



Fructose-1, 6-diphosphatase Deficiency Presenting as Glycogen Storage Disease

Authors

Dr Vrushali S. Joshi¹, Dr Suresh D. Gangane², Dr Murlidhar Mahajan³

¹Ph.D, Navigene Genetic Science Pvt. Ltd, 25-27, Gaurav Garden Complex, Opp. Sai Petrol Pump, Mira-Bhyandar Road, Miraroad, Thane 401 107

Email: vrushali.joshi@navigene.in; svrushalij@gmail.com

²MBBS, MD (Anatomy), Prof.& Head Dept. of Anatomy, Terna Medical College, Sector No. 12, Phase II, Nerul, Navi Mumbai 400 706

Email: gangane@gmail.com

³MBBS, MD (Pediatrics), Apollo Clinic, Mumbai

Email: mahajanmdm@yahoo.co.in

Corresponding Auhors

Dr Vrushali S. Joshi

C-8, Brindavan CHS, Koldongari Lane No. 2, Sahar Road, Andheri (E), Mumbai -400 069

Email: Vrushali.joshi@navigene.in; svrushalij@gmail.com

Abstract

Six year male child presented with hypoglycaemia and metabolic acidosis having history of recurrent episodes of hypoglycemic convulsions and more than ten hospital admissions for similar complaints. First episode occurred on day second of life with refusal to feed, respiratory distress, hypoglycemia and metabolic acidosis. Based on the examination and investigations results at that time, Glycogen Storage Disorder (GSD) was suspected. Treatment and management for the same was started. Various investigations done during remission period were normal except elevated blood lactate. At 6 years index case had episode of hypoketotic hypoglycaemia with metabolic acidosis. Considering this presentation GSD was ruled out. Fatty acid oxidation disorder was suspected and metabolic work up done which was normal. During current episode advanced metabolic investigations—urinary gas chromatography—mass spectrometry analysis revealed increased excretion of lactate, glycerol-3-phosphate and glycerol suggesting Fructose 1, 6-diphosphatase deficiency (FDPD). Management for FDPD was started immediately and was monitored closely. In last 3 years the child has not a single episode and is attending normal school. Therefore, it is important to subject the child to advance and comprehensive metabolic investigation such as urinary GC-MS profiling as soon as possible in such cases. The timing for collection of sample is key in many such disorders as the metabolic abnormality might not show up during remission and hence the blood & urine sample collection during the episode is of utmost importance to arrive at a diagnosis. An accurate diagnosis through timely collection of sample, advanced metabolomics techniques and targeted management regime can go a long way in determining the outcome and prognosis in such cases.

Key words: Fructose-1, 6-diphosphatase Deficiency, hypoglycaemia, hepatosplenomegaly, metabolic acidosis

Fructose-1, 6-diphosphatase deficiency (FDPD) is an autosomal recessive disorder of carbohydrate metabolism. Majority of glucose and fructose entering the body is converted to fructose 1,6-diphosphate and fructose 6-phosphate. This in turn allows endogenous glucose formation from gluconeogenic amino acids alanine, glycine and glycerol or lactate. Deficiency of FDPD results in disturbed gluconeogenesis. It causes life-threatening episodes, hypoglycemia and metabolic acidosis in new-borns, infants or young children^[1]. Here, we present a case initially diagnosed as glycogen storage disease, than suspected fatty acid oxidation disorder, but turned out to be F DPD deficiency.

Six year six months male child, a product of a non-consanguineous marriage, born by full term vacuum delivery with birth weight 3.5 kilograms was referred for urinary organic acid profiling. He cried immediately after birth. Family history was not significant. On day two of life he had respiratory distress and refusal to feed. Investigations showed severe hypoglycaemia (18 mg/dL), high lactate levels (144.5 mg/dL; NR: 5.7-22.0 mg/dL) and high pyruvate levels (0.8 mg/dL; NR: 0.3-0.7 mg/dL). Metabolic acidosis was noted with raised lactate to pyruvate ratio (108.6) and elevated blood ammonia (106; NR: 30-86). Urinary galactose test was positive. Next three consecutive days test for urinary reducing substances was positive without sugar and ketones with acidic pH (6.0). Ultra sonography of the skull was normal and that of abdomen showed splenomegaly (4.5 cms). Glycogen storage disorder (GSD) was suspected and patient was put on lactodex.

