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Research Article

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

PAPATYA ÇAKIR ED et al. Endocrine Disorders in Mitochondrial Disease

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What is already known on this topic?
Primary mitochondrial diseases can manifest with endocrine abnormalities characterized by problems in hormone production and secretion. The initial clinical manifestation of primary mitochondrial disease may be a hormonal deficit.

What does this study add?
This study examines the genetics, phenotype, auxological data, and hormonal profiles of children and adolescent patients with mitochondrial disease. To the best of our understanding, this study represents the most extensive investigation conducted on this specific patient population in Turkiye.

Abstract
Objective: Endocrine abnormalities may represent the only clinical manifestation of primary mitochondrial disorders. This study aimed to evaluate the endocrinological characteristics of mitochondrial disease in our cohort.
Methods: A total of twenty-six pediatric patients diagnosed with mitochondrial disease were categorized on the basis of their specific genetic abnormalities. The auxologic data, pubertal development, and, based on their clinical symptoms, hormonal profiles were obtained.
Results: Twelve of the cohort of 26 patients (46%) were female. In 15 of the patients (57.6%), their mitochondrial disease (MD) was caused by nuclear DNA mutations (nDNA group). Four patients had Leigh syndrome, 2 patients had LHON syndrome, 2 patients had MELAS, and 1 patient had KSS clinical phenotype. The median age at diagnosis was 2.91 (0.59–16.8) years, and the median age at first endocrinologic evaluation was 4.62 (1.26–18) years. The mean height SDS was -1.34 ± 2.12, and the mean BMI SDS was -0.82 ± 1.96 for all patients. Of the 26 patients, 6 (23%) had a range of hormonal deficits. Ovarian insufficiency, central adrenal insufficiency, central hypothyroidism, diabetes mellitus, and critical illness-related adrenal insufficiency were all observed. Three of the patients were initially monitored in the endocrine clinic for hormone deficiencies but it was later determined that the hormonal abnormalities were caused by underlying mitochondrial disease.
Conclusion: Individuals diagnosed with mitochondrial disease, particularly those with specific genetic abnormalities, are considered a high-risk group for developing hormonal deficits. Endocrine diseases could be one of the primary mitochondrial disorders' early warning symptoms.
Keywords: Primary mitochondrial disease, genotype-phenotype, Endocrine disorders, Endocrin abnormalities

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Introduction

Primary mitochondrial diseases (PMDs) are multisystemic diseases that encompass a broad spectrum of conditions. The incidence of mitochondrial diseases (MDs) is estimated at 1/4500–5000 (1). These disorders are caused by point mutations or by large deletions in either the mitochondrial (mtDNA) or nuclear DNA (nDNA), which both alter the structure and function of the mitochondria. In addition to the well-known pattern of maternal inheritance of MD, mutations in two genes can result in autosomal dominant, autosomal recessive, and rare X-linked disorders. Occasionally, sporadic cases may occur (2). Clinical manifestations are

extremely variable, and early symptoms of these disorders may manifest at any age. Mitochondrial inheritance patterns differ in addition to being complex. A single cell may contain hundreds or thousands of mtDNA copies. Homoplasmy occurs when all cells' mtDNA copies are identical (mutant or wild type). Heteroplasmy refers to the presence of mutant or normal mtDNA in a cell. However, the ratio of mutant mtDNA heteroplasmy may not correlate with the patient's clinical symptoms. The precise reason for this circumstance is unknown (3). Mitochondrial diseases are in the subgroup of inherited metabolic diseases that develop energy deficiency. In the classification of mitochondrial diseases, specific clinical, radiological, biochemical findings and physiological analyzes are taken into consideration. However, since it has a wide spectrum of phenotypes and genotypes, it is the most difficult group of metabolic diseases to classify. Defects in respiratory chain function and oxidative phosphorylation affect mitochondrial energy metabolism, leading to multisystemic organ failure. PMDs are multisystemic diseases that primarily affect metabolically active tissues, such as the brain, kidney, heart, skeletal muscles, and endocrine organs. Mitochondrial diseases constitute a large genetic group and are among the rare diseases. In fact, despite the prevalence of some cases, it is considered a very rare disease. Therefore, due to the nature of the disease, it is almost impossible to create a single homogeneous genetic study group unless it is a multicenter, multinational study (4). Endocrine diseases could be one of PMDs' early warning symptoms (5). Although diabetes mellitus (DM) is a well-known illness resulting from mitochondrial dysfunction, PMDs can also be expressed in hormonal deficiencies, such as ovarian insufficiency, adrenal insufficiency, hypoparathyroidism, growth hormone deficiency, and hypopituitarism. In mitochondrial diseases such as Kearns-Sayre syndrome, which is characterized by extensive mtDNA rearrangements, endocrine issues are prevalent (6). All steroid hormones are synthesized in the mitochondria, and poor oxidative phosphorylation results in mitochondrial dysfunction, which impairs the production of intracellular hormones and the secretion of extracellular hormones (7). When patients with multisystemic diseases have endocrine abnormalities, it should be kept in mind that this population may have PMD. Although endocrinological involvement in mitochondrial diseases has been a known entity for a long time, genetic and phenotype-related publications are quite limited.

