resected neck lymph nodes metastatic PTC was found. The findings in this patient were interpreted as ectopic PTC with metastatic disease. El Bouhmadi et al. (17) reported on their experience with a 36-year-old female treated for hypothyroidism for one year. She presented with PTC of the thyroid with extracapsular extension and with a lateral neck mass revealing PTC in lateral ectopic thyroid tissue. No lymph node tissue and no lymph node metastasis were found and the patient received ablative radioactive iodine treatment after total thyroidectomy.

In the present case the question was whether the lateral neck mass could represent a lymph node metastasis, even if the primary thyroid tumor could not be found. The finding of a lymph node metastasis in absence of a thyroid tumor has been reported previously. Yamashita et al. (18) shared the case of a 66-year-old woman who presented with a swelling of the right upper neck, just below the parotid in the absence of a primary thyroid tumor. Fine-needle aspiration revealed a PTC that was treated with total thyroidectomy, excision of the neck mass together with the superficial lobe of parotid gland along with extended lymph node dissection. Pathological examination revealed that the neck mass was a fusion of two lateral lymph nodes with metastasis of PTC. No papillary carcinoma was found in the five-millimeter slices of the thyroid. Based on a hyalinized image, the possibility of spontaneous disappearance of the papillary thyroid microcarcinoma was postulated. She was diagnosed with lymph node metastasis of a micro-PTC, supported by histopathological report. Adjuvant ablative radioactive iodine treatment was given and no recurrence or metastasis was reported 24-month after surgery. Li et al. (19) also found lymph node metastasis of a PTC without a primary thyroid tumor. They reported on a 27-year-old woman who underwent left thyroidectomy and lymph node resection for suspected malignancy in a thyroid node and enlarged cervical lymph nodes. Intraoperative frozen section examination, as well as the final histopathological examination of the suspected nodule in the left thyroid, only showed benign thyroid, whereas two of the 15 resected lymph nodes showed PTC metastasis. Subsequent right thyroidectomy was refused, and the reported follow-up of two years duration was uneventful.

There are reports of tumor regression in PTC, which may explain the missing primary thyroid tumor in metastasized PTC. Shim et al. (20) confirmed the hypothesis of spontaneous remission of PTC, even in lymphatic metastasis. These

authors reported a 58-year old woman already treated with total thyroidectomy along with adjuvant ablative radioactive iodine for PTC without lymph node involvement. During her follow-up she presented with enlarged cervical lymph nodes and fine-needle aspiration confirmed metastatic PTC. As she refused to undergo surgery she was followed-up. One year later no abnormal lymph nodes could be identified. During the next nine years no abnormalities were identified and the follow-up was uneventful.

The present case, along with these case reports illustrates the dilemma of diagnosing a thyroid malignancy without a primary thyroid tumor as it is difficult to distinguish between ectopic malignancy and metastatic disease. However, this distinction is important because metastatic disease necessitates additional treatment.

In the present case, a total thyroidectomy was performed, as this is the cornerstone of the treatment of pediatric PTC. In addition, histopathological examination may show the presence of a thyroid microcarcinoma that was missed during the diagnostic process. In our opinion total thyroidectomy is necessary in the case of histological confirmed PTC, regardless of location, as imaging modalities may miss microcarcinoma within the thyroid gland. Furthermore, additional thyroid stimulating hormone suppression therapy and follow-up with serum thyroglobulin measurements will only be therapeutically reasonable if a total thyroidectomy has been performed. In the present case we did not perform a lymph node dissection. In some adult cases of ectopic PTC in thyroglossal or branchial cleft cysts, cervical lymph node dissections were performed, to various extents (7,12,21). In these cases clinical examination was not suspicious for lymph node involvement and histological analysis of the resected lymph nodes could not detect any lymphatic spread. Lymphatic spread of ectopic PTC is possible, but in our opinion, a preventive neck dissection without any clinical or radiological indication of lymph node involvement should not be performed, and especially not in children, as additional lymph node dissection is a known risk factor for permanent post-operative hypoparathyroidism (22). The decision not to perform a lymph node dissection in our patient was supported by post radioactive iodine therapy scintigraphy did not show any signs of (lymphatic) metastases. However, ultrasound to follow-up lymph node status and measurement of thyroglobulin levels should be included in the follow-up of these patients (13).

