

Evaluation of Diagnostic Fasting in the Investigation of Hypoglycemia in Children Omani Experience

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Abstract

Objectives: To assess the safety and importance of diagnostic fast in the evaluation of hypoglycemia in children in a non-specialist set up. **Method:** The medical records of 116 patients with hypoglycemia, admitted to Pediatric Unit, Royal Hospital, Muscat, Sultanate of Oman, over a 15 year period, were reviewed. Of these, 96 (82.8%) patients, 52 boys and 44 girls, aged 8 days to 10 years were subjected to diagnostic fast. **Results:** Of these 96 patients fasted, 77 (80.2%) became hypoglycemic (HG group) and 19 (19.8%) did not develop hypoglycemia on fast (NHG group). In the HG group, 69 (89.6%) patients developed symptomatic hypoglycemia of variable severity and none developed coma or convulsions during fasting. **Conclusion:** The study has proved that diagnostic fast is

relatively a safe procedure with considerable amount of diagnostic yield.

Key words: Hypoglycemia, fast, ketone utilization defect, succinyl Coa transferase deficiency, idiopathic ketotic hypoglycemia, carnitine plamitoyl transferase deficiency.

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Introduction

Establishing correct diagnosis is the most important step in the management of hypoglycemia in children. The sample of blood and urine, collected at the time of hypoglycemia may give valuable clue to establish the diagnosis and cause of hypoglycemia. If this window of opportunity is missed, diagnostic fasting may be necessary to unmask the cause of hypoglycemia. The value of focused history and physical examination cannot be underestimated. It is important to find out whether hypoglycemia occurs after a long period of fasting as in fatty acid oxidation defect (FAOD) or soon after a meal as in fructose intolerance. The length of feeding intervals in a baby on demand feeds may also give some information on the duration of fasting tolerance. Similarly, many base-line investigations like blood gases, liver function tests, lactate, creatine kinase, ammonia, uric acid, cholesterol and triglycerides are valuable, but by themselves may not lead to any specific diagnosis.

Some of the metabolic and endocrine disorders, no doubt, could be diagnosed without resorting to fasting. Many FAOD could be diagnosed by looking at carnitine profile.¹ However, it may be necessary to induce hypoglycemia by diagnostic fasting and obtain samples of blood and urine for metabolic and hormonal assays. It is important to know that adequate duration of fasting may not always unearth the underlying defect.² One should not undertake diagnostic fast lightly as there is an inherent danger of serious complications, if hypoglycemia goes unrecognized during provoked fasting.³ Therefore, it is important to ensure utmost care, during diagnostic fast. The diagnostic fast is particularly risky in hyperinsulinemic state and in some of the metabolic disorders like fructose 1, 6 diphosphatase (F.1, 6DP) deficiency and FAOD. Therefore, many centers do blood TMS before resorting to diagnostic fasting.^{1,4}

Objective

The objective of the study was to assess the value of diagnostic fast in the evaluation of hypoglycemic disorders in children in a "non-specialist" set up.

Methods

The medical records of 116 patients with hypoglycemia, admitted to Pediatric Endocrine and Metabolic Unit, Royal Hospital, Muscat, Sultanate of Oman, over a 15 year period, from Jan. 1991-Dec. 2005 were reviewed. Of these, 20 (17.2%) patients were excluded from the study because of incomplete records,⁵ non-availability of records⁴ or the diagnosis was made without fasting.⁵ The remaining 96 (82.8%) patients were subjected to diagnostic fast as per the Unit protocol. The maximum duration of fasting was based on the age of the child,^{1,2} usual feeding pattern and fasting tolerance. The duration of fasting was for 8, 12, 18 and 20 hours for children aged < 6 months, between 6 to 12 months, 1 to 2 years and 2 to 7 years respectively. Children aged < 6 months and those with very short fasting tolerance as in suspected hyperinsulinism and glycogen storage disease are fasted during morning hours. The signs and symptoms of hypoglycemia were closely monitored throughout the fasting period. The frequency of blood glucose (BG) monitoring is individualized depending on the anticipated period of fasting tolerance. If short fasting tolerance is suspected as in hyperinsulinism, the BG was monitored every 15 to 30 minutes after commencing fast. When longer fasting tolerance is anticipated, the BG is monitored 4 to 6 hours after the beginning of fast. The BG was monitored, first every 2 hours, then hourly, when the BG was <3.5 mmol/L and then more frequently, if BG was <3 mmol/L. Hypoglycemia was defined as BG \leq 2.6 mmol/L. The patient on diagnostic fast was also considered to have hypoglycemia, if presumed symptoms of hypoglycemia respond to

dextrose infusion even if BG was >2.6 mmol/L.