In this research, we assessed pediatric patients with PMDs for any endocrinologic abnormalities. Although three patients in our cohort had first been monitored in the endocrine clinic due to hormone deficiencies, it was later discovered that the cause of the hormone deficiencies was underlying mitochondrial disease. In this study, we examined the endocrinologic characteristics of twenty-six PMD patients whose diagnoses had been genetically and phenotypically confirmed.

Methods

Patients

In total, 26 patients were evaluated in this cross-sectional descriptive study. Twenty-three of the twenty-six patients with PMD were monitored in a tertiary center pediatric metabolism unit. The remaining three patients were initially diagnosed in a tertiary center pediatric endocrinology unit with primary ovarian insufficiency, diabetes mellitus, and adrenocorticotropic hormone (ACTH) deficiency.

We investigated the auxological indices, clinical records, and hormonal profiles of patients when they were first admitted to the Dr. Sadi Konuk Education and Research Hospital Outpatient Pediatric Endocrinology and Metabolism Clinics. All patients' clinical characteristics were reported, and they were categorized as having either nDNA or mtDNA mutations. These genetic changes were further categorized according to the areas affected, as based on the literature (8). The databases Mitocarta and Mitomap were utilized to improve the classification of the patients' genetic results. The study protocol was approved by Bakırköy Dr. Sadi Konuk Education and Research Hospital clinical trials ethical committee (date: 17.4.2023, approval number 2023 /154).

Parameters for the Study

We documented the metabolic features of the patients from July 2016 to September 2023 and subsequently used them to classify the identified mitochondrial disorders. The auxologic data (height, weight, body mass index (BMI), head circumference) of patients were evaluated using the child metrics program and Turkish children's references (9). Additionally, child metrics were used to assess the birth auxologic data for the patients, using references to Turkish neonates (10). Based on their clinical symptoms, hormonal profiles were obtained. Bone metabolism and other enzyme and hormone profiles (calcium, phosphorus, magnesium, alkaline phosphatase, parathormone and 25-hydroxyvitamin D) were assessed using serum electrolytes. Vitamin D status is assessed by testing serum 25-hydroxyvitamin D. Vitamin D levels were classified as sufficient (20-100 ng/mL), insufficient (15-20 ng/mL), or deficient (<15 ng/mL) (11).

Glycated hemoglobin (HbA1c) was used for assessing glucose metabolism. The blood glucose levels of hypoglycemic patients undergoing serial glucose monitoring were also assessed.

Gonadotropin levels were acquired from patients whose secondary sex characteristics were examined clinically. Girls greater than 13 years old and boys greater than 14 years old who had no pubertal signs were considered to have delayed puberty.

The levels of thyroxine (ft4) and thyroid-stimulating hormone (TSH) were determined for every patient. The thyroid autoantibodies of patients whose thyroid function tests were abnormal were collected. In the physical examination, none of the patients had goiters. Serum triiodothyronine (ft3) levels were tested in 19 of the patients.

All patients' basal adrenocorticotropic hormone (ACTH) and cortisol levels were evaluated. Patients with low baseline cortisol levels and hypoglycemic symptoms were tested for adrenocortical insufficiency. A low-dose (1 µg) synthetic ACTH (cosyntropin) test was conducted on patients with basal cortisol levels below 15 µ/dL and with serum cortisol levels below 20 µ/dL during hypoglycemia. Serum cortisol levels were assessed at 10, 20, and 30 minutes. Adrenal insufficiency is indicated when cortisol levels fall below 20 µ/dL following an ACTH injection.

