

A Case of Galactosaemia with an Atypical Presentation

Case History and Biochemical Investigations

A baby girl was born at full term by Caesarean section due to foetal distress, with a good Apgar score (9/10) and a normal birth weight of 3.9kg (75-91st percentile). She was breast-fed and was on lactose-containing feeds from birth.

3 Months – At the Local DGH

In a routine check-up it was noted that she had poor weight gain (body weight at this point was 4.3 Kg) and her growth had fallen to the 0.4th centile of the growth chart. She was immediately seen by the local paediatrician. On examination she had mild motor delay and hepatomegaly but was otherwise normal. Mother reported no difficulty in feeding. The preliminary laboratory investigations were as follows:

Blood gases	Normal
Renal function	Normal
LFTs	Normal, no jaundice
Full blood count	Macrocytic anaemia detected
Urine organic acids	No abnormality detected (NAD)
Plasma amino acids	NAD

7 Months – At a Specialist Centre

The growth arrest was further investigated. At this point her growth remained on the 0.4th centile (body weight stayed at 4.3 Kg). She continued to receive lactose-containing feeds. On examination the baby was mildly jaundiced with hepatomegaly, hypotonic with mild motor delay, and developed a grade 3/6 systolic murmur. The paediatrician also indicated the presence of chubby cheeks and mild dysmorphic facial features, which are often associated with glycogen storage diseases, mucopolysaccharidoses or congenital defects of glycosylation. The results of haematological, general biochemical and metabolic investigation are as follows:

Haematology

Macrocytic anaemia detected	
Red cell B ₁₂ & folate	Normal
Sickle cell disease screen	Negative
Prothrombin time	Normal

Biochemistry

Blood:

Total bilirubin	20	μmol/L	(<18)
ALP	474	U/L	(60-330)
ALT	45	U/L	(12-47)
AST	89	U/L	(20-65)
Alpha-fetoprotein (AFP)	684	kU/L	(0-10)
CK		Normal	
LDH		Normal	
Cortisol (9 am)		Normal	
TFT		Normal	
Acylcarnitine profile		NAD	
Plasma amino acids		NAD	

Urine:

- Excessive urinary excretion of retinal-binding protein (RBP) and N-acetyl D-glucosaminidase (NAG) was indicative of proximal distal tubule dysfunction or injury.
- There was albuminuria, a generalised aminoaciduria and a moderately increased urinary excretion of calcium.
- Reducing substances - positive
- Sugar chromatography - galactose >50 mmol/L (0 – 1)

Urine Organic Acids	NAD
Urine Glycosaminoglycans (GAG)/creatinine	NAD

Comments:

Macrocytic anaemia was again detected. Since there was no evidence of vitamin B₁₂/folate deficiency it was likely to be a result of poor nutrition. There were some abnormalities in LFTs but the clotting studies were normal. The increase in AFP indicated a liver abnormality and in neonates AFP usually remains high until the liver problem is resolved. The abnormal RBP and NAG results were consistent with proximal renal tubule dysfunction or injury and may explain the generalised aminoaciduria. The sugar giving a positive result on testing for urine reducing substances was confirmed, by paper chromatography, to be mainly galactose at a high concentration of over 50 mmol/L.

Differential diagnoses

Three possible diagnoses were considered:

1. **Fanconi-Bickel Syndrome** is an autosomal recessive disorder caused by a mutation on the gene encoding Glut2, a glucose transporter protein. The defective Glut2 transporter leads to hepatorenal glycogen accumulation, impairment of glucose and galactose uptake and utilisation hence their leakage into urine. Patients typically present at 3-10 months with vomiting, fever, growth failure, rickets, and later with hepatomegaly, proximal renal tubule dysfunction, hyperglycaemia, glycosuria, galactosuria, a moon face and short stature. Treatment involves a galactose-restricted and diabetic-like diet with vitamin supplementations and should be instigated in all suspected cases even before confirmation of diagnosis.

This diagnosis was later excluded on the basis of normal 24-hour plasma glucose and lactate profiles which suggest normal glucose utilisation and metabolism.