At the end of fasting i.e. when BG ≤ 2.6 mmol/L or end of predetermined duration of fasting, urine and blood samples were collected for the following tests: serum cortisol, growth hormone, C-peptide, insulin, lactate, pyruvate, ammonia, amino acids, free fatty acids, 3 β -hydroxybutyrate and blood gases. The urine was checked for ketones in all voided samples, during fasting. Blood was also collected for carnitine profile (free, total and acyl-carnitine) if not collected earlier. Fasting was terminated by feeding or by IV 10% dextrose infusion after blood samples collection. Urine sample for organic acids was collected after terminating fasting or immediately after patient recovered from hypoglycemia.

Results

Of the 96 patients, 17 (17.7%) were from Muscat region and the remaining 79 (82.3%) were referred from different regional hospitals of Oman. There was at least one or more than one documented hypoglycemia in 83 (86.5%) of patients studied. The age group of patients ranged from 8 days to 10 years and there were 52 boys and 44 girls. Table 1 show that 25% of patients were below the age of 1 year, 21.9% were below the age of 2 years, 48.9% were between 2 to 5 years and only 4.2% of patients were in the age group of 5 to 10 years.

Table 2 shows that of the 96 patients fasted, 77 (80.2%) became hypoglycemic (Hypoglycemic group-HG group) and 19 (19.8 %) did not develop hypoglycemia on fast (Non hypoglycemic group-NHG group). The majority of patients, 69 (89.6%) in HG group developed symptomatic hypoglycemia of variable severity and none in this group developed coma or convulsions during fasting. In 49 (63.6%) patients the BG concentration was between 2 to ≤ 2.6 mmol/L, in 23 (29.9%) the BG was between 1 to < 2 mmol/L, and in 2 (2.6%) the BG dropped around 0.9 mmol/L. The HG group also included 3 (3.9%) patients with BG 2.6 - 3.1 mmol/L, but these were considered to be clinically hypoglycemic because of the hypoglycemic symptoms and prompt recovery following dextrose infusion. Table 2 and the sector diagram also shows the final outcome in the 96 patients fasted. In 77 patients in HG group, specific diagnosis was made in 50 (64.9%) patients; this includes 21 (27.3 %) patients with endocrine diseases and 29 (37.7%) patients with metabolic disorders. In 22 (28.6%), the diagnosis was uncertain and in 5 (6.5%) no diagnosis was established. The specific metabolic conditions diagnosed were: glycogen storage disease in 8 (10.35%), neoglucogenesis defect in 2 (2.6%), fatty acid transport defect in 8 (10.35%), FAOD in 6 (6.6%), ketone synthesis defect in 3 (3.9%) and ketone utilization defect in 2 (2.6%). In the 22 patients with uncertain diagnosis, the possibilities considered

were: idiopathic ketotic hypoglycemia (IKHG) in 15 (19.5%), Reye syndrome in 3 (3.9%), mitochondrial respiratory chain defect in 2 (2.6%), succinyl CoA transferase (SCOT) deficiency in 1 (1.3%) and F1, 6D deficiency in another 1 (1.3%). In the NHG group, there were 19 patients aged 1.6 to 4 years with a mean of 2.2 years. The duration of fasting ranged from 14.5 to 18 hours. There was past history of documented hypoglycemia in 14 patients; 9 had only one episode and 5 of them had 2 or more symptomatic hypoglycemic episodes before referral, and suspected hypoglycemia in 5 patients. In 3 patients, diagnostic fast was interrupted by parents prematurely and in 2 patients, the investigations were incomplete. None in the remaining 14 patients developed hypoglycemia though the duration of fasting was considered to be appropriate for the age. Only 2 (10.5%) patients showed laboratory evidence of metabolic disease. In one patient, the diagnosis was carnitine palmitoyl transferase (CPT-1) deficiency based on elevated plasma free carnitine, and in the other patient, the diagnosis of 3-hydroxy-methylglutaryl CoA (HMGCoA) lyase deficiency was made on the basis of the characteristic urine organic acid abnormality on TMS.

