Focal Congenital Hyperinsulinism as a Cause for Sudden Infant Death

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Case Report

Title: Focal Congenital Hyperinsulinism as a cause for Sudden Infant Death

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Focal Congenital Hyperinsulinism as a cause for Sudden Infant Death

Abstract

Congenital hyperinsulinism (CHI) is the commonest cause of persistent and severe hypoglycaemia in infancy due to unregulated insulin secretion from pancreatic beta cells. Prompt early diagnosis is important, as insulin reduces glucose supply to the brain, resulting in significant brain injury and risk of death. Histologically, CHI has focal and diffuse forms; in focal CHI, an inappropriate level of is secreted from localised beta-cell hyperplasia. We report a four-month old boy, who presented with sudden illness and collapse without a recognised cause and died. Post-mortem examination revealed pancreatic histopathology compatible with focal CHI. Immunofluoresence-staining showed limited expression of $p57^{kip2}$ beta-cells reinforcing the diagnosis. Mutation testing for

genes associated with CHI from DNA from the focal lesion was negative. This case highlights the recognition of focal CHI as a possible cause for sudden infant death. In children dying suddenly and unexpectedly, post-mortem pancreatic sections should be carefully examined for focal CHI.

Key words: congenital hyperinsulinism, hypoglycaemia, post-mortem, sudden infant death, pancreas, insulin

Running head: Focal CHI and Sudden Infant Death

Introduction

Congenital hyperinsulinism (CHI) is the commonest cause of persistent and severe hypoglycaemia in infancy. CHI typically presents in the neonatal period, although cases in infancy and even childhood have been reported [1]. These infants typically present with symptoms of hypoglycaemia, with detectable/inappropriate insulin and C-peptide levels for hypoglycaemia, along with suppressed beta-hydroxybutyrate and free fatty acids. Prompt diagnosis is important, as the presence of insulin inhibits ketone formation, resulting in significant brain injury if the hypoglycaemia is persistent or profound [2]. Acute management involves provision of high concentration glucose, either in feeds or parenterally, and glucagon as an emergency measure. Long-term medical management includes diazoxide and octreotide as first-line and second-line options respectively. Failing response to medical therapy, pancreatectomy may be required [3].

CHI refers to a heterogenous group of conditions, with genetic aetiology in around 40% of patients with mutations in 11 known genes, those affecting the ATP sensitive K^+-channel (*ABCC8/KCNJ11*) being most frequent [4, 5]. Histologically, CHI can be classified broadly into focal and diffuse forms. In the diffuse form, hyper-functioning pancreatic beta cells with nucleomegaly are distributed throughout the pancreas, whereas in the focal form there is nodular hyperplasia of islet cells surrounded by normal tissue [6].

We report a four-month old male infant who died in hospital after presentation with an acute illness and collapse. Pancreas histology at post mortem revealed an isolated focal lesion in keeping with focal CHI.

Case Report

A four-month old boy presented to hospital unresponsive.

The background to this episode was birth at 34+4 weeks of gestation following normal vaginal delivery, with a weight of 1.84kg (9^{th} centile) and satisfactory Apgar scores (8 at 1 minute and 9 at 5 minutes). He spent 10 days on the neonatal unit, requiring antibiotics for suspected sepsis. He had received phototherapy for unconjugated hyperbilirubinaemia. Hypoglycaemia was identified by point of care testing during this neonatal period on more than one episode and was promptly treated with frequent feeds, although a venous sample for confirmation was not obtained and the specific value of glucose was not recorded in the case notes. The cause for hypoglycaemia at the time was not investigated. Hypoglycaemia was corrected for inadequate glucose intake on the

background of prematurity, as per the unit policy. Sepsis was presumed to be a cause for hypoglycaemia and treated robustly, although blood cultures were negative indicating that infection was an unlikely aetiology. Following discharge from the neonatal unit, he had reportedly been well until just prior to representation.

Prior to representation, the parents had noted blood in his nappy. Balanitis was diagnosed, and treated with oral antibiotics in the community. The following day he fed poorly, and suffered from vomiting and diarrhoea. His condition deteriorated rapidly and he became unresponsive. He was rushed to hospital, where he was noted to be hypothermic, pale, with circulatory failure. There were no external signs of bleeding. Initial blood tests showed profound hypoglycaemia (serum glucose <1.0mmol/L), acidosis (pH 6.96) and pancytopaenia (haemoglobin 56g/L, white cell count 1.6 $x10^9$/L, platelet count 49 $x10^9$/L). Despite extensive resuscitative measures, including ventilatory support, fluid boluses, inotropes and antibiotics, he died within 4 hours of presentation.

