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CLINICAL REPORT

Pyruvate dehydrogenase deficiency disease detected by the enzyme activity of peripheral leukocytes

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Abstract

Background: Pyruvate dehydrogenase complex (PDHC) deficiency is a common neurodegenerative disease associated with abnormal mitochondrial energy metabolism. The diagnosis of PDHC is difficult because of the lack of a rapid, accurate, and cost-effective clinical diagnostic method.

Methods: A 4-year-old boy was preliminarily diagnosed with putative Leigh syndrome based on the clinical presentation. PDHC activity in peripheral blood leukocytes and a corresponding gene analysis were subsequently undertaken. Sodium pyruvate 1-^{13}C was used for the analysis of PDHC activity in peripheral leukocytes. The genes encoding PDHC were then scanned for mutations.

Results: The results showed that the corresponding PDHC activity was dramatically decreased to 10.5 nmol/h/mg protein as compared with that of healthy controls (124.6 ± 7.1 nmol/h/mg). The ratio of PDHC to citrate synthase was 2.1% (control: 425.3 ± 27.1). The mutation analysis led to the identification of a missense mutation, NM_000284.4:g214C>T, in exon 3 of *PDHC*.

Conclusion: The peripheral blood leukocyte PDHC activity assay may provide a practical enzymatic diagnostic method for *PDHC*-related mitochondrial diseases.

KEYWORDS

Leigh syndrome, leukocyte, PDHA1, pyruvate dehydrogenase complex (PDHC)

1 | INTRODUCTION

The pyruvate dehydrogenase complex (PDHC) is an important metabolic enzyme, which catalyzes oxidative decarboxylation of pyruvate to acetyl-coenzyme A (acetyl-CoA). The complex consists of three enzymes: pyruvate dehydrogenase (E1, EC1.2.4.1), dihydrolipoamide transacetylase (E2, EC2.3.1.12), and dihydrolipoamide dehydrogenase (E3, EC1.8.1.4). The E1 enzyme is a heterotetramer containing two α and two β subunits and has a thiamin pyrophosphate binding site (Chun et al., 1995; Patel et al., 2014). The gene (*PDHA1*) encoding the E1α subunit is localized on the X chromosome (Cardozo et al., 2000). The clinical presentation of PDHC deficiency is heterogeneous and differs among patients. Common clinical presentations include neonatal encephalopathy with lactic acidosis, mild systemic acidosis, nonprogressive infantile encephalopathy and childhood intermittent-relapsing encephalopathy, Leigh syndrome and Leigh-like syndrome, and relapsing ataxia (Patel et al., 2012).

PDHC deficiency occurs most commonly due to mutations in the *PDHA1* gene encoding E1α. Mutations in this gene result in an accumulation of pyruvate and lactic acid and are responsible for most cases of PDHC deficiency (OMIM 312170) (Chun et al., 1995; Lissens et al., 2000; Quintana et al., 2010). PDHC deficiency is a mitochondrial disorder, which leads to impaired energy metabolism and a wide range of clinical symptoms. Mutational variations and the degree of X inactivation in females are responsible for the different clinical manifestations in patients with pyruvate dehydrogenase (PDH) deficiency (Bhandary & Aguan, 2015). It has been reported that a family with pyruvate dehydrogenase complex deficiency to a level C>t substitution at nuclear position 407 in exon 4 of the X-linked E 1 α gene (Tulinius et al., 2005).

The diagnosis of PDHC deficiency mainly depends on a PDHC activity assay and an analysis of PDH-related genes Progressive neurological degeneration and elevated lactate and pyruvate raise a suspicion of PDHC deficiency. Further measurements of PDHC activity and gene analysis are essential to confirm the diagnosis (Bhandary & Aguan, 2015). Skin fibroblasts and muscle are most frequently used for measuring PDHC activity and gene analysis (Quintana et al., 2010; Steller et al., 2014). According to our recent research, we hypothesized that an assay based on PDHC activity in peripheral blood leukocytes could aid the diagnosis of PDHC deficiency. In this study, we describe the diagnosis of PDHC deficiency based on PDHC activity in peripheral blood leukocytes and relative gene analysis.