After two months, urinary screening tests- Dinitrophenyl hydrazone (DNPH; ketones), Ferric chloride (ketoacids) and Ninhydrine (amino acids) were positive. Benedict's test for reducing substances and urinary thin layer chromatography for sugars were negative. Serum lactate and ammonia levels were elevated. Serum phenylalanine (2.45 mg/dl, NR < 4 mg %) and GALT enzyme (3.49, NR > 2.4 U/g Hb) were

normal. Same treatment was continued. At the age of five months workup for GSD was done. Serum Glutamate Oxalo Transferase (35 NR- 0-40 IU/L) and Serum Glutamate Pyruvate Transferase (43; NR: 0- 45 IU/L) were normal. After 6 hours of fasting serum cholesterol 153 (150-250 mg/dl) and serum triglyceride 64 (40-165 mg/dl) were normal. Based on these results, fructose loading test and liver enzyme assay was recommended. Same treatment was continued. At six months of age patient was hospitalized for fructose challenge test, but due to unavailability of pure fructose test could not be done. During remission period urine organic acid analysis done was normal. At 11 months he was hospitalized for respiratory tract infection and had severe metabolic acidosis with lactic acidosis. Serum triglyceride level was elevated and was 200 mg/dl. He was advised to avoid starvation and to have carbohydrates, fruits and fruit juices.

Glucagon challenge test (0.1 mg /kg) done at 22 months of age was normal with urine ketones negative in presence of hypoglycaemia. Considering investigation results and clinical presentation, fatty acid oxidation defect was suspected and GSD Type I was ruled out. He was put on oral carnitine (100 mg/kg/day) and discharged. Patient was advised for urinary organic acid and blood carnitine profile for fatty acid disorders. Both these investigations done were normal. Patient was again and again hospitalized at 2 years 6 months, 3 years, 5 years and 6 years with similar presentation. At 6 years 6 months he was hospitalized and referred to metabolic expert. Overall picture was hypoketotic hypoglycaemia with persistent metabolic acidosis with hepatosplenomegaly. Presentation was consistent with metabolic disorder. Differential diagnosis considered was Multiple acyl CoA-Dehydrogenase deficiency/mitochondrial disorders. Comprehensive urinary metabolic profiling by GC-MS covers the disorders of fatty acid, organic acids, amino acids, mitochondrial disorders, carbohydrate related conditions and other neurometabolic disorders. Considering this

advantage of urine analysis, urine sample was sent for comprehensive metabolic profiling using GC-MS technology. This time sample was collected before starting treatment. Urinary analysis using modified MILS (qualitative: values of metabolites are in terms of peak area ratio) method^[2] showed elevation of lactate(control value -2.709), 3-hydroxybutyrate (3HB, control value-0.041), glycerol(control value-0.93), glycerol-3-phosphate (g3p; control value-0.165) and 4-hydroxyphenyllatate (4HPL; control value-1.967). This metabolic profile was suggestive of Fructose-1, 6-diphosphatase deficiency (FDPD). Meanwhile treatment was started and genetic counselling was offered to the family. Complete avoidance of fructose and prolonged fasting was recommended. Confirmatory diagnosis by lymphocyte enzyme assay or molecular diagnosis was not feasible due to financial issues. In the recent follow up it came to know that patient is doing well and attending normal school with good academic performance. First case of FDPD was reported by Baker and Winegrad^[3]. Incidence is around 1: 20,000 live births with both genders equally affected. In carbohydrate related inborn errors of metabolism (IEM) glucose, galactose and fructose, metabolism is disturbed. Any enzyme defect in the pathway of glycolysis, gluconeogenesis, or oxidative phosphorylation will result in a clinical presentation. Clinical presentation of GSD and FDPD is similar, mainly recurrent hypoglycaemia. Endogenous glucose production in case of these two disorders get severely hampered^[4]. During fasting, endogenous glucose production is from hepatic gluconeogenesis and glycogenolysis^[5]. Because of insufficient G6Pase activity, G6Phosphate cannot be converted into free glucose, but G6Phosphate is metabolized to lactic acid or incorporated into glycogen. The chief biochemical alteration in FDPD is hypoglycemia, while secondary abnormalities are hyperlactatemia, metabolic acidosis, hyperlipidemia, and hyperuricemia. The elevated blood lactate level causes metabolic acidosis. A prolonged fast can induce lactic acidosis with

