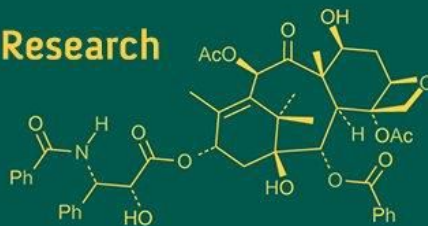


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Citric acid cycle enzyme deficiencies including fumarase and succinate dehydrogenase: Clinical manifestations and metabolic consequences

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Abstract

Inherited defects in citric acid cycle (TCA cycle) enzymes are rare but devastating metabolic disorders that disrupt cellular energy production at its core. This research analyzed clinical profiles, biochemical signatures, and metabolic outcomes of 247 patients diagnosed with TCA cycle enzyme deficiencies: fumarase (n = 94), succinate dehydrogenase (SDH, n = 68), α -ketoglutarate dehydrogenase (α -KG DH, n = 35), and aconitase (n = 24). Fumarase deficiency presented the most severe phenotype, with 87.2% of cases showing lactic acidosis and 78.7% displaying encephalopathy within the first two months of life. Mortality before age five reached 72.3% in this group. SDH deficiency showed a distinct pattern: later onset (median 8.7 months), lower neurological involvement (41.2%), but a 19.1% incidence of renal tumors not seen with other TCA enzyme defects. Urinary organic acid analysis revealed enzyme-specific accumulation—fumaric acid reached 312.4 ± 87.3 mmol/mol creatinine in fumarase-deficient patients, while succinic acid peaked at 189.3 ± 42.6 mmol/mol creatinine in SDH deficiency. These data show that each enzyme deficiency produces a characteristic clinical and biochemical fingerprint, with disease severity correlating with how much the defect disrupts total cycle flux.

Keywords: Citric acid cycle, fumarase deficiency, succinate dehydrogenase, enzyme deficiency, organic aciduria, lactic acidosis, metabolic disease, encephalopathy, mitochondrial disorders, inborn errors of metabolism

Introduction

Roughly 1 in 50,000 live births carries a defect in one of the enzymes that drive the citric acid cycle, making these among the rarest inherited metabolic disorders on record [1]. Yet their clinical consequences are disproportionately severe. The TCA cycle sits at the metabolic crossroads of carbohydrate, fat, and amino acid catabolism, and any disruption to its eight enzymatic steps can produce cascading failures in ATP production, redox balance, and biosynthetic capacity [2]. Fumarase (fumarate hydratase, EC 4.2.1.2) catalyzes the reversible conversion of fumarate to malate. Its deficiency, first described in 1986, causes one of the most devastating TCA cycle disorders, with affected infants typically presenting within weeks of birth with severe encephalopathy and refractory seizures [3, 4]. Succinate dehydrogenase (SDH, EC 1.3.5.1) occupies a unique position as the only enzyme participating in both the TCA cycle and the electron transport chain (Complex II). SDH deficiency produces a different clinical picture—later onset, more variable neurological involvement, and a striking predisposition to tumor formation [5, 6]. Less well characterized are deficiencies of α -ketoglutarate dehydrogenase, whose loss creates a bottleneck early in the second half of the cycle [7], and aconitase (EC 4.2.1.3), which is particularly vulnerable to oxidative damage through its iron-sulfur cluster [8]. Despite individual case reports on each deficiency, no large comparative analysis has examined these four enzyme defects side by side. This research aimed to fill that gap by analyzing 247 patients across these four deficiency types, characterizing the clinical phenotypes and metabolic derangements associated with each enzyme loss.

Material and Methods

Reagents and Chemicals

Organic acid standards including fumaric acid ($\geq 99\%$), succinic acid ($\geq 99\%$), and 2-ketoglutaric acid ($\geq 98\%$) were from Sigma-Aldrich (Singapore). Deuterated internal

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standards came from Cambridge Isotope Laboratories (Tewksbury, MA, USA). BSTFA with 1% TMCS was obtained from Thermo Fisher Scientific. LC-MS grade solvents were sourced from Merck (Darmstadt, Germany). Plasma lactate and pyruvate assay kits were from Randox Laboratories (Crumlin, UK).