A Coroner's post-mortem examination was conducted. Autopsy revealed a male infant with normal growth, with crown heel length (59cm) and weight (6.08kg) between 50th and 75th centiles for his corrected age for gestation (2 months and 3 weeks). No congenital abnormalities of the internal organs were identified at autopsy. A male karyotype was noted in keeping with male genitalia. Post mortem examination did not identify liver or kidney tumours; there were no clinical features of Beckwith Wiedemann syndrome. A widespread petechial rash was present. Bone marrow histology showed a severely left shifted marrow with immature precursor cellular profile, thus raising the possibility of sepsis/acute infective terminal illness. However, no positive bacterial culture or tissue inflammation was ever identified.

The most significant finding was found on pancreas histology. This revealed a localised (focal) expansion of islets interspersed within normal pancreatic tissue suggestive of focal CHI. Targeted next generation sequencing of DNA isolated from pancreatic tissue did not reveal any coding mutations or changes in the copy number of the ATP sensitive K^+ channel genes, *ABCC8* and *KCNJ11*. Coding mutations and partial/whole gene in *GLUD1*, *GCK*, *HADH*, *HNF4A*, *HNF1A*, *INSR* and *TRMT10A* were also excluded. Immunohistochemistry and immunofluorescence studies were performed on 5µm-thick sequentially sectioned tissue slides (heat-mediated antigen retrieved) as previously described [6,7]. Insulin (Abcam 1:1000 rabbit), glucagon (RTU Biogenex PA039-5PSG) and somatostatin (Dako 1:2000 A00566) staining were performed to examine islet cell expression. All slides were digitised as previously described [6,7]. Figure 1A illustrates the histological characteristics of the pancreas, with the focal domain associated with islet cell hyperplasia, in contrast to the non-focal region. Figure 1B demonstrates the immunological characteristics - how the focal lesion is associated with a marked expansion of insulin-expressing islet cells and the location of glucagon-expressing cells within the lesion. Glucagon expression is localized to the peripheral regions of the focal islets. Figure 2 shows the expression and localization of islet insulin, glucagon and somatostatin within the lesion and the non-lesion domains of the pancreas. Note the expansion of insulin-positive cells within the lesion and how the non-β-cells are located around the periphery of the islet and islet-like structures. To address the mechanism of islet β-cells expansion we assessed the expression of $p57^{kip2}$ by immunohistochemistry (1:50, mouse; Thermo Scientific, UK), also demonstrated in Figure 2. $P57^{kip2}$ is normally expressed in β-cell nuclei where it acts as a negative repressor of the cell cycle. Focal CHI is caused by the loss of heterozygosity on Ch.11p15 and loss of $p57^{kip2}$ expression. This was confirmed in tissue samples from the lesion domain.

On the balance of probabilities the cause of death was felt to be related to the focal lesion in the pancreas most probably resulting in hypoglycaemia.

Discussion

We have reported focal CHI as a probable cause of death in a child with infantile hypoglycaemia, presenting with sudden illness and collapse. Focal CHI is well recognised as a cause for early and late presenting hypoglycaemia [8]. While neonatal hypoglycaemia is detected early when the child is still in hospital, infantile hypoglycaemia due to late presenting CHI can be missed and the diagnosis therefore delayed. We have highlighted in our case report an extreme scenario where focal CHI was not diagnosed until after post-mortem examination.

The incidence of focal CHI as a cause of sudden unexpected infant death has not been reported. It is important to raise awareness that focal CHI may cause hypoglycaemia severe enough to cause death from delayed recognition. For histopathologists, it is important to consider the possibility of focal CHI when examining pancreatic sections in post-mortem examinations.

It was not possible to establish the diagnosis of CHI in the neonatal period as samples for serum insulin had not been drawn at the time of hypoglycaemia. Further, samples for glucose and insulin were not analysed in the post-mortem period as measurements several hours after death would be too unreliable. However, the diagnosis of focal CHI in our case was comprehensively confirmed by standard examination of pancreatic sections, followed by immunofluorescence staining for insulin and $p57^{kip2}$. Although a genetic aetiology was not ascertained, the diagnosis of focal CHI was unequivocal.