2 | MATERIALS AND METHODS

2.1 | Patient

The patient was a 4-year-old boy and the first child of healthy and consanguineous parents. The child was born after an uneventful pregnancy, and his mother did not report alcohol or tobacco use during pregnancy. The patient had a normal delivery. The child's parents, grandparents, and relatives were healthy. Motor and mental retardation were obvious. The child could sit unaided at the age of 10 months and walk unsupported at the age of 17 months. Exercise intolerance was apparent. Deterioration, with weakness and ataxia, were observed from the time he was 18 months old and progressively worsened over time. Afterward, he was admitted to local hospitals many times, with complaints of ataxia, weakness, and motor regression. Metabolic acidosis was a prominent presentation during the disease course. All the child's motor abilities gradually disappeared and were completely absent 1 year later. The child died of multiorgan failure when he was 4 years old. Brain magnetic resonance imaging showed hypointensity on T1-weighted images and hyperintensity on T2-weighted images of the bilateral globus pallidus, in addition to symmetrical abnormality, compatible with a diagnosis of Leigh syndrome (data not shown).

2.2 | PDH and mitochondrial respiratory chain activity assay

Peripheral venous blood (5 ml) was added to 20 ml of lysis buffer (0.1 mmol/L of EDTA.K_2, pH 8.0), placed for 15 min until the erythrocytes broke up, and then centrifuged at 1500× *g* for 10 min. The supernatant was discarded, and the pellets (the leukocytes) were collected. The leukocytes were washed with saline twice and then centrifuged at 2,000 × g for 5 min. The pellets were collected, frozen, and stored in −80℃.

The leukocytes were suspended in a suspension buffer at a ratio of 1:50 (v/v) (0.1 mg/mL of digitonin, 25 mmol/L of sucrose, 75 mmol/L of mannose, 1 mmol/L of EDTA-K_2, and 10 mmol/L of Tris, pH 7.2). They were then disrupted by 20 passes in a homogenizer with a tight-fitting Dounce homogenizer. The homogenate was centrifuged for 10 min at 1000× *g*. The supernatant was then collected and centrifuged for 10 min at 10,000 × g. The collected pellets of mitochondria were stored at −80℃ for 6 months. The mitochondria were suspended in phosphate buffer and broken by sonication for 30 s. The total protein level was detected by the Bradford method (Ghafourian et al., 2016).

Assays for mitochondrial respiratory chain complexes were performed in peripheral leukocytes using spectrophotometry. The activity of each complex was expressed both as the rate (nmol/min/mg of mitochondrial protein) and ratio of the rate of citrate synthase, as previously described (Ma et al., 2011).

The isotope-labeling method was used to detect the PDHC enzyme activity of the mitochondria (Overmyer et al., 2018). Briefly, PDHC lysate from 50 μg of leukocyte mitochondria was added to 80 mmol/L of phosphate buffer (pH 7.5) containing 1 mM $MgCl_2$, 10 mmol/L NAD^+, 2 mg/mL coenzyme A, 1 mmol/L thiamin pyrophosphate, and 1 mmol/L of sodium pyruvate 1-^{13}C. The enzyme mixture was kept in the dark for 60 min for reaction. Lys-C in an enzyme:protein ratio of 1:100 was added and incubated with gentle rocking at room temperature for 15–18 hours. The sample was centrifuged at room temperature for 5 min at 10,000× *g* to pellet any insoluble material. The sample was desalted using a Sep-Pak cartridge size to a size ~20 times greater than the protein sample. Following Sep-Pak instructions, the peptide was eluted into a new 2-ml microcentrifuge tube with 1 mL of elution buffer A, followed by 1 ml of elution buffer B. The sample was dried in a SpeedVac. Dried samples were resuspended in 0.2% (vol/vol) formic acid at approximately 1 μg/μl concentration. A quantity of 2 μg of peptides was injected into a nano-LC column for LC–MS/MS analysis. A PDHC enzyme mixture inactivated by boiling was

used as a control. The enzyme activity is measured in nmol/h/mg protein. The enzyme kinetics of mitochondria citrate synthase was determined. Briefly, 5 μg of PDHC crude enzyme from human leukocyte mitochondria were added to Tris-HCl buffer (pH 8.0) containing 100 μmol/L of DTNB, 50 μmol/L of acetyl-CoA, 0.1% (w/v) Tritonx-100, and 250 μmol/L of oxaloacetic acid. The mixture was scanned immediately at 412 nm for 10 min, and changes in the absorption values were recorded. The activity of the complex was expressed both as the rate (nmol/min/mg of mitochondrial protein) and as the ratio of PDHC to citrate synthase.