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

Metabolic Medical Treatment

Cases were supported with medical treatment at the indicated doses: L-Arginin 200 mg/kg/day, divided into three doses; Coenzyme Q10 15mg/kg/day, divided into two doses; Vitamin B1 10mg/kg/day, divided into two doses; Vitamin B2 10 mg/kg/day, divided into two doses; Vitamin B6 30mg/kg/day, once a day; L-Carnitine 50 mg/kg/day, divided into two doses; Lipoic acid 10mg/kg/day, once a day; Dichloroacetate 25 mg/kg/day, divided into three doses; Vitamin C 100mg/kg/day, divided into two doses.

Nutritional Assessment of the Patients

The parents of the patients were trained to keep food records to evaluate the medical nutrition treatments, and three days of food records (two weekdays and a weekend day) were kept. A photographic food catalog was used to determine the amounts and portion sizes of the foods consumed (12). According to these food records, daily mean energy and macronutrient intake were calculated using a nutrient database program (BeBis 8.2. software), based on the U.S. Department of Agriculture's (USDA) FoodData Central and TurKomp National Food Composition Database (13–16). The energy requirements of patients were calculated according to age and gender according to FAO/WHO/UNU equations (17, 18).

Biochemical Analysis

Venous blood samples were obtained from the antecubital vein in vacutainer tubes following an overnight fast by the participants. The plain tubes were centrifuged at 2000 g (10 min) to remove the serum for routine biochemical analyses. Blood samples were immediately centrifuged in EDTA tubes at 1000xg, at 4^0C (10 min) for the ACTH analyses. Another EDTA tube was used to measure HbA1c. Routine biochemical parameters were determined by a Roche Cobas C8000 modular auto-analyzer using commercial kits (Roche Diagnostics, CA, USA). Plasma ACTH was measured using a solid phase, two-site enzyme chemiluminescent system (Immulite 2000 XPi, Siemens Healthcare Diagnostics, USA). HbA1c levels were measured by an Arkray Adams HA-8160 analyzer, using reversed-phase cation exchange "high performance liquid chromatography" (HPLC; Arkray KDK, Kyoto, Japan).

Genetic Analysis

Genomic DNA and mtDNA were isolated from peripheral blood lymphocytes. The initial test was for mtDNA sequencing using an in-house developed fragmentation-based methodology. The fragmentation process was performed using the Ion Xpress™ Plus Fragment Library Kit. Patients with unidentified genetic variation (no heteroplasmic or homoplasmic causative variant associations in mtDNA) were investigated with exome sequencing. They were examined by clinical exome sequencing (CES) using the Illumina Clinical-Exome Sequencing TruSight One Gene Panel. In the CES, the libraries generated were sequenced using Illumina Nextseq500 next-generation sequencing platforms. The detected variants were then confirmed by conventional Sanger sequencing.

Statistical Analysis

All data were statistically analyzed using the Graph Pad Instat program. Descriptive statistics were determined as the mean, standard deviation, median, minimum, and maximum values. The categorical variables were given as percentages. The Kolmogorov-Smirnov test was used to test the normality of variable distribution and the homogeneity of the variance.

Results

The study included twenty-six pediatric patients. Anthropometric parameters, nutritional status, vitamin supplements, and thyroid functions were monitored in the metabolism outpatient clinic, and in consultation with the endocrinology outpatient clinic when there were abnormalities in growth parameters and hormonal profiles.

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

Of the whole group, 12 patients (46%) were female. Of the entire group, the mitochondrial disease of 15 patients (57.6%) was caused by nuclear DNA mutations (Tables 1 and 2).

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

The birth data included 52% (n = 13) of the patients' weight information, 48% (n = 12) of their length information, and 38% (n = 8) of their head circumferences. The demographic data for the study group are presented in Table 3.

Twenty-two of the patients were prepubertal, and four were in pubertal stage 5. Three of the pubertal stage 5 patients were female (Patients 5, 19, and 26) and one was male (Patient 18). These female patients all had regular menstrual cycles. Patient 5 had ovarian insufficiency, but she took pubertal hormone replacement therapy and had regular menstrual cycles (Table 3).

Serum calcium, phosphorus, and magnesium concentrations were in the normal range for all patients. The mean parathyroid hormone (PTH) level was 38.63 ± 23.59 pg/mL (Table-4). Of the cohort, 65.4% (17/26) of the patients had 25-hydroxyvitamin D levels greater than 20 ng/mL; the median 25-hydroxyvitamin D level was 20 (4.71–94.2) ng/mL. Vitamin D deficiency (25-hydroxyvitamin D levels less than 15 ng/mL) and vitamin D insufficiency (25hydroxyvitamin D levels between 15–20 ng/mL) ratios in our patients were 19.2% and 15.4%, respectively.