In most cases of focal CHI, diagnosis is made following the identification of paternal heterozygous mutations in *ABCC8/KCNJ11* in peripheral blood DNA and confirmation by 18-fluoro-dopa PET-CT scanning [9]. In the presented case, a diagnosis of CHI was not suspected at any stage of life and the above investigations of gene mutation testing and imaging were not undertaken. Focal CHI was identified only at post-mortem on histology.

Conclusions

This case demonstrates the consequences and severity of focal CHI; if not recognised, investigated and treated, focal CHI can lead to death. This case also highlights the need for careful assessment of the pancreas in post-mortem examination in children dying suddenly and unexpectedly.

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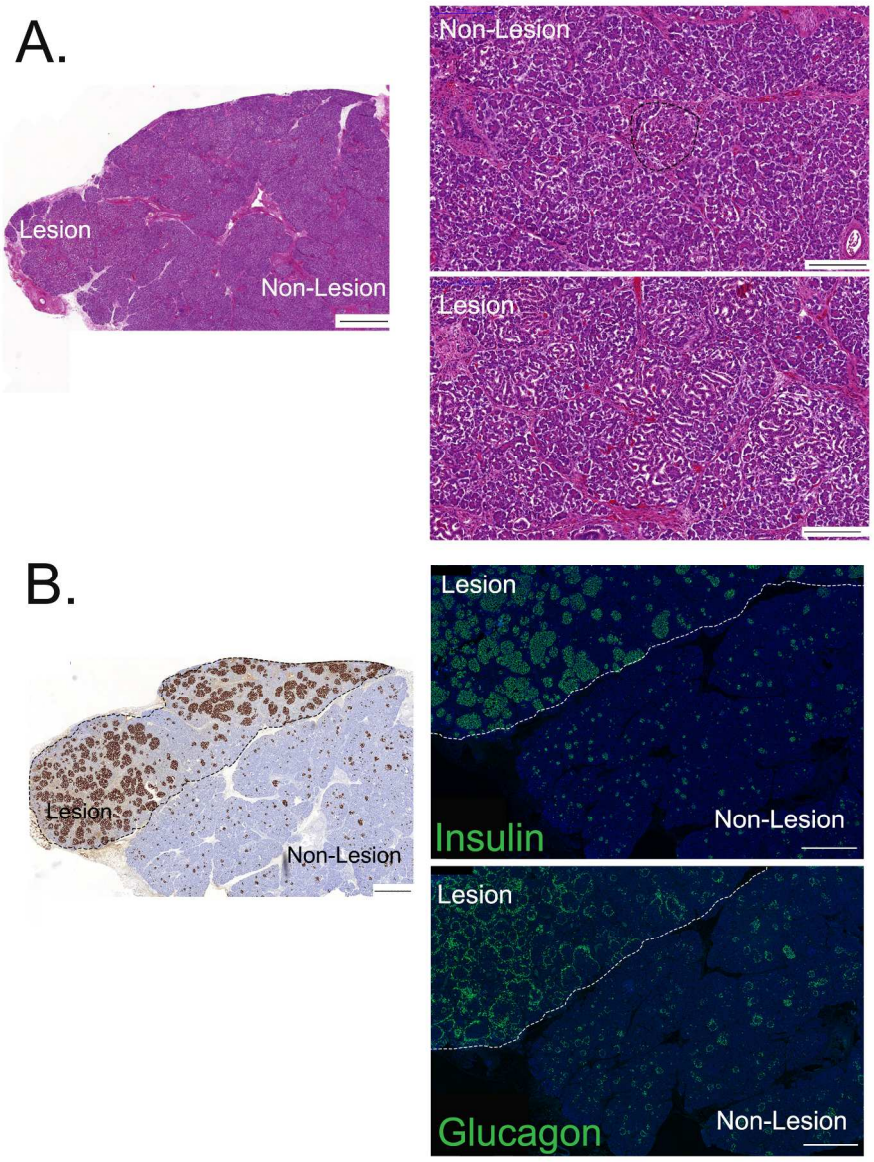

Figure 1. Histological and immunological characteristics of the pancreas. Panel A high and low power H&E images of the pancreas. The focal domain 'Lesion' is associated with islet cell hyperplasia. In the non-focal region – 'Non-Lesion', islets can be readily observed, indicated by dotted region. Panel B illustrates the expression of insulin by immunohistochemistry in the tissue block. Note the enrichment of insulin-expressing cells in the designated lesion domain compared to the expression of insulin in the remainder of the pancreas, Panel A low power image (x5), scale bar 1mm; high power images (x40), scale bars 200 µm. Panel B magnification 2.5X, scale bars 1mm.