2. **Mucopolysaccharidoses (MPS)** are characterised by high concentrations of dermatan, heparan or keratan sulphate in urine and may present with dysmorphic features. The initial screening test for MPS in our laboratory is urinary glycosaminoglycan (GAG) quantitation. Urinary GAG concentration was normal in this baby. GAG electrophoresis was not performed because the screen was negative and the clinical features were not typical. The diagnosis was also effectively excluded on the basis that galactosuria is not a presenting feature of MPS.
3. **Congenital defects of glycosylation** are another group of disorders that may present with dysmorphic features. Transferrin electrophoresis was carried out which revealed an 'atypical distribution of transferrin glycoforms' which is only seen in three clinical conditions: carbohydrate-deficient glycoprotein syndromes, untreated hereditary fructosaemia and untreated galactosaemia. This particular finding, together with the presence of galactosuria and the proximal renal tubular dysfunction, pointed towards a diagnosis of galactosaemia.

Confirmatory tests

- Red cell galactose-1-phosphate uridylyl transferase (Gal-1-PUT) activity = 1.0 μmol/hr/g Hb (normal 18-40; heterozygotes 9-15; homozygotes 0-6)

- Red cell galactose-1-phosphate = 4.49 $\mu\text{mol/g Hb}$ (non-galactosaemic <0.1)
- Red cell galactokinase = normal activity

A significant deficiency of Gal-1-PUT activity and a significant elevation in red cell galactose-1-phosphate were detected. **These results were consistent with a diagnosis of galactosaemia due to a deficiency in Gal-1-Put activity.**

Overview of the Disease and Confirmatory Tests

Classical galactosaemia, caused by a deficiency of Gal-1-PUT, has an incidence of 1 in 45,000 in the UK. Patients with this disorder typically present during the first week of life, with poor feeding, poor weight gain, vomiting, diarrhoea, lethargy, hypotonia, hepatomegaly and jaundice. Cataracts, encephalopathy, bleeding or excessive bruising may also be present. Improvement is usually seen on commencement of intravenous fluid infusion and a galactose-free formula. Hepatic dysfunction is typical and is characterised by abnormal clotting functions and LFTs, conjugated hyperbilirubinaemia, and raised plasma amino acids. Galactosuria and glycosuria are present in most cases, with albuminuria or aminoaciduria due to impairment in glomerular and proximal tubule functions. The abnormal galactose metabolism is characterised by elevations in plasma galactose, red cell galactose-1-phosphate and urine/blood galactitol. It is essential that samples for these investigations should be taken while the patient is on lactose-containing diet because absence of galactose intake will produce false negative results. Haemolytic anaemia is another finding. Patients are susceptible to septicaemia especially E coli infections.

Long-term clinical outcomes are variable and in many cases unrelated to dietary compliance. Neurodevelopmental, speech and cognitive dysfunctions are common in children. During puberty, female patients tend to develop hypergonadotrophic hypogonadism and ovarian failure.

In this case, although the baby has the classical form of galactosaemia, she had a delayed onset with much milder clinical symptoms. It was unfortunate that urine reducing substances were not included as part of the initial investigation at the local hospital, but her liver function tests did not show any abnormality at that time. By seven months galactosuria and renal tubule dysfunction were detected. Interestingly her AST level was not as substantially deranged as would have been expected in classical galactosaemia. Her anaemia was not of the haemolytic type which is typical in this disorder, and she did not develop severe metabolic crises such as hypoglycaemia or metabolic acidosis. The fact that the galactose-free diet was instigated as a first line treatment for a suspected diagnosis of Fanconi-Bickel syndrome probably prevented further deterioration of the course of the disease. Several lines of investigation for MPS, CDGS and GSD were initiated due to the presence of a 'moon face' and mild dysmorphic features in this child. However, while any dysmorphic features should not be overlooked, they are often difficult to assess and can be misleading in very young children. The crucial finding was the galactosuria which pointed specifically towards the diagnosis of galactosaemia.

Evidence of a reduced Gal-1-PUT activity, normal galactokinase, and an increased galactose-1-phosphate in the red cells are sufficient to support a diagnosis of classical galactosaemia. Presence of one of the common mutations for the disorder confirms the diagnosis but DNA analysis was considered unnecessary in this case. It is worth noting that certain GAL-1-PUT assays use whole blood in a linked enzyme format employing endogenous red cell glucose-6-phosphate dehydrogenase (G6PD). False positive results may be obtained in patients with G6PD deficiency or if EDTA blood samples are analysed. The latter is due to chelation of magnesium ions which are required as a cofactor in the G6PD reaction. Samples taken within 6 weeks of a blood transfusion may give rise to false negative results.