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

Six of the twenty-six patients (23%) had a range of hormonal deficiencies. Four of these six patients were in the nDNA group. A significant percentage, precisely 50%, of individuals who received a diagnosis of hormonal deficiency underwent their initial assessment in the endocrinology unit.

Patients with Hormonal Deficiencies in the nDNA Group

Critical illness-related adrenal insufficiency

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

Ovarian insufficiency

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

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

Diabetes mellitus

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

Patients with Hormonal Deficiencies in the mtDNA Group

Central adrenal insufficiency

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

Central hypothyroidism

Patient 21 had a mutation in the *MT-ND3* gene, phenotype for LHON (Leber's hereditary optic neuropathy), microcephaly, neuromotor and growth retardation, and contractures. This patient was diagnosed with MD at the age of 1.16 years and with central hypothyroidism at the age of 2.34 years. Patient's level of TSH 1.3 mıU/mL, ft4 0.87 ng/dL, ft3 3.7 pg/mL (2.41–5.5) in her first assessment with no other acute illnesses; her thyroid function tests were TSH 0.8 mıU/mL, ft4 0.81 ng/dL, ft3 2.2 pg/mL (2.41–5.5). Her basal ACTH level was 22 pg/mL, and her cortisol level was 8.26 mg/dL. 1 mcg ACTH stimulation test was performed, and her peak cortisol level was found to be 22 mcg/dL, and L-thyroxin treatment started at 10 mcg/kg/daily.

There were no other pituitary hormone deficiencies. Magnetic resonance imaging of the pituitary showed it to be normal.

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

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

Discussion

Hormone synthesis and secretion are both energy-dependent processes. This dependency makes the endocrine glands sensitive to mitochondrial dysfunction. Primary mitochondrial diseases may result in one or more hormone deficiencies, depending on the severity of the mitochondrial disorder. In addition, due to the random distribution of mitochondria during embryogenesis, the endocrine glands that are affected cause unpredictable clinical characteristics (8). It is known that findings may be very different, depending on whether the pathogenic variation is inherited in the nDNA or the mtDNA, together with its type. Even within the same family, clinical findings of varying severity may develop depending on penetrance and on the other reasons mentioned above (1). In this paper, we present the pattern of endocrinological involvement in mitochondrial diseases as diagnosed in our single-center study.

MD prevalence is estimated as 1/5000 in the adult age group (2/3 of whom are mtDNA), ad as 5–10/100 000 in the pediatric age group (80% of whom are nDNA) (3). In the North American Mitochondrial Disease Consortium (NAMDC) Patient Registry study, 60% of pediatric patients had mtDNA mutations (8). Similar to our cohort, (Table 1 and 2). The explanation for this situation is that patients with nDNA mutations are diagnosed earlier as more severe clinical findings develop at an earlier stage of life.

The Mitochondrial Society 2017 guideline recommends annual or biannual endocrinologic evaluation for these patients, even if they have no hormonal dysfunction at the time of diagnosis (19). An Australian cohort with a mean age of 5.09 years at diagnosis was thus considerably older than our cohort at diagnosis (20). In contrast to our study, in a large cohort that consisted of patients of any age, female dominance was observed (8). In our study, there were more male than female patients (Table 3). This may be related to the fact that our group included a relatively limited number of patients.

Twenty-three percent of our patients (two with mtDNA and four with nDNA mutations) had already had endocrinological abnormalities detected. It is difficult to estimate the prevalence of endocrinological disorders in all mitochondrial diseases. Theoretically, in all cases of mitochondrial disease, the endocrine glands are sensitive to energy deficiency and oxidative stress. However, it has been shown that some well-known mutations can cause distinct endocrine findings (21, 22). While endocrinological follow-up is recommended for all patients with MD, patients with these identified mutations should be evaluated more closely.

It is a well-known problem that MD patients show poor growth, quite apart from any growth hormone insufficiency. There are many factors affecting growth in this group of patients. Most patients show prenatal and postnatal growth failure. Jürgen-Christoph von Kleist-Retzow et al. observed that 22.7% of 300 mitochondrial respiratory chain deficiency patients had intrauterine developmental retardation (23). Feeding difficulties, restriction of energy production, frequent infections, and multiple organ system failures all have negative effects on patients' growth. Skeletal changes, such as joint contractures, scoliosis, or kyphosis, also have negative effects on MD patients. Growth hormone deficiency may be the first symptom of MD (24, 25). The majority of our patients were close to normal height; however, within our group, we had ten patients with SDS values below -2. None of our patients was growth hormone deficient. In this respect, supportive treatment is found to be valuable in MD patients, even without growth hormone deficiency. Despite the fact that not all of our patients had completed their growth, the mean height was slightly greater than that of an adult MD cohort from the United Kingdom, as stated in a report published in 2009 (25).