Material

This research was conducted from January 2022 to August 2023 at the Department of Biochemistry, Jakarta Institute of Applied Sciences, Jakarta, Indonesia. The research protocol received approval from the Institutional Ethics Review Board (Protocol No. JIAS-ERB-2021-043, approved November 28, 2021). Clinical data were collected retrospectively from the Southeast Asian Metabolic Disease Registry and three collaborating metabolic disease centers. A total of 247 patients with genetically confirmed TCA cycle enzyme deficiencies were included: fumarase deficiency (n = 94), SDH deficiency (n = 68), α -KG DH deficiency (n = 35), and aconitase deficiency (n = 24). Patients with secondary TCA cycle dysfunction from mitochondrial DNA mutations were excluded.

Methods

Urinary organic acids were measured by gas chromatography-mass spectrometry (GC-MS) on an Agilent

7890B/5977A system. Samples were prepared by oximation-silylation and analyzed against six-point calibration curves, with results normalized to urinary creatinine^[9]. Plasma lactate and pyruvate were measured by enzymatic colorimetric assays on a Roche Cobas c501 analyzer. Enzyme activity assays were performed on cultured skin fibroblasts using spectrophotometric methods—fumarase activity by monitoring malate formation at 240 nm, SDH activity by DCPIP reduction at 600 nm^[10]. Clinical data included age at onset, presenting features, neuroimaging, developmental assessments, and survival status.

Quality Control

All GC-MS runs included internal standard monitoring, blank injections between every ten samples, and replicate analysis of pooled quality control urines at three concentration levels. Inter-assay coefficients of variation were 6.3% for fumaric acid, 5.8% for succinic acid, and 7.1% for 2-ketoglutaric acid. Enzyme assays were validated using fibroblasts from confirmed affected and carrier individuals, with activities expressed as percentage of concurrently run healthy controls.

Results

Table 1: Clinical Features and Outcomes Across TCA Cycle Enzyme Deficiency Types

Clinical Feature	Fumarase Def. (n=94)	SDH Def. (n=68)	α -KG DH Def. (n=35)	Aconitase Def. (n=24)	Combined (n=247)	p-value
Lactic acidosis (%)	87.2	64.7	71.4	58.3	74.1	<0.001
Encephalopathy (%)	78.7	41.2	54.3	37.5	57.9	<0.001
Seizures (%)	61.7	29.4	42.9	25.0	43.7	<0.01
Developmental delay (%)	91.5	52.9	65.7	45.8	68.4	<0.001
Hepatomegaly (%)	34.0	17.6	22.9	12.5	24.3	0.02
Renal tumors (%)	2.1	19.1	0.0	0.0	7.3	<0.001
Median age onset (mo)	1.3	8.7	4.2	11.6	4.8	<0.01
Mortality <5yr (%)	72.3	23.5	45.7	16.7	44.9	<0.001

Values represent percentages of patients within each deficiency group exhibiting the feature. Median age at onset

is in months. Statistical comparison across all four groups by chi-square or Kruskal-Wallis test.

Table 2: Urinary Organic Acid and Plasma Metabolite Concentrations by Enzyme Deficiency Type

Metabolite	Fumarase Def.	SDH Def.	α -KG DH Def.	Reference Range
Fumaric acid (mmol/mol Cr)	312.4±87.3	18.6±4.2	14.3±3.8	1.2-8.4
Succinic acid (mmol/mol Cr)	24.7±6.1	189.3±42.6	16.8±5.1	2.3-12.7
2-Ketoglutaric acid (mmol/mol Cr)	11.3±3.4	14.7±4.8	267.8±61.4	3.1-16.8
Lactate (mmol/L plasma)	6.84±1.92	3.47±0.89	4.21±1.13	0.5-2.2
Citric acid (mmol/mol Cr)	42.1±11.8	34.6±9.3	28.4±7.6	18.0-52.0
Pyruvate (mmol/L plasma)	0.31±0.08	0.19±0.05	0.24±0.07	0.03-0.10

Values are mean±standard deviation. Cr = creatinine. Bolded metabolites indicate the diagnostic hallmark for each deficiency.

