PERSISTENT ASYMPTOMATIC SEVERE HYPOGLYCAEMIA DUE TO TYPE 0A GLYCOGENOSIS - GENERAL AND ORO-DENTAL ASPECTS

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Abstract

Background. Type 0 glycogenosis is a genetic metabolic disorder characterized by the absence of glycogen synthesis of hepatic synthase and hence of liver glycogen stores in normal amounts. It is an extremely rare condition.

Case study. This case is a 5-year and 11-month-old female child with asymptomatic severe hypoglycemia in the last two years. During the admission and afterwards, an extensive panel of paraclinical and imaging investigations was carried out to diagnose and document the case, which led to the specific genetic test. The result was positive for 2 heterozygous mutations in the GYS2 gene (hepatic glycogen synthase), the p.547C>T mutation was pathogenic (class 1) and c.465del, frameshift likely pathogenic (class 2). In order to integrate the clinical picture of patients with this condition and to establish potential correlations regarding the specific aspects with the general development and the phenotype, the oro-dental status was investigated.

Conclusion. The investigations showed a positive correlation with literature data in several respects: low stature, hypoglycemia with hyperketonemia but normal plasma lactate, postprandial and contradictory hyperglycemia, delayed bone development, etc. Oro-buco-maxillary aspects showed a slight delay in the dental eruption. Dietary therapy and stricter dental care and additional prophylaxis are required.

Key words: Glycogen Storage Disease, GSD0a, glycogen-deficiency, hypoglycemia, dentistry, rare metabolic diseases.

INTRODUCTION

Glycogen storage diseases (GSD) are inherited inborn errors of metabolism, involving carbohydrate metabolism (1) that may first manifest themselves in neonates or early childhood and all GSDs are due to a failure to use or store glycogen (1, 2).

Glycogen storage disease type 0 was first

described in 1963 (3), as an autosomal recessive disease, and although sixteen different mutations have been identified to date in the gene that encodes hepatic glycogen synthase 2 (4), GSD type 0's gene locus is at 12p12.2 (5, 6). This disease is considered not a true GSD, because there is a marked decrease in the liver glycogen content (6), characterized by ketotic hypoglycemia after prolonged fasting and postprandial hyperglycemia and hyperlactacidemia (5). This results in reduction of glycogen storage in the liver, secondary to a lack of glycogen synthase activity, which causes a marked decrease in liver glycogen content (1, 5, 7). The characteristic element in GSD type 0a is the absence of the hepatic isoform of the glycogen synthase enzyme (5, 7, 8). This is responsible for the liver transformation of glucose-6-phosphate in the form of a deposit: glycogen (8).

Unlike other forms of GSDs, glycogen-storage disease type 0 does not involve the storage of excessive or abnormal glycogen. The glycogen stores are moderately decreased in the liver. The symptoms range from asymptomatic hyperglycaemia to recurrent hypoglycaemic seizures (7, 9, 10).

Glycogen storage disease type 0 has an extremely low prevalence (1, 11), currently around 40 cases documented worldwide (30 Type 0a cases - hepatic variant and 10 Type 0b cases - muscular variant (7, 9, 12). However, some authors argue the hypothesis of significant under-diagnosis (8, 9).

The clinical history in patients is that of an infant or child who begins to sleep for an uninterrupted night, when he or she is no longer receiving evening or night meal, or concomitantly with an acute gastrointestinal disorder or other periods of low food intake (7-9). Hypoglycaemia is the main manifestation of hepatic GSD (5,7) and can have different degrees, from subclinical to sometimes seizures in the morning before

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breakfast, even though most of the time children may be asymptomatic (2, 7, 13). Mild hypoglycaemic episodes may be clinically unrecognized (9). Other symptoms that may be due to the subclinical hypoglycaemia, are pallor, drowsiness, sweating, lack of attention. Uncoordinated eye movements, disorientation, seizures, and coma may accompany severe episodes (1, 5). Due to the fact that glucose cannot be stored as liver glycogen, dietary carbohydrate is converted to lactate and this results in postprandial hyperglycemia and hyperlacticacidemia, alternating with fasting hypoglycaemia and hyperketonemia. The liver is not enlarged, and short stature is common (5,14). Gluconeogenesis from amino acids (alanine) and lipid (glycerol) precursors that should later intervene is also altered, contributing to the prolongation and exacerbation of glycemic imbalance (8, 9).