194x264mm (300 x 300 DPI)

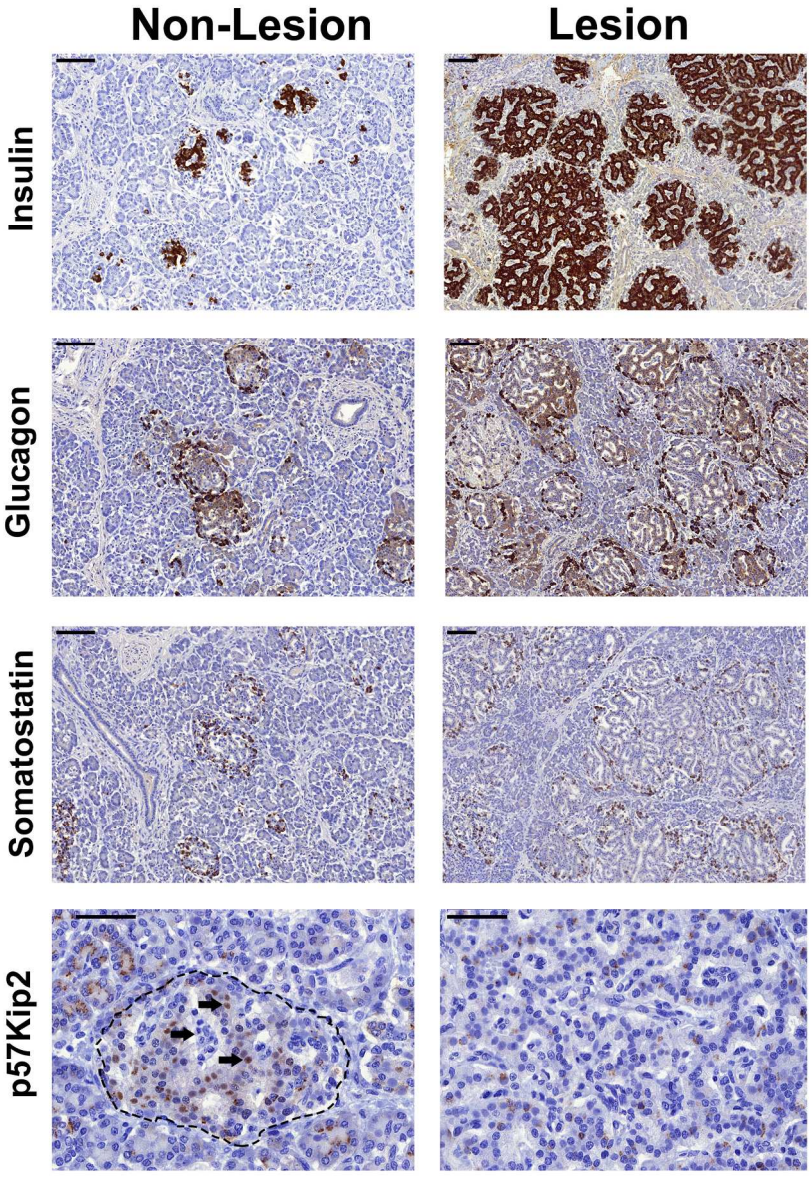

Figure 2. Islet hormone expression in the focal and non-focal domains of the CHI pancreas. In the non-lesion domains of the tissue insulin expression is largely found within islet structures with a central location within the islet domain. Glucagon and somatostatin expression is localized to the periphery of the islets. The focal lesion is characterized by extensive expression of insulin-positive cells, with glucagon- and somatostatin-expressing cells around the peripheral structures of the extended structures. Islets outside of the focal lesion have nuclei localization of p57kip2, whereas islet cells within the lesion do not express p57kip2. Images 20-40X magnification; Scale bars: 100 μm for all images, except p57kip2; 50 μm.

158x230mm (300 x 300 DPI)