Even though the current Dutch guideline for the treatment of differentiated thyroid cancer in children clearly states the indication for post-surgery adjuvant ablative radioactive iodine treatment in all patients, its application in the present case is arguable (13). In thyroid carcinomas of less than one centimeter limited to the gland without signs of lymphatic metastasis, postoperative radioactive iodine ablation can be omitted in close consultation with the multidisciplinary team (13). However, no matter whether the papillary carcinoma in the lateral neck lesion is classified as a thyroid tumor extending outside the thyroid gland or as lymphatic metastasis, in both cases there would be an indication for post-surgery adjuvant ablative radioactive iodine treatment.

Conclusion

We report a 15-year-old girl in whom an asymptomatic lateral neck mass turned out to be a PTC without (histological) evidence of a primary carcinoma in the thyroid gland. In the absence of a primary tumor, we hypothesized that the PTC found in the lateral neck mass emerged from lateral ectopic thyroid tissue or alternatively could be a lymph node metastasis of a now completely remitting primary thyroid tumor. The treatment consisted of a total thyroidectomy followed by postoperative adjuvant ablative radioactive iodine treatment. Cases presenting with an inconclusive lateral neck mass should be discussed in a multidisciplinary consultation and should be treated with caution taking the possibility of thyroid related etiologies into account.

Ethics

Informed Consent: Informed consent was obtained from the patient and their family member.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Marijke E.B. Kremer, A. S. Paul van Trotsenburg, Anton F. Engelsman, Esther Edelenbos, Arantza Farina-Sarasqueta, Joost van Schuppen, José C.C. Koppes, Joep P.M Derikx, Christiaan F. Mooij, Concept: Marijke E.B. Kremer, Koppes, Joep P.M Derikx, Christiaan F. Mooij, Data Collection or Processing Marijke E.B. Kremer, Analysis or Interpretation: Marijke E.B. Kremer, A. S. Paul van Trotsenburg, Anton F. Engelsman, Esther Edelenbos, Arantza Farina-Sarasqueta, Joost van Schuppen, José C.C. Koppes, Joep P.M Derikx, Christiaan F. Mooij, Literature Search: Marijke E.B. Kremer, Christiaan F. Mooij, Writing: Marijke E.B. Kremer, A. S. Paul van Trotsenburg, Joep P.M Derikx, Christiaan F. Mooij.

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CASE REPORT

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Worsening of Congenital Hypothyroidism After Start of Carob-bean Gum Thickened Formula: Is There a Link? A Case Report

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What is already known on this topic?

Infant formulas containing soya bean extracts, as well as soy-containing baby foods, some drugs used to manage infants with recurrent regurgitation and specific medications for infant colic, are related to altered thyroid function in early childhood. Moreover, carob-beans used to thicken formula milk are reported to increase its viscosity and thickened formula with locust (carob) bean gum, slows gastric emptying in infants.

What this study adds?

This is the first case report to provide evidence of a possible effect of carob-bean gum thickened formula on the absorption of levothyroxine. We recommend caution and frequent thyroid function evaluation if these products are given to young patients with congenital hypothyroidism.

Abstract

Congenital hypothyroidism (CH), if not correctly treated with levothyroxine (L-T4), may cause permanent intellectual disability. If patients treated with L-T4 do not achieve good thyroid stimulating hormone (TSH) control, the possibility of poor compliance and/or poor absorption of L-T4 should be investigated. We describe an infant with CH whose thyroid hormone levels worsened after she started a carob-bean gum thickened formula. A baby girl was diagnosed with CH by newborn screening [confirmatory venous blood TSH was 496.0 µIU/mL and free thyroxine (fT_4) was 0.13 ng/dL]. Five weeks after beginning replacement L-T4 treatment (10.6 µg/kg per day), thyroid function normalized (TSH 2.72 µIU/mL, fT_4 2.08 ng/dL). However, five weeks later, thyroid function test results had worsened (TSH 31.1 µIU/mL, fT_4 1.27 ng/dl), which worsened further (TSH 44.8 µIU/mL, fT_4 1.16 ng/dL) even after L-T4 dose was increased (10.9 µg/kg per day). Anamnesis disclosed that she had started a carob-bean gum thickened formula to combat gastroesophageal reflux disease rather than regular type 1 formula milk. The anti-reflux milk formula was discontinued. Fourteen days later her TSH level dropped to 0.38 µIU/mL and fT_4 increased to 2.68 ng/dL, allowing the L-T4 dose to be reduced (from 10.9 µg/kg per day to 8.0 µg/kg per day). These findings suggest that carob-bean gum thickened formula may affect absorption of L-T4. If such formulas are used, we recommend a more frequent evaluation of thyroid function. In CH infants, inexplicably high TSH levels may be caused by gastrointestinal disorders or the interference from drugs or other substances, including some types of milk formula, which impair L-T4 absorption.