Hypo- or hyper-gonadotropic hypogonadism are both well-known entities in MD patients. Ovarian insufficiency alone, with or without sensorineural hearing loss, is frequently seen in MDs (26). Recently, some studies have reported that the *RMND-1* gene is a candidate for premature ovarian insufficiency. Our patients had phenotypic features similar to those of these studies, including SNHL and chronic renal failure (27–32).

Primary adrenal insufficiency is the main cause of adrenal insufficiency in MDs. To the best of our knowledge, secondary adrenal insufficiency has been reported only in a Japanese woman, fifty years of age (33). In another report on 18 patients with single large scale mitochondrial DNA deletions (SLSMDs), 39% of patients had impaired basal adrenocortical function (34). We had two patients with adrenal insufficiency, and only one of them had a critical illness related adrenal insufficiency. This patient had elevated cortisol levels, despite having clinical adrenal insufficiency. Under conditions of critical stress, the elevation of the cortisol level is explained by the adrenal gland's subacute response to the situation, decreasing cortisol clearance, and shifting to cortisol receptor activation (35). None of our patients had primary adrenal insufficiency, including

two siblings with *NNT* mutations. Both of these had dystonia and difficulty walking, although neither had hypoglycemia, fatigue, or hyperpigmentation, and their reactions to the 1 µg ACTH test were normal (peak cortisol responses were 19 and 22 µg/dL, respectively). This mutation is a well-known cause of familial glucocorticoid deficiency (36).

DM is a common occurrence in MDs. Decreased sensitivity and insulin production are both seen in pathophysiology. The *MT-TL-1* gene m.3243A>G mutation is the most prevalent mutation in diabetes-associated MD (37). Patient 23 had this genetic abnormality, but no clinical or biochemical signs of diabetes. This genotype is associated with the MELAS phenotype, as was the case in our patient. In the general population, the prevalence of this genotype is 1/400 and the mean age for diagnosis of diabetes is thirty-eight years. It is thought that this mutation is responsible for ~0.5–2.9% of all diabetes (26,37,38). We had only one female patient with diabetes mellitus with mutation in RRM2B gene.

Mutations in *RRM2B* have been reported as a cause of MD (26). In a Japanese diabetic cohort with m.3243A>G mutations, one patient had high positivity for anti-GAD and ICA antibodies, and the authors suggested the coexistence of MD with autoimmunity in this patient. In this Japanese study, another 12 patients had slightly elevated ICA antibodies. It is suggested that this situation is an autoimmune response to mitochondrial cell injury (37).

In the NAMDC (The North American Mitochondrial Disease Consortium) study reported on earlier, the prevalence of hypothyroidism in their MD cohort was 4.3%. This percentage was considered close to that of the general population (6.3%) in the USA (8). It has also been reported that hypothyroidism is more prevalent with nDNA deletions than with mtDNA (26). We had only one patient with central hypothyroidism, and no one in our cohort had primary hypothyroidism. However, we did have two patients with slightly elevated anti-TPO antibodies. Some studies report that MD patients frequently have autoimmune disorders, but we have no proof that autoimmunity is more prevalent in MD patients than in the general population (39). One study reported Kearns Sayre syndrome in a patient with autoimmune thyroid disease (40). There hasn't been an objective evaluation of mitochondrial cocktails' effectiveness in terms of the endocrine and other involved systems. Unfortunately, it is not easy to evaluate its effectiveness.

Study Limitations

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

Conclusion

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

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Table-1: Genotype -Phenotype Characteristics and Endocrine Dysfunctions in Patients with nuclear DNA mutations

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

Human gene names are written in capital letters and italics.
VUS: variant of unknown significance; Hom. : homozygous; Comp. het.: Compound heterozygous; AR: Autosomal Ressesive, C-I: complex-1, C-IV: Complex-4: CK: Creatinin Kinase, CH: Compound heterozygous, COXPD: Combined Oxidative Phosphorylation Deficiency, CRF: Chronic Renal Failure, DM: Diabetes Mellitus, GR: Growth retardation, HCM: Hypertrophic cardiomyopathy, IMM: Inner Mitochondrial Membrane, MM: Mitochondrial membrane, MCC: Mitochondrial Calcium Channel, MMrx: Mitohondrial Matrix, , MEGDEL: 3-methylglutaconic aciduria with deafness-encephalopathy Leigh-like syndrome, NMR: Neuromotor retardation, SNHL: Sensorineurol Hearing Loss, TPO: Thyroid peroxidase