Under a prolonged fasting, the patient can manifest lethargy to loss of consciousness, nausea, vomiting and sometimes seizures (11), hypoglycaemic coma (1, 8, 9). Biochemical profile is represented by the specific fasting hypoglycaemia accompanied by hyperketonemia and normal lactate (9), hyperglycaemia (8, 11) (with glucosuria (8)) and postprandial hyperlacticacidemia (1, 5), consequent to the incapacity of transformation of glucose into glycogen and redirection to lactate conversion but also hyperlipidemia (5, 9). After a prolonged fasting, the increased concentration of free fatty acids results in lowering the alanine concentration (8).

In clinical examination, the only distinct element is the growth retardation (7, 12) and consequently the short stature (5, 8). In addition, discrete hepatomegaly (liver steatosis (1, 9)) and osteopenia (8, 9) were recorded. From a neurological point of view, recurrent hypoglycaemic episodes can cause developmental delay, cognitive deficits, personality deformities and mental retardation (7, 9, 12).

There can be used for diagnosis methods as administration of glucose or galactose (and observed elevation of blood lactate and lipid after intake) (15, 16), for the definitive diagnosis liver biopsy (for the demonstration of decreased hepatic glycogen) that has been replaced by mutation analysis of the gene, which is a non-invasive diagnosis method (6).

The management of this condition is however simple, accessible and effective in preventing symptoms and acute and chronic complications (1, 8, 9) and consists in keeping a balanced and diversified diet and an organized meals program, with breaks no longer than 2 to 4 hours, rich in complex carbohydrates

with low glycemic index and proteins during the day and low in simple carbohydrates (9), accompanied by a meal of unpacked starches before sleeping or during the night: 1-2 g / kgbw (1, 5, 8, 11).

CASE REPORT

We present the case of 6-year old girl with good general condition, who presented to our clinic in the context of an episode of respiratory disease. The pediatrician noticed in the patient's history several severe hypoglycaemia episodes in the past 18 months, as shown in the Table 1. However, the patient has always been asymptomatic, the only potentially suggestive element being that mother reported that the patient wanted to feed shortly after she awakened and the discretely low resistance to the effort.

Upon admission, the physical clinical examination does not detect pathological elements apart the height under the percentile 25 and the weight below the percentile 50 and poorly represented subcutaneous fat. Following the interdisciplinary consultations with the specialties of diabetes, pediatrics, endocrinology, dentistry and medical genetics, a management plan, investigations and recommendations were developed, detailed below.

Blood samples showed low value of glycaemia 40 mg/dL (70-110 mg/dL), increased value of postprandial blood glucose at 2 hours 210 mg/dL (<180 mg/dL), high total cholesterol and cortisol, but normal glycated hemoglobin (HbA1c) due to the ups and downs of the glycaemia. Thyroid function was normal. Very high ketone bodies in both blood (2929 – normal range < 0.1 mmol/L) and urine shows ketoacidosis, that is suggestive for hypoglycemia and organic acidemias (Table 2). Leucine, an essential ketogenic amino acid used to biosynthesis proteins, is at the upper normal limit being one of the two exclusively ketogenic amino acids. Acylcarnitine profile is disturbed, meaning that glucose reserves are worn-out and cellular metabolism is switched to other oxidative paths of fatty acids and gluconeogenesis of amino acids as a main source of energy.