Keywords: Congenital hypothyroidism, L-thyroxine, treatment, carob-bean gum thickened formula, gastro-intestinal absorption, gastroesophageal reflux, case report

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Introduction

Congenital hypothyroidism (CH) is a relatively frequent endocrine disorder in which thyroid hormone (TH) deficiency, if not promptly diagnosed and correctly treated with L-thyroxine (L-T4) replacement, may lead to permanent intellectual disability (1). Below three years of age, adequate TH levels are essential for normal brain development. In CH patients treated with L-T4, poor compliance and/or poor absorption of L-T4 may cause a poorer developmental outcome compared to patients with satisfactory compliance and uncompromised absorption (2).

To achieve better developmental outcome, THs should return to normal range rapidly, preferably within the first 14 days of therapy (2). Screening programs for CH are used in many countries, allowing L-T4 treatment to be started early, generally when the disease is still asymptomatic (1). High starting doses of L-T4, between 10 and 15 mg/kg are the treatment of choice and determine both auxological (3) and neurological improvement (4). Nevertheless, initial L-T4 dose should vary based on formulation used, thyroid stimulating hormone (TSH) and TH levels (5). In the presence of poor TSH control, it is advisable to first investigate the correct method of administration and intake of L-T4, since caregivers make mistakes that can often be identified (2). However, it has been shown that gastrointestinal disorders, as well as several medications and other substances, may affect L-T4 absorption in infants (2,6).

Proton-pump inhibitors, aluminium-containing antacids, iron and calcium salts, herbal remedies and nutritional supplements may worsen L-T4 absorption, modifying gastric pH or binding with L-T4 to create insoluble complexes (2,7,8,9). Although few data have been reported, there may also be interference from infant milk formula, which, due to its composition rich in fats, proteins and lactose, inhibits the absorption of L-T4 in the small intestine (10). In a small number of historic case reports, soy infant formulas and soy-containing baby foods were shown to alter L-T4 absorption in the first years of life (11). After weaning, if L-T4 is taken with food, a higher dose may be needed to maintain euthyroidism (5,8).

The liquid formulation of L-T4 is currently the most widely used in children with CH, given the greater absorption of oral solutions compared to tablet form L-T4 (12). Moreover, some L-T4-treated children with CH achieve euthyroidism only if they are treated with a combined therapy of L-T4 and liothyronine (L-T3), probably because of insufficient peripheral conversion of thyroxine (T4) to thyronine (T3) (13).

Endocrinologists and pediatricians must ensure that parents are appropriately informed about the correct method of L-T4 administration and the possible interference of some drugs, supplements and foods. Parents also need to be made aware of the potential negative consequences for growth and especially neurological development of their child in case of insufficient replacement (10). Thickened formulas are increasingly used in patients with gastroesophageal reflux. The availability of calcium, iron, and zinc tends to be significantly impaired by products thickened with locust (carob) bean gum compared to non-thickened infant formulas (14).

In this case report, an infant with CH whose TH levels worsened after a carob-bean gum thickened formula was introduced for gastroesophageal reflux disease (GERD) is presented for the first time. The possible interference of infant milk formula with L-T4 treatment is discussed.

Case Report

A baby girl was referred for diagnosis with by newborn screening with a blood spot TSH of 186.57 µIU/mL. At the first evaluation, anamnesis showed that the patient was born to non-consanguineous, healthy, young parents. There was no family history of thyroid disorders. Pregnancy was uncomplicated and the child was born by cesarean delivery at 39 weeks of gestation. Her birth weight was 3.140 g [-0.36 standard deviation score (SDS), according INeS chart, 2010], length 49.0 cm (-0.41 SDS, according INeS chart, 2010) and head circumference 34.5 cm (0.44 SDS, according INeS chart, 2010). Apgar scores were 9 at one minute and again 9 at five minutes. Clinical examination at 8 days of age revealed icterus, little spontaneous motor activity, a hoarse cry and cool, dry, rough and thick skin. Her abdomen was distended with an umbilical hernia. At that time she was fed breast milk supplemented with regular type 1 formula milk (80 mL for seven meals a day). On confirmatory blood evaluation, also on day 8, TSH was 496.0 µIU/mL (normal value for age: 0.70-11.0 µIU/mL), free T4 (fT_4) was 0.13 ng/dL (normal value for age: 1.24-3.89 ng/dL), and free T3 (fT3) was 1.05 ng/dL (normal value for age: 2.73-8.46 ng/dL), confirming the diagnosis of CH. Further testing showed anti-thyroid peroxidase, anti-thyroglobulin and TSH receptor antibodies (TRAbs) were negative. Urinary iodine excretion was normal. Ultrasound examination of the neck was consistent with thyroid agenesis (Figure 1).