TABLE 1 Data of the pyruvate dehydrogenase complex (PDHC) activity

	Patient	Control (average ± SD)	Reference range
PDHC (nmol/h/mg protein)	10.3	124.6 ± 7.1	54.4–189.4
Citrate synthase (nmol/min/mg protein)	443.1	425.3 ± 27.1	300.5–498.4
PDHC/citrate synthase (%)	2.3	29.3	18.1– 38.0

2.3 | PDHC gene analysis

Total genomic DNA was extracted from the peripheral leukocytes by the proteinase-K-chloroform method. Exon 3 of the patient's *PDHA1* gene (NM_000284.4 → NP_000275.1) was amplified from 1 μl of genomic DNA by the polymerase chain reaction (PCR). PCR was performed in a 30-μl system containing 1 μl of DNA template, Ex Taq DNA polymerase, 10×Ex buffer, dNTPs, and primers. The forward and reverse synthetic oligonucleotide primers for exon 3 were as follows: GTAAAACGACGGCCAGTTATACTTTCCAAAATAGCTG and CAGGAAACAGCTATGACCTAAAGCCCAATAAGTACAA, respectively. The reaction was carried out with an initial denaturation step at 94℃ for 5 min, followed by 35 cycles at 94℃ for 40 s, 55℃ for 40 s, and 72℃ for 60 s, and a final elongation step at 72℃ for 5 min. Sequence analysis was performed using ABI-3730XL.

3 | RESULTS

3.1 | Activities of mitochondrial respiratory chain complexes in peripheral blood leukocytes

The activities of mitochondrial respiratory chain complexes I–V were detected in peripheral blood leukocytes. These activities were in the normal range. The details are shown in Table 1.

3.2 | Activities of PDH and gene sequence analysis

The results of both routine blood tests and routine urinalysis were normal. Blood amino acid, acylcarnitine, and urinary organic acid analysis were normal. Serum lactate was increased to 5.2–6.9 mmol/L (normal range: 0.5–2.4 mmol/L). Serum pyruvate was also higher than that of a normal control, ranging from 0.32–0.41 mmol/L (normal range: 0.03–0.1 mmol/L).

The PDHC activity in peripheral leukocyte mitochondria was 10.3 nmol/h/mg protein (reference range: 54.4–189.4 nmol/h/mg protein). The ratio of PDHC to citrate synthase (%) was 2.1 (reference range: 18.1–48.0). The PDHC activity was only 8.3% of the controls, and the ratio of PDHC to citrate synthase was 8.3% of the controls. They all decreased markedly as compared with these parameters in the controls (Table 1).

Sequencing of exon 3 of the *PDHA1* gene revealed a missense mutation, NM_000284.4:g214C>T, in the proband. As a result of this mutation, arginine was replaced with cysteine in position 72 of the protein (Figure 1).

3.3 | Controls

Based on the value of PDH and activities of the mitochondrial complex (I–V) in peripheral leukocytes in 200 control participants (age range: 1–12 years, 108 females, 92 males), statistical analyses were performed using SPSS software (SPSS, IBM Company, Chicago, IL, USA). Each complex activity was stable and showed a normal distribution. Reference ranges (mean ± 1.96 standard deviation), including 95% of normal enzyme activities, were determined for these enzymes (Tables 1 and 2).

4 | DISCUSSION

In cases of PDHC deficiency, the citric acid cycle is impaired, and the production of the principal fuel (cellular ATP) is decreased (Alston et al., 2017). Although the clinical spectrum of PDHC deficiency varies widely, neuromuscular and neurological degeneration are frequently the most dominant characteristics. Leigh syndrome is one of the phenotypes in mitochondrial diseases, characterized by symmetrical necrosis of the basal ganglia, thalamus, and brainstem (Leigh et al., 2015). Brown and Salti reported that PDHC deficiency found in males with neurological diseases often manifested as Leigh syndrome (Bhandary

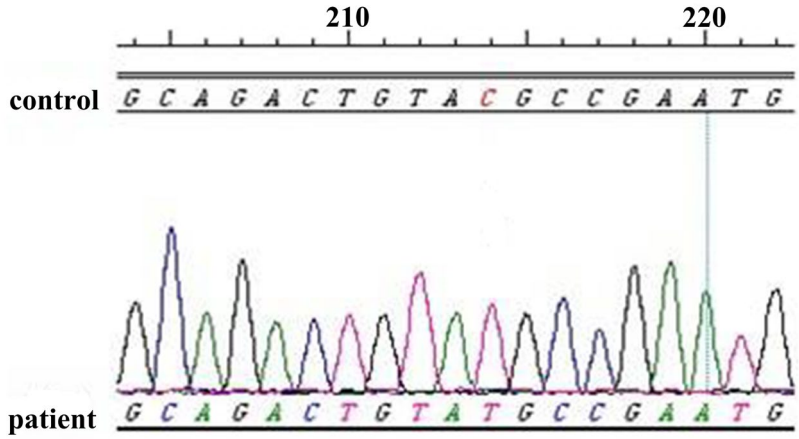

FIGURE 1 Sequencing results of exon 3 of *PDHA1* gene (GenBank reference sequence and version number: NM_000284.4:g214C>T)