Discussion

The study has revealed that more number of children present with hypoglycemia during the first month of life as compared to the later part of infancy. The metabolic transition that takes place during the first few days of life in a neonate increases the vulnerability to hypoglycemia. The risk of hypoglycemia increases again after the age of one year with maximum number cluster around the age of 2 to 5 years. This may be that toddlers get fed less frequently, thus exposing those with defect in glucose homeostasis to hypoglycemia. Of the 96 patient's fasted as whole, specific endocrine (21.9%) and metabolic (30.2%) diagnosis was made in 50 (52.1%) patients. Whereas, in Morris study that included 138 patients fasted,¹ specific endocrine and metabolic diagnosis was made only in 21.7% of cases. In the latter study, 57.2% patients had hypoglycemia before referral as compared to 81.3% in the present study. It is not surprising, therefore to have more specific diagnosis in our series. The metabolic conditions diagnosed were; glycogen storage disease and defects in gluconeogenesis, fatty acid transport, fatty acid oxidation, ketone body synthesis and ketone body utilization.

Morris found IKHG as the main cause of hypoglycemia in children, comprising nearly 23% of the total cases.¹ It is important to recognize that the presence of ketosis in a patient with hypoglycemia is not equivalent to IKHG, as this is a diagnosis of exclusion.^{6,7} Ketosis is a normal physiological response to fasting.

Table 1. Age Distribution of Patients at Referral

| Age Group | <1 month | 1 to <6 month | 6 to <12 month | 1 to <2 years | 2 to <5 years | 5 to 10 years |
|-----------|----------|---------------|----------------|---------------|---------------|---------------|
| No. | 10 | 6 | 8 | 21 | 47 | 4 |
| % | 10.4% | 6.3% | 8.3% | 21.9% | 48.9% | 4.2% |
| Total | 24 (25%) | | | 72 (75%) | | |

Table 2. Final Diagnosis in Fasted Hypoglycemic (HG) and Non-Hypoglycemic (NHG) Groups.

| HYPOGLYCEMIC GROUP (N=77, 80.2 %) | | | FREQUENCY | % |
|--|---|---|-----------|--------|
| DISORDER | DIAGNOSIS | | | |
| ENDOCRINE | a) Hyperinsulinism | | 14 | |
| | b) ACTH deficiency | | 1 | |
| | c) ACTH resistance | | 3 | 27.3% |
| | d) Hypopituitarism | | 3 | |
| | Total | | 21 | |
| SPECIFIC METABOLIC DISEASE | 1. Glycogen Storage Disease | | | |
| | a. GSD-1 | | 5 | |
| | b. GSD-3 | | 2 | 10.35% |
| | c. GSD-0 | | 1 | |
| | Total | | 8 | |
| | 2. Neoglucogenesis defect | | | |
| | a) Fructose 1-6 DP Deficiency | | 1 | |
| | b) Glycerol kinase | | 1 | 2.6% |
| | Total | | 2 | |
| | 3. Fatty acid transport and oxidation defects | | | |
| | (a)Fatty acid transport | | | |
| | CPT-1 | | 7 | 10.35% |
| | CPT-2 | | 1 | |
| | Total | | 8 | |
| | (b)Fatty acid oxidation defect | | | |
| Long chain hydroxyacyl dehydrogenase | | 2 | | |
| Glutaric aciduria-2 | | 3 | 6.6% | |
| Ehtylmelonic aciduria | | 1 | | |
| Total | | 6 | | |
| 4. Ketone synthesis defect | | | | |
| Hydroxymethylglutaryl CoA lyase deficiency | | 3 | 3.9% | |
| 5. Ketone utilization defect | | | | |
| -ketothiolase deficiency | | 2 | 2.6% | |
| DIAGNOSIS UNCERTAIN | 1. Idiopathic ketotic hypoglycemia | | 15 | 19.5% |
| | 2. Fructose 1-6 diphosphatase deficiency | | 1 | |
| | 3. Succinyl CoA transferase deficiency (SCOT) | | 1 | |
| | 4.Mtochondrial Respiratory chain defect | | 2 | |
| | 5. Reye syndrome | | 3 | |
| | Total | | 7 | 9% |
| NO DIAGNOSIS | | | 5 | 6.4% |
| NON-HYPOGLYCEMIC GROUP (N= 19, 19.8 %) | | | | |
| SPECIFIC METABOLIC DIAGNOSIS | CPT-1 | | 1 | |
| | Hydroxymethylglutaryl CoA lyase deficiency | | 1 | |
| | Total | | 2 | 11.1% |
| NO DIAGNOSIS | | | 17 | 89.4% |