hypoglycemia in patients with FDPD deficiency because of impaired gluconeogenesis.

The presentation of the disorder is typical metabolic acidosis, lactic acidosis and hypoglycaemia. Present case had a typical presentation as reported in the literature^{[6], [7]}. In between episodes patients are generally normal, but mild acidosis may be present and the same was observed in this case. Index case was initially diagnosed as 'Glycogen Storage Disease' as reported^[8], where adult patient was hospitalized 30 times, before the correct diagnosis. This can be due to the similar clinical presentation and the disturbed pathways. Diagnosis of the FDPD is based on the invasive procedures and enzyme assay from lymphocytes and molecular analysis, which is not easily available in developing countries. Ning and co-workers^{[9], [10]} reported urinary GC-MS method for the detection of this disorder. Elevated urinary glycerol-3-phosphate and glycerol during the episode are the important and specific markers of this disorder. In present case, though the urinary GC-MS analysis was carried out twice (previously) and the analysis was done by our centre using modified Matsumoto's method, both the time the metabolic profile was normal. The bio-chemical diagnosis of this disorder is difficult when the sample is collected during remission. When urine sample was collected at the time of episode and before starting the treatment, diagnosis could be done while in two previous episodes urine sample was collected at the time of remission. That is why correct diagnosis was not possible.

Due to urease pre-treatment and modified MILS method, it is possible to simultaneously diagnose the disorders of organic acids, amino acids and even carbohydrate related. At the same time it is necessary to collect the appropriate sample at an appropriate time for the accurate diagnosis. This will help for treatment, management and genetic counselling to family.