TABLE 2 Enzyme assay results of mitochondrial respiratory chain complexes activities

	I		II		I+III		IV		V	
	Patient	**Control**	**Patient**	**Control**	**Patient**	**Control**	**Patient**	**Control**	**Patient**	**Control**
Enzyme rate[a]	212.5	218.0 ± 30.4	79.1	71.8 ± 12.9	703.9	692.5 ± 88.7	84.4	80.1 ± 15.4	175.8	196.9 ± 43.1
Ratio to citrate synthase (%)	48.0	49.8 ± 4.7	17.9	18.3 ± 2.9	158.9	158.5 ± 15.5	19.0	18.3 ± 3.1	39.7	44.8 ± 9.8

[a]Enzyme rate showed as nmol/min/mg of mitochondrial protein.

& Aguan, 2015; Salti et al., 1979). In most patients, PDHC deficiency is manifested in the first few months. Although mutations in the X-linked E1α gene are mainly the cause of PDHC deficiency, the course of the disease is generally very similar among boys and girls. However, some severe symptoms tend to affect boys more than girls, and mortality among boys with PDHC deficiency is proportionately greater than that of female mortality (K. P. Patel et al., 2012).

PDHC deficiency is a common cause of congenital lactic acidosis (Chow et al., 1987). In this study, the presentation of the patient was in line with that of previous reports of Leigh syndrome. The child presented with motor and mental retardation, ataxia, and elevated pyruvate and lactate, all of which pointed to an energy metabolic disorder. A leukocyte PDHC enzyme assay revealed an activity deficiency in enzyme activity. Subsequently, gene analysis confirmed the enzymological diagnosis of PDHC deficiency.

Many mutations resulting in PDHC deficiency have been found. A previous study showed that exon skipping, insertion of duplicate sequences, and missense mutations in the X-linked E1α subunit of the *PDHA1* gene resulted in PDHC deficiency (Chun et al., 1995). Many studies noted that the severity of the deficiency in affected girls was largely dependent on X-chromosome inactivation patterns in the brain. In the present case, the enzyme assay revealed a NM_000284.4:g214C>T mutation of exon 3 in *PDHA1*. As reported previously, this missense mutation in *PDHA1* results in the transformation of arginine into cysteine in highly conserved regions and can lead to PDHC deficiency (Chun et al., 1995; Lissens et al., 2000).

To date, most studies have used cultured skin fibroblasts and muscle for PDHC activity and PDHA1 mutation assays (Chun et al., 1995; Quintana et al., 2010). In a previous case, the activity of PDHC in lymphocytes and muscle (homogenate and mitochondria) was 20% lower than that of a healthy (Bonne et al., 1993). The present study is the first case of a patient with PDHC deficiency to be diagnosed based on a peripheral blood leukocyte PDHC activity assay. In this paper, we measured the activity of mitochondrial PDHC in peripheral leukocytes. The enzyme activity was 8.3% lower than that of the controls, and the ratio of PDHC to synthase also decreased (8.3% lower than that of the controls). To confirm this deficiency, *PDHA1* sequence analysis was performed in leukocytes, and one mutation, NM_000284.4:g214C>T of exon 3, was detected. The presence of the NM_000284.4:g214C>T mutation confirmed that Leigh syndrome was the cause of the PDHC deficiency in peripheral leukocytes in our patient. The use of lymphocytes in PDHC activity assays and in sequencing analysis of PDHA1 is rare. In the present study, we utilized an easily obtainable material and a convenient method (peripheral blood leukocytes) to check the PDHC activity and PDHA1 sequence. The peripheral blood leukocyte PDHC activity assay described herein may be useful in the clinical setting in the diagnosis of PDHC deficiency.

5 | CONCLUSIONS

In conclusion, PDH deficiency often lacks specific clinical manifestations, Among diagnostic methods, the analysis of enzyme activity in the clinical laboratory is the most important. In the present study, sodium pyruvate 1-13C was used for the analysis of PDHC activity in peripheral leukocytes. The measurement of peripheral blood leukocyte PDHC activity may provide a practical enzymatic diagnostic approach for PDHC-related mitochondrial diseases.

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CONFLICT OF INTEREST

There are no conflicts of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the ethics committee of Qinghai University Affiliated Hospital. Written informed consent was provided by the participant.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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