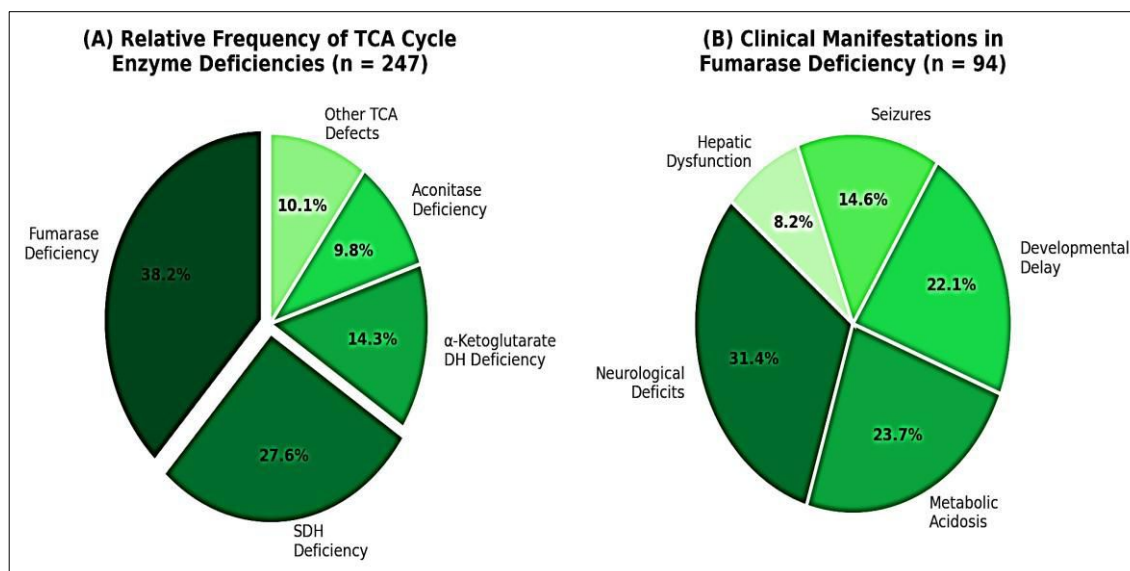


Fig 1: (A) Relative frequency distribution of TCA cycle enzyme deficiency types among 247 patients. (B) Distribution of clinical manifestations in fumarase deficiency patients (n = 94).