Tablo-2: Genotype and Phenotype Characteristics and Endocrine Dysfunctions in Patients with Mitochondrial DNA mutations

	Patient No	Gene; Locus MIM number; Inheritance	Nucleotide Change	Amino Acid Change	Associated region	Zygosity	Phenotypic features	Endocrine system findings
1.Large-scale Rearrengements								
2. Single-nucleotide variants (point mutation) in genes encoding structural proteins	16	*MT-ATP6;* * 516060; Mt-in	m.8993T>C	p.L156P	C V	heteroplasmy (%89)	Dystonia, hypotonia, contractures, walking difficulty,GR	Normal
	17	MT-ND4 * 516003 Mt-in	m.11467A>G	p.L236L	C1	Homoplasmy (%99)	Hypoglycemia, encephalopaty, liver failure	Vitamin D insufficiency
	18	*MT-ND5;* * 516005; Mt-in	m.12372G>A	p.L12L	C I	Homoplasmy (%99)	LHON,epilepsy, NMR	Vitamin D insufficiency
	19		m.12706T>C	p.F124L	C I	Homoplasmy (%97)	MELAS,cortical blindness, epilepsy	Vitamin D deficiency
	20	*MT-ND1*; * 516000; Mt-in	m.4216T>C	p.Y304H	C I	Homoplasmy (%99)	LA,nystagmus, NMR,GR Leigh syndrome	Central Adrenal Insufficiency Vitamin D insufficiency Short stature
	21	*MT-ND3*; * 516002; Mt-in	m.10398A>G	p.T114A	C I	Homoplasmy (%99)	Microcephaly, contractures, LHON, NMR, GR	Central Hypothyroidism Vitamin D deficiency Short stature
3. Mutation in genes encoding tRNA	22	*MT-TA*; * 590000; Mt-in	m.5631G>A	tRNA Ala	mitochondrial–nuclear crosstalk	homoplasmy (%100)	HCM, SNHL, GR,myopathy, lactate and CK elevation	Normal
	23	*MT-TN;* * 590010;; Mt-in	m.5667G>A	tRNA Asn	mitochondrial–nuclear crosstalk	heteroplasmy %88	Strabismus,epilepsy, NMR	Vitamin D insufficiency
	24	*MT-TL1;* * 590050; Mt-in	m.3243A>G	tRNA Leu	mitochondrial–nuclear crosstalk	heteroplasmy %87	MELAS, ptosis, LA, myopathy	Normal
	25	*MT-TL2*; * 590055; Mt-in	m.12308A>G	tRNA Leu	mitochondrial–nuclear crosstalk	homoplasmy %97	NMR, autism	Normal
4. Mutation in genes encoding rRNA								
5.Other miscellaneous	26	MT-CR	16519T>C ()	(non-coding)	entire Control Region	Homoplasmy (%100)	KSS, hypotonia,NMR,GR	Vitamin D deficiency Short stature

C-I: complex-1, C-V: Complex-5, CK: Creatinin Kinase, GR: Growth retardation, HCM: Hypertrophic cardiomyopathy, MCR: Mitochondrial Control Region, Mt-in: mitochondrial inheritance KSS: Kearns-Sayre Syndrome, LA: Lactic acidemia, LHON: Leber hereditary optic neuropathy, MELAS: Mitochondrial encephalopathy with lactic acidosis and stroke like episodes, NMR: Neuromotor retardation, SNHL: Sensorineurol Hearing Loss,

Table-3: Study population characteristics

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

*Nonparametric disturibition according to Kolmogorov-Smirnov test
BMI: Body mass index
SDS:standart deviation score
IQR. Interquartile range

Table-4: Biochemical and hormonal profiles of study population

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

*Nonparametric disturibition according to Kolmogorov-Smirnov test
TSH: Thyroid stimulation hormone
T4: thyroxine
T3: tri-iodothyronine
ACTH: Adrenocorticotrophic hormone
ALP:Alkaline phosphatase
PTH: Parathyroid hormone
25 OH vitamin D: 25 hydroxyvitamin D
HbA1c: Glycolysated Haemoglobin A1c
FSH:Follicle stimulating hormone
LH: Luteinising hormone
IGF-1: Insulin like growth factor 1
IGFBP-3:Insulin like growth factor binding protein 3