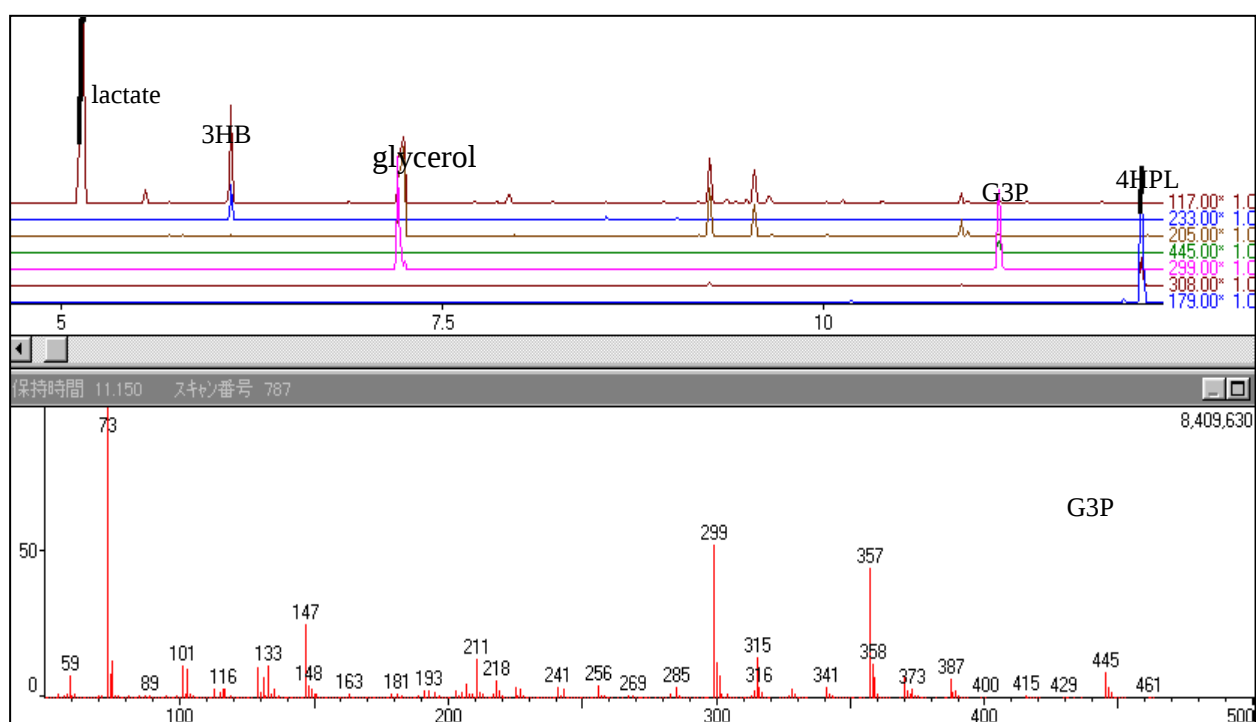


Figure1: Mass spectrum and MIC showing marker metabolites lactate, 3-hydroxybutyrate (3HB), glycerol and G3P (Glycerol -3- phosphate) and 4HPL (4-hydroxyphenyllactate)

Acknowledgement

We would like express our sincere thanks to all referring clinicians, patients and their parents for their kind help and support for such studies. We are also grateful to our colleagues for their encouraging support.

References

1. Emery JL, Howat AJ, Variend S, Vawter GW (1996): Investigation of inborn errors of metabolism in unexpected infant deaths. *Lancet* 8601:29-31
2. Matsumoto I and Kuhara T (1996): A new chemical diagnostic method for inborn errors of metabolism by mass spectrometry-rapid, practical and simultaneous urinary metabolites analysis. *Mass Spectrometry Reviews*. 15:43-57
3. Baker L, and Winegrad AI (1970): Fasting hypoglycemia and metabolic acidosis associated with deficiency of hepatic fructose-1, 6-bisphosphatase activity. *Lancet* II. 13-16
4. Huidekoper HH, Visser G, Ackermans MT, Sauerwein HP, Wijburg FA (2010): A potential role for muscle in glucose homeostasis: in vivo kinetic studies in glycogen storage disease type 1 and fructose-1, 6-bisphosphatase deficiency. *J. Inherit Metab Dis*. 33:25-31
5. Browning JD, Baxter J RN, Satapathi S and Burgess SC. (2012): The effect of short-term fasting on liver and skeletal muscle lipid, glucose, and energy metabolism in healthy women and men. *J Lipid Res*. Mar; 53(3),557-586
6. Takagi D, Ben-Ari J, Nemet D, Zeharia A, Eliakim A. 2012): Recurrent infantile hypoglycaemia due to combined fructose-1, 6-disphosphatase deficiency and growth hormone deficiency. *Pediatr Endocrinol Metab*. 7: 1-3
7. Moon S, Kim JH, Han JH, KoSh, Ahn YB, Kim JH, et al. *Metabolism*. (2011) Novel compound heterozygous mutations in the fructose-1, 6-disphosphatase gene cause hypoglycaemia and lactic acidosis 60(1):107-13

8. Krishnamurthy V, Eschrich K, Boney A, Sullivan J, McDonald M, Kishnani PS. et al.(2007):Three successful pregnancies through dietary management of fructose-1, 6-bisphosphatase deficiencyJ. Inherit MetabDis.Oct; 30 (5): 819
9. Iga M, Kimura M, Ohura T, Kikawa Y, Yamaguchi S. (2000): Rapid simplified and sensitive method for screening of fructose-1, 6-disphosphatase deficiency by analyzing urinary metabolites in urease/direct preparations and gas chromatography- mass spectrometry in the selected-ion monitoring mode. J. Chromatography B Biomed Sci App. 746 (1): 75-82
10. Ning C, Kuhara T and Matsumoto I. (1997): Chemical diagnosis and metabolic studies for Inborn errors of Carbohydrates Metabolism by GC/MS simultaneous urinary analysisJ. Kanazawa Med. Univ. 22:163-171