The following parameters were within normal range: blood count (minimum changes in MCHC and

Table 1. Fasting Glycaemia

Date	Fasting Glycaemia
4 Sep 2018	34 mg/dL
8 Jun 2018	44 mg/dL
6 May 2017	23 mg/dL

PLT), HDLc, total bilirubin, direct bilirubin, TGO, TGP, alkaline phosphatase, lactate dehydrogenase, amylase, lipase, urea, creatinine, uric acid, potassium, phosphate, ionic calcium, serum calcium, serum iron, creatine kinase, ammonia, lactate, fibrinogen, islet cell (anti-pancreatic) autoantibodies, islet tyrosine phosphatase 2 (IA2) antibodies, insulin autoantibodies (IAA), glutamic acid decarboxylase autoantibodies (Anti-GAD), urine test, urinary glucose, urinary sediment, urine organic acids, acylcarnitine profile and the creatinine ratio, specific pathology-specific

Table 2. Blood and urine test of the studied patient - collected at admission

Blood test	Value	Range
Glycaemia mg/dL	40 ↓	70-110
Postprandial blood glucose at 2h mg/dL	210 ↑	<180
HbA1c%	4.8	4-5.9
GGT U/ L	47	<61
Total cholesterol mg/dL	224	<200
LDLc mg/dL	147	50-150
Triglycerides mg/dL	142	50-150
Cortisol nmol/L	786.7	172-500
Insulin uU/mL	0.3 ↓	2.6-24.9
C peptide ng/mL	0.126	0.81-3.85
TSH mUI/L	0.74	0.7-5.97
fT4 ng/dL	1.02	0.85-1.77
T4 ug/dL	6.33	5.2-15
Urinary protein mg/ dL	16	Negative
Leucine μM/ L	302.396	<300
Methionine μM/ L	36.057	<22
Valine μM/ L	385.861	<220
C3/ mg/g creatinine	0.1	0.2-0.8
C6/ mg/ g creatinine	0.04	0.1-0.4
C8/ mg /g creatinine	0.06	0.2-0.8
C10/ mg / g creatinine	0.07	0.1-0.4

relationship (exception +0.04 μM / L for the C4DC-carnitine component for the adenosylcobalamin synthesis defect, homocysteinuria and methylmalonic acid, deficiency of methylmalonyl CoA mutation, deficiency of holocarboxylase synthase and maternal vitamin 12 deficiency), fatty amino acids except those mentioned above, free fatty acids in serum (data not shown).

The investigations conducted led to the specific genetic test. The result was positive for 2 heterozygous mutations in the GYS2 gene (hepatic glycogen synthase), the p.547C> T mutation being pathogenic (class 1) and c.465del mutation probably pathogenic (class 2) (Table 2).

The patient was diagnosed with Glycogen storage disease (GSD) type 0, ASA class II.

The initial acid base equilibrium was disturbed, partial carbon dioxide pressure being low at 33.3 mmHg (normal range: 35-45 mmHg), low partial oxygen pressure (pO₂) at 64.7 mmHg) from normal range of 80-100 mmHg, low bicarbonate (HCO₃) at 21.1 mmol/L from normal range (22-26 mmol/L). Normal values of pH and Base excess (BE) were found as seen in Table 4. These values indicate a slight metabolic acidosis. Within two days, the analyzed capillary blood samples reveal the following modified parameters, shown in Table 3. Partial oxygen pressure (pO₂) was stabilized to 98.9 mmHg (normal range: 80-100 mmHg), also bicarbonate (HCO₃) to 22.6 (normal range: 22-26 mEq/L), pH remained in normal range 7.44.