The patient immediately started therapy with 32.2 µg/day (10.6 µg/kg per day) of liquid L-T4. After five weeks of L-T4 treatment, TSH values had normalized at 2.72 µIU/mL

(normal value: 0.40-4.0 µIU/mL) with fT_4 values of 2.08 ng/dL (normal value for age: 1.24 - 3.89 ng/dL). However, five weeks later, blood tests revealed lower fT_4 values (1.27 ng/dL) associated with an increase in TSH to 31.1 µIU/mL, without any clinical findings of hypothyroidism and anamnesis suggested good compliance. Furthermore, parents reported that they waited at least 30 minutes between the milk meal and the administration of L-T4. Consequently, the L-T4 dose was raised to 50.0 µg/day (10.9 µg/kg per day) in line with the increase in body weight. One week later, TSH had again increased to 44.8 µIU/mL (normal value: 0.40-4.0 µIU/mL), while fT_4 had decreased further to 1.16 ng/dL although it remained in the normal range for age of 0.8-1.9 ng/dL. The patient was not taking any medications other than L-T4. Anamnesis revealed that to supplement breast milk, the patient had been given a carob-bean gum thickened formula (170 mL for 7 meals a day) rather than regular type 1 formula milk for approximately three weeks as treatment for GERD. When this anti-reflux milk was discontinued, after only two weeks, the TSH level dropped to 0.38 µIU/mL, and her fT_4 level increased to 2.68 ng/dL. L-T4 treatment was reduced to 8.0 mg/kg per day, with normalization of thyroid function (Figure 2).

Discussion

This case suggests that carob-bean gum thickened formula may influence the absorption of L-T4. We therefore suggest additional caution when the use of these products in patients with CH is necessary. If such formula is used, we recommend evaluating thyroid function more frequently. To the best of our knowledge, this is the first published report of a potential interference of this type of anti-reflux milk on the absorption of L-T4. Therefore, further studies are needed to confirm these findings.

Some drugs used to manage infants with recurrent regurgitation, such as acid lowering agents or increasing gastric pH, reduce the intestinal absorption of L-T4 (15). With different mechanisms, specific medications for infant colic, such as simethicone, alter the bioavailability of L-T4 (16).

Various thickening agents derived from cereals, such as polysaccharide from glass rice and carob-bean gum, which are all sources of dietary fibre, are commonly used in the treatment of GERD and failure to gain weight in infants and children (17). Cow's milk formulas with added thickening agents, again including carob-beans or galactomannan, have long been commercially available. Carob-beans thicken formula milk, increasing its viscosity. The beans have a high sugar content (48-56%: mainly sucrose, glucose, and fructose), and a low protein (3-4%) and fat content (0.2-0.6%). They are also rich in dietary fibre and minerals, including calcium and iron (18). Milk thickening agents with carob-bean gum improve clinical symptoms of GERD in infants (19), even if data from rat studies have suggested a reduction in gastric emptying rate with a slowed passage of food from the stomach to the upper small intestine (20). In one study on infants with gastroesophageal reflux, a thickened formula with commercially available concentrations of locust (carob) bean gum slowed gastric emptying (21). Another study revealed a 1000-fold increase in meal viscosity, with a significant delay in gastric emptying (22). However, in a study involving 20 full term Thai infants without pathological gastroesophageal reflux there was no significance difference in gastric emptying half time (17). In adult humans, the addition of locust bean gum to a semisolid meal significantly delayed the gastric emptying rate (23).