As discussed previously, the diagnosis of galactosuria requires that the patient is on galactose- or lactose-containing feeds. If results of the initial urine sugar analysis or enzymological tests are equivocal, the following should be considered:

- Attempts can be made to establish carrier status of the parents by red cell GAL-1-PUT activities but the test is unreliable for this purpose, a negative result does not exclude the diagnosis.
- DNA analysis by PCR for the common Q188R mutation. However the absence of the mutation does not exclude the diagnosis.
- Quantitation of urinary galactitol (a product of galactose by the action of a non-specific enzyme aldose reductase) has been proposed as another biochemical test for galactosaemia. It is excreted unchanged in the urine and its levels correlate well with red cell GAL-1-PUT activity. In rare occasions, this test is used to diagnose galactosaemia in equivocal cases.

Prenatal diagnosis is possible by detecting Gal-1-PUT activity in cultured amniotic fluid cells or chorionic villi (CV) biopsies, or by measuring galactitol concentration in the amniotic fluid supernatant. DNA analysis on a CV biopsy is possible provided that the genotype of the index case is known.

Treatment & Monitoring

The treatment of this disorder is to follow a galactose-restricted diet. Symptoms and signs usually regress in one to two weeks following commencement of the diet. The patients are monitored for dietary compliance by quantitation of red cell galactose-1-phosphate. The need for frequent monitoring is questioned because it reflects only the galactose intake in the previous 24 hours and because the levels do not correlate with long-term clinical outcomes. Female patients are followed up with appropriate endocrine testing during puberty to determine whether hormone replacement is required to maintain normal pubertal development or, in later life, as treatment for infertility. Ophthalmologic assessments are made regularly for the detection of cataracts. Management therefore involves a multidisciplinary approach, and it is essential that patients are referred to specialist centres.

Genetics

Ethnic origin is the dominating factor in strategies for mutation analysis. In the Caucasian population the commonest mutations are Q188R and K285N which account for 60% and 25% of the classical galactosaemic patients. Of interest to this case is that this baby is of African-American origin. It has been reported that in this population group a significant proportion of galactosaemics have much milder clinical presentations, and that almost 50% of the mutant GAL-1-PUT gene are attributed for by a S135L mutation. This S135L mutant allele, when transfused into various animal and yeast cell models, exhibited tissue specificity in GAL-1-PUT activity, which might explain the milder presentation of classical galactosaemia.

Summary

- Galactosaemia can present late with much milder clinical features than the classical form of the disease.
- Abnormalities in LFTs, particularly AST, were not as deranged as is usual in the classical form of the disorder
- Although diagnosis was not established until 7 months of age, this baby girl has, to date developed no major problems except for a mild motor delay
- Isoelectric-focusing of transferrin shows a distinctive pattern in untreated galactosaemia this can be a useful indicator for the disorder.
- Milder forms of classical galactosaemia are more common in African and African-American populations, attributed for by the S135L mutant allele
- This case highlights the importance of collecting the basic samples for the metabolic workup i.e. frozen plasma accompanied by a urine sample. In the case of galactosaemia a simple urine reducing substance test holds the key for the diagnosis

Patient Confidentiality

Permission has been obtained from the consultant responsible for the patient's care.

Useful references

Henderson H, Leisegang F, Brown R, & Eley B. The clinical and molecular spectrum of galactosaemia in patients from the Cape Town region of South Africa. *BMC Pediatr* 2002;2:1-6

Ridel KR, Leslie ND, Gilbert DL. An updated review of the long-term neurological effects of galactosemia. *Pediatr Neurol*. 2005 Sep;33(3):153-61. Review

Tyfield LA. Galactosaemia and allelic variation at the galactose-1-phosphate uridylyltransferase gene: a complex relationship between genotype and phenotype. *Eur J Pediatr* 2000;159(Suppl 3):S204-7

Walter JH, Collins JE, & Leonard. Recommendations for the management of galactosaemia. *Arch Dis Child* 1999;80:93-96

Yager C, Wehrli S & Segal S. Urinary galactitol and galactonate quantified by isotope-dilution gas chromatography-mass spectrometry. *Clin Chim Acta* 2006;336:216-224