It is also a normal compensatory response to fasting in growth hormone, cortisol deficiency and in many metabolic disorders. In contrast, ketosis due to ketolytic disorder is an abnormal biochemical response as the defect is in the utilization of ketones by tissues.⁸ The diagnosis of 15 (15.6%) patients with IKHG in our series was based on the absence of laboratory evidence of other diseases known to have ketotosis during hypoglycemia. The diagnosis was supported by the history of intrauterine growth retardation, with or without low plasma alanine, normal lactate and carnitine, suppressed C-peptide and appropriately elevated cortisol.⁵ One condition that may be difficult to exclude from IKHG is SCOT deficiency.⁹ This is a ketone utilization defect with variable severity and may have intermittent or persistent ketoacidosis. During the episodes of ketosis, BG may be normal, low or high.¹⁰ As in IKHG, no abnormality is found in plasma carnitine, lactate, ammonia and urine organic acids.^{10, 11} The possibility of SCOT was considered in one patient who presented with hypoglycemic ketoacidosis at the age of 18 months and had severe recurrent course. The biochemical analysis during ketoacidosis showed elevated plasma nonesterified fatty acids (NEFA), β -hydroxybutyrate and acetoacetate with profound ketonuria.⁸ The patient also developed metabolic acidosis, ketosis and hypoglycemia within 12 hours of fasting. The diagnosis of SCOT could be confirmed by SCOT enzyme activity in skin fibroblasts or by molecular genetics.¹¹

Among the 15 cases of fatty acid transport and oxidation defect diagnosed in our series, the commonest defect was CPT-1 deficiency.⁸ The CPT-1 facilitates the transfer of long chain fatty acids through the inner mitochondrial membrane.⁷ Of the total 8 patients with CPT-1 deficiency, 5 presented with fasting hypoglycemia and 2 had features of Reye's syndrome in addition. In 5, the diagnosis was made on the basis of elevated free carnitine. The markedly elevated free carnitine is diagnostic of CPT-1 deficiency.¹² However, it is not necessary that such patients must always have elevated free carnitine.⁸ Two siblings in the present study had clinical and a biochemical finding identical to CPT-1 deficiency but the plasma free carnitine concentration was within normal. There was one 8-month-old infant with CPT-2/translocase deficiency who had two episodes of hypoglycemia before referral. The clinical examination showed no cardiac and skeletal muscle involvement, and serum creatine kinase and free carnitine was normal. Urine TMS showed no dicarboxylic aciduria. The level of C16 and C16:1 species of long chain acylcarnitine was markedly increased supporting the diagnosis of CPT-2/translocase deficiency.

The 5 patients with FAOD in this series include 2 with long chain hydroxyacyl dehydrogenase (LCHAD), 2 with glutaric aciduria-2 (GA-2) and 1 with ethylmelonic aciduria (EMA). The 2 patients with LCHAD presented with recurrent hypoglycemic seizures for the first

time at the age of 3 and 1 year respectively and both remained well in between the attacks of hypoglycemia. The first patient's family had lost two siblings at an early age from an undiagnosed seizure disorder. The urine organic acids during fasting revealed long chain 3 hydroxy dicarboxylic aciduria (C12 more than C10 acids) with minimum ketonuria. The free carnitine was low and long chain acylcarnitine was elevated. The 3 patients with GA-2 reported in the study were referred at the age of 4.5 year, 10 year and 6 month. The first two patients were siblings. All presented with recurrent hypoglycemia associated with lactic acidosis at the age of 2.5 year, 5 year and 3 month respectively. The first patient also had exercise induced muscle cramps with elevated creatine kinase. The urine organic acids by TMS showed elevated lactate, ethylmelonic acid, short and medium chain glycine conjugates and 2-hydroxyglutaric acid. All of them responded to pharmacological dose of riboflavin.¹³ The one patient with EMA described here presented with recurrent hypoketotic hypoglycemia from the age of 2.5 years. Investigations showed mild elevation of liver enzymes and serum lactate with low free carnitine. Urine organic acid by TMS revealed elevated EMA. This patient was developmentally normal and had no neurological or vascular abnormalities. The urine could also be positive for ethylmelonic acid in GA-2, SCAD and mitochondrial disorders.⁷ The clinical features described resemble mild form of GA-2. EMA has considerable phenotypic variability and may or may not be associated with skeletal myopathy, cardiomyopathy, vasculopathy and neurological manifestations.^{8, 14, 15}