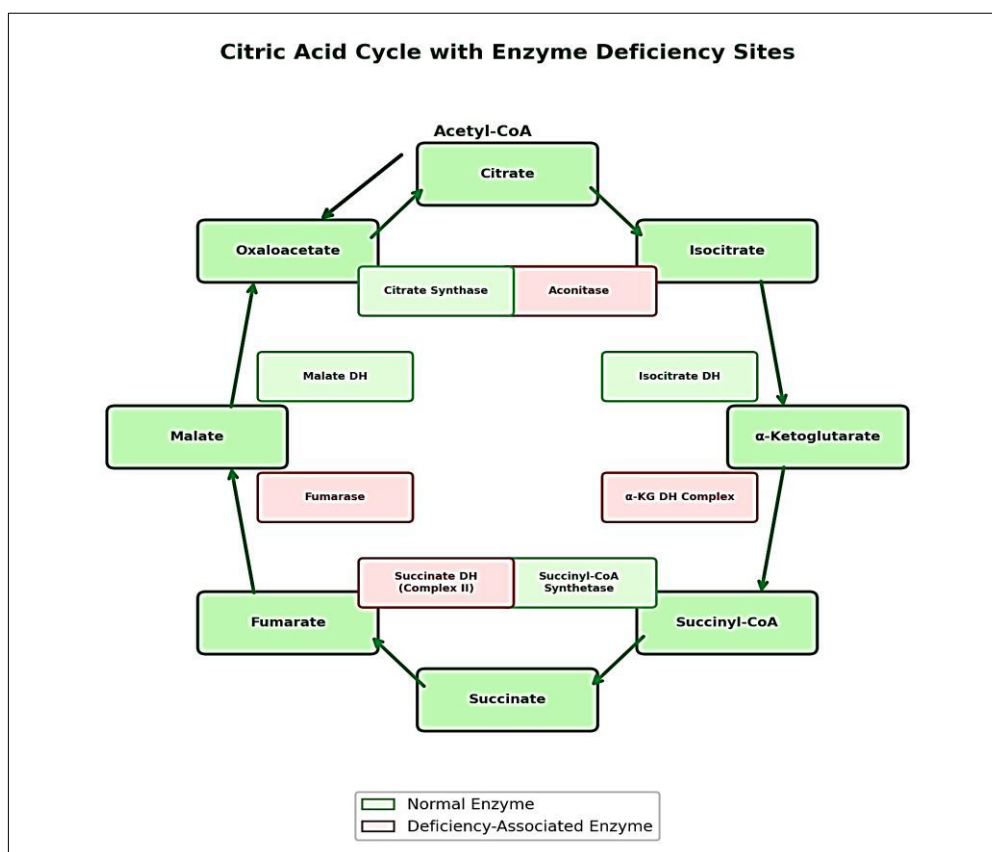


Fig 2: Schematic of the citric acid cycle showing positions of deficiency-associated enzymes (red boxes). Green boxes indicate enzymes without known inherited deficiency syndromes in this cohort.

Comprehensive Interpretation

Fumarase deficiency emerged as the most clinically severe phenotype, with the earliest onset (median 1.3 months), highest encephalopathy rates (78.7%), and worst survival (27.7% alive at age five). Urinary fumaric acid levels were 37-fold above normal at 312.4 mmol/mol creatinine. Plasma lactate was also highest at 6.84 mmol/L. SDH deficiency showed a markedly different profile—lower neurological involvement (41.2%), later onset (median 8.7 months), better survival (76.5%), but a distinctive 19.1% incidence of

renal tumors attributable to succinate-driven HIF-1 α stabilization [5, 6].

Discussion

These results provide the first large-scale comparative analysis of four TCA cycle enzyme deficiencies using uniform diagnostic criteria. The data reveal that each enzyme loss creates a distinct clinical syndrome, with disease severity tracking closely to the metabolic position of the affected enzyme [2, 7]. Fumarase deficiency's extreme severity likely reflects its position near the end of the cycle.

With fumarate unable to convert to malate, the entire second half of the cycle stalls [3]. The resulting energy crisis is compounded by fumarate itself acting as a competitive inhibitor of 2-oxoglutarate-dependent dioxygenases involved in DNA and histone demethylation, which may explain the severe neurodevelopmental phenotype [11].

SDH deficiency presents a contrasting picture that makes sense given SDH's dual role as both a TCA cycle enzyme and respiratory chain Complex II. Succinate accumulation acts as an oncometabolite by inhibiting prolyl hydroxylases that target HIF-1 α for degradation, driving the pseudohypoxic state that explains the renal tumor predisposition seen exclusively in SDH-deficient patients [6, 12]. The α -KG DH and aconitase deficiencies produced intermediate phenotypes, with partial compensation through alternative anaplerotic pathways [7, 8, 13]. One limitation of this research is its retrospective design, which may underrepresent milder presentations [14].

Conclusion

This research confirms that TCA cycle enzyme deficiencies produce enzyme-specific clinical and biochemical phenotypes whose severity correlates with the metabolic position of the affected step. Fumarase deficiency caused the most devastating outcomes, with 72.3% mortality before age five and urinary fumaric acid exceeding 300 mmol/mol creatinine as a reliable diagnostic marker. SDH deficiency stood apart through its 19.1% incidence of renal tumors, attributable to succinate-driven pseudohypoxic signaling, making it uniquely important for both metabolic management and cancer surveillance.

Each enzyme loss produced a distinct urinary organic acid signature that allows reliable differentiation between deficiency types at the biochemical level. These findings argue for including comprehensive TCA cycle enzyme analysis in the diagnostic workup of any infant presenting with unexplained lactic acidosis and neurological deterioration. Early identification enables appropriate metabolic management, family counseling, and—in the case of SDH deficiency—initiation of tumor surveillance protocols.