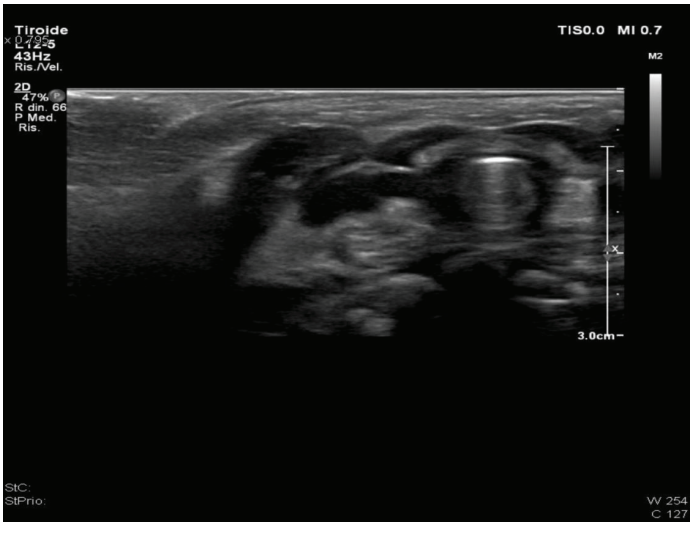

Figure 1. Ultrasound of the thyroid gland showing the absence of thyroid tissue

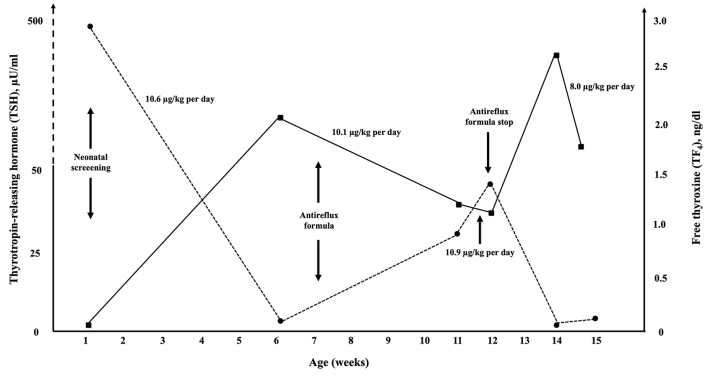

Figure 2. Trend in fT_4 and TSH, illustrating the effect of anti-reflux formula on their values

fT_4: free T_4, TSH: thyroid stimulating hormone

To date the correlation between formula milk and L-T4 absorption is not well clarified, but it would seem that composition of infant formula milk, rich in fat, protein and lactose, may reduce it. Consequently larger L-T4 doses are required to maintain euthyroidism (7). One study reported that in adults, cow's milk may reduce L-T4 absorption by nearly 8%. There are no data in the literature regarding breast milk, although it is possible that it also decreases L-T4 bioavailability (24,25).

The effect of soy bean-containing infant formulas, as well as soy-containing baby foods, on thyroid function in early childhood is better understood (11,26,27,28,29,30,31). Soy-based formula is often an alternative to milk protein formula in cases of allergies or intolerances. Two historic case reports described two infants fed with soy-based formula, one of whom had cretinism and goitre at 10 months of age, and the other was asymptomatic but with persistently high TSH levels despite high doses of L-T4. In both cases, thyroid function normalized after discontinuation of the soy-based diet and appropriate L-T4 dose adjustment (11,28). Other cases of soy-induced goitre in infants have been reported (29,30). Infants fed soy formula have prolonged increase of TSH when compared to infants on non-soy formula and they may need more frequent TSH measurements and increased L-T4 doses to achieve good control of thyroid function (31).

Of note, certain herbal remedies and nutritional supplements contained in formula milk, including iron, calcium and fibre, may also impair L-T_4 absorption (2,9), although these were not used in the presented case.

Conclusion

Carob-bean gum thickened formula appears to impair the absorption of L-T4. Therefore, we recommended caution and frequent thyroid function evaluation if these products are given to patients with CH. In infants who need increasing doses of L-T4, it is essential to investigate the method of administration first, as this is usually the cause of poorer thyroid function test results, and the medical history, including current medicines and diet, before modifying the drug dosage. Furthermore, parents and caregivers should be educated about factors that may interfere with L-T4 therapy in order to prevent or reduce potential irreversible neurodevelopmental damage.

Ethics

Informed Consent: Written and signed consent has been obtained from the patient's parents.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: Stefano Stagi, Concept: Stefano Stagi, Design: Claudia Signorino, Data Collection or Processing: Marta Ferrari, Analysis or Interpretation: Marta Ferrari, Literature Search: Stefano Stagi, Claudia Signorino, Giovanna Municchi, Writing: Claudia Signorino.

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