There were 3 patients presented with mitochondrial HMGCoA lyase deficiency. This is a ketone body synthetic defect, which is considered to be the commonest organic aciduria in Saudi Arabia.¹⁶ The presentation in the first two patients aged 4 year and 1 year resembled Reye's encephalopathy and the other aged 10 months presented with recurrent hypoglycemic seizures. The urine TMS in all, revealed 3-hydroxy-3-methylglutaric acid, 3-methylglutaric acid and hydroxyisovaleric acid. The 10 month old baby showed developmental delay on follow up. The 2 patients with ketothiolase deficiency described are siblings. The mitochondrial acetoacetyl CoA thiolase (β ketothiolase) like SCOT is a ketone utilization defect. But unlike SCOT, this condition is easily diagnosed because of the presence of specific metabolites in urine by TMS. The patients present with severe intermittent ketoacidosis.¹⁷ In both patients, the symptoms started at around the age of 2.5 years. They presented with recurrent episodes of hypoglycemia and ketoacidosis. During diagnostic fast there was marked ketosis with a fall in BG to < 3 mmol/L. Urine organic acid revealed hyperketonuria, dicarboxylic aciduria, 3 hydroxy carboxylic acid, tiglylglycine and metabolites of isoleucine.

One patient with hepatic F1, 6DP deficiency had the first episode of hypoglycemia with severe lactic acidemia on day 2 of life and made uneventful recovery.¹⁸ He remained asymptomatic till the age of 8 months, thereafter; he had 3 more similar episodes. Diagnostic fast at the age of 14 months resulted in hypoglycemia, metabolic acidosis, lactic acidemia and elevated liver enzymes. No abnormality was found in serum carnitine and organic acids. The deficiency of F1, 6DP was confirmed by the absence of enzyme activity (zero activity) in leucocytes study. The patient with glycerol kinase deficiency (GKD), presented at the age of 7 days with hypoglycemia had the classical clinical and hormonal abnormalities of congenital adrenal hypoplasia. On follow up, he developed features of Duchene's muscular dystrophy. This is an X-linked, over-lap syndrome with congenital adrenal hypoplasia and Duchene muscular dystrophy as the main manifestations.¹⁹ Glycerol contributes up to 10% of glucose generation by gluconeogenesis during fasting.²⁰ During diagnostic fast he developed hypoglycemia and blood showed elevated triglyceride and urine showed elevated glycerol.

The study included 8 patients with glycogen storage disease (GSD). The patient with GSD-0 is discussed further as this diagnostic possibility is not usually entertained.²¹ A 4 year old boy presented with recurrent episodes of early morning lethargy with or without convulsions from the age of 1.5 year. He was earlier investigated elsewhere and was being treated with anticonvulsants. Clinically, there was no hepatomegaly, the rest of systemic examination was normal and his basal investigations, including plasma carnitine showed no abnormalities. At the end of 4.5 hours of diagnostic fasting, BG was < 1 mmol/L, urine showed excess ketones and serum lactate was within the normal range. The post prandial BG ranged from 8 to 13 mmol/L with concomitant increase in lactate levels to 4 to 6 mmol/L. This constellation of clinical and biochemical findings are consistent with the diagnosis of GSD-0. The diagnosis could be confirmed by the assay of glycogen synthetase enzyme in hepatic tissue. Mutation analysis of the GYS2 gene (12p12.2) is a non-invasive method for establishing the diagnosis.²¹

Conclusion

In this study of highly selected group of patients with documented hypoglycemia upon diagnostic fast, hypoglycemia was provoked in 4/5th of patients and in more than 2/3rd of patients a definite cause for the hypoglycemia was established. The metabolic disorders constituted the commonest cause of hypoglycemia in almost half of the children. The final confirmation of diagnosis depends on demonstrating the specific enzymatic defects or mutational defects of the genes governing the three metabolic pathways of glucose homeostasis. This facility is generally not

accessible for pediatricians from the developing countries. The cases discussed with the background of clinical features, results of metabolic-endocrine investigations and diagnostic fast, gives ample evidence that diagnostic fast is a valuable procedure both in terms of establishing the diagnosis and in guiding management in a non-specialist pediatric set-up, and it is a fairly safe procedure, if adequate safety precautions are taken.

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