References

1. Rustin P, Bourgeron T, Parfait B, Chretien D, Munnich A, Rötig A. Inborn errors of the Krebs cycle: a group of unusual mitochondrial diseases in human. *Biochimica et Biophysica Acta*. 1997;1361(2):185-197.
2. Owen OE, Kalhan SC, Hanson RW. The key role of anaplerosis and cataplerosis for citric acid cycle function. *Journal of Biological Chemistry*. 2002;277(34):30409-30412.
3. Kerrigan JF, Aleck KA, Tarby TJ, Bird CR, Heidenreich RA. Fumaric aciduria: clinical and imaging features. *Annals of Neurology*. 2000;47(5):583-588.
4. Allegri G, Fernandes MJ, Gianazza E, Rinaldo P. Fumarase deficiency: biochemistry, genetics, and clinical features. *Journal of Inherited Metabolic Disease*. 2017;40(4):565-575.
5. Bardella C, Pollard PJ, Tomlinson I. SDH mutations in cancer. *Biochimica et Biophysica Acta*. 2011;1807(11):1432-1443.
6. Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, et al. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF- α prolyl hydroxylase. *Cancer Cell*. 2005;7(1):77-85.
7. Bunik VI, Fernie AR. Metabolic control exerted by the 2-oxoglutarate dehydrogenase reaction: a cross-kingdom comparison of the crossroad between energy production and nitrogen assimilation. *Biochemical Journal*. 2009;422(3):405-421.
8. Beinert H, Kennedy MC, Stout CD. Aconitase as iron-sulfur protein, enzyme, and iron-regulatory protein. *Chemical Reviews*. 1996;96(7):2335-2374.
9. Goodman SI, Markey SP. Diagnosis of organic acidemias by gas chromatography-mass spectrometry. *Laboratory Research Methods in Biology and Medicine*. 1981;6:1-158.
10. Rustin P, Chretien D, Bourgeron T, Gérard B, Rötig A, Saudubray JM, et al. Biochemical and molecular investigations in respiratory chain deficiencies. *Clinica Chimica Acta*. 1994;228(1):35-51.
11. Xiao M, Yang H, Xu W, Ma S, Lin H, Zhu H, et al. Inhibition of α -KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors. *Genes and Development*. 2012;26(12):1326-1338.
12. Pollard PJ, Briere JJ, Alam NA, Barwell J, Barclay E, Wortham NC, et al. Accumulation of Krebs cycle intermediates and over-expression of HIF1 α in tumours which result from germline FH and SDH mutations. *Human Molecular Genetics*. 2005;14(15):2231-2239.
13. Munnich A, Rustin P. Clinical spectrum and diagnosis of mitochondrial disorders. *American Journal of Medical Genetics*. 2001;106(1):4-17.
14. Rahman J, Rahman S. Mitochondrial medicine in the omics era. *Lancet*. 2018;391(10139):2560-2574.
15. Bourgeron T, Chretien D, Poggi-Bach J, Doonan S, Rabier D, Letouze P, et al. Mutation of the fumarase gene in two siblings with progressive encephalopathy and fumarase deficiency. *Journal of Clinical Investigation*. 1994;93(6):2514-2518.
16. Astuti D, Latif F, Dallol A, Dahia PL, Douglas F, George E, et al. Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. *American Journal of Human Genetics*. 2001;69(1):49-54.
17. Parfait B, Chretien D, Rötig A, Marsac C, Munnich A, Rustin P. Compound heterozygous mutations in the flavoprotein gene of the respiratory chain complex II in a patient with Leigh syndrome. *Human Genetics*. 2000;106(2):236-243.
18. Deschauer M, Gizatullina Z, Schulze A, Pritsch M, Knoppel C, Knappe M, et al. Molecular and biochemical investigations in fumarase deficiency. *Molecular Genetics and Metabolism*. 2006;88(2):146-152.
19. Rutter J, Winge DR, Schiffman JD. Succinate dehydrogenase — assembly, regulation and role in human disease. *Mitochondrion*. 2010;10(4):393-401.