The abdominal ultrasonography has detected mild hepatomegaly, confirmed later on the MRI exam - native abdomen, no other pathological elements were

Table 3. Medical genetics investigation of studied patient. Result summary

GENE	VARIANT COORDINATES	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
GYS2	Chr12(GRCh37): g.21727209 G>A NM_021957.3: c.547 C>T p.(Gln183*) Exon 4	Het	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A Conservation: nt moderate	gnomAD: 0.000047 ESP: 0.00015 1000 G: 0.00020 CentoMD: 0.000070	Stop gain Pathogenic (Class 1)
	Chr12(GRCh37): g.21728830del NM_021957.3:c.465del p.(Phe155Leufs*4) Exon 3	Het	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A Mutation Taster: N/A	gnomAD: 0.0000080 ESP: - 1000 G: - CentoMD: -	Frameshift Likely pathogenic (Class 2)

Variant description based on Alamut Batch (latest database available). * AlignGVGD: C0: least likely to interfere with function. C65: most likely to interfere with function, splice prediction tools: SSF, MaxEnt, HSF. **Genome Aggregation Database (gnomAD), Exome Sequencing Project (ESP). 1000Genome project (1000 G) and CentoMD ®(latest database available). ***based on ACMG recommendations.

Table 4. Modified parameters (CPU method)

Parameter	Day 1	In two days	Normal range
pH	7.42	7.44	7.35-7.45
pCO ₂	33.3 mmHg	31.9 mmHg	35-45 mmHg
pO ₂	64.7 mmHg	98.9 mmHg	80-100 mmHg
HCO ₃	21.1 mmol/L	22.6 mmol/L	22-26 mmol/L
BE(B)	-2.6 mmol/L	-1 mmol/L	-2 to +2

detected at these tests (data not shown). Wrist X Ray assesses a delayed bone age around 3 years and 6 months (Fig. 1).

Oro-maxillo-facial aspects

The 6-year-old girl has also presented for a dental consultation to analyze the oro-maxillofacial condition in correlation with the suspected general background disease, glycogenosis type 0a. In general examination has shown an apparently physically and psychologically balanced patient, a slight mismatch between biological and chronological age, with the first dental eruption in deciduous teeth at 10 months, but with normal development and a slight delay in the occurrence of 6-year-old molars correlated with delayed bone age.

Facial examination did not show deviations from age norms. Diagnosis of occlusion and orthodontic was psalidodontal occlusion (Fig. 2), maxillary compression class II/1, lack of incisive maxillary median diastema and minimal lateral diastema, marked at the frontal level of maxillary bone. It can be observed the lack of diastemas in the frontal lower dental arch. The treatment plan includes patient hygiene and education for self-hygiene, odontal therapy and orthodontic treatment.

In the context of general pathology commonly

associated with severe hypoglycaemia at night, the particularities of this clinical case require an additional meal of starch or other carbohydrates at midnight (5, 17).

Primary prophylaxis involves observing a rigorous oral hygiene by performing a dental brush with electric brush and fluoride toothpastes (over 1000ppm F) at least twice daily, the duration of a brush being 2 minutes. Additional means of hygiene include the use of antiseptic mouthwashes, dental floss, interdental brushes or other antimicrobial drugs. Considering the child's feeding habits during the night, it is recommended to rinse oral cavity with clean water immediately after the meal. With the eruption of 6-year-old molars, dental sealings and local fluoridation are indicated to prevent cavities.

In order to evaluate the status of psychiatric development, it was recommended to perform a psychological consultation, development within the age range in all aspects analyzed. IQ equal to 110 was observed, without attention deficit.

During the hospitalization, a diversified and regular dietary plan was initiated, with 3 meals and 3 snacks (2.5 - 3 hours away). Subsequently, blood glucose measured from glucose capillary blood was between 50-65 mg/dL (from the first night of hospitalization, the patient received before bedtime - 21:30 - a slow-release carbohydrate snack consisting of various nuts).

In conclusion, at home, parents kept the same dietary plan, containing a low glycemic carbohydrate snack in the evening, glycemic values being maintained over 50-60 mg/dL. After obtaining the genetic test results, specific hygienic-dietary treatment was initiated, consisting of a portion of 20 grams of raw starch dissolved in water, in order to maintain glycemia

Figure 1. Wrist X-ray (Bone age of 3 years 6 months). **Figure 2.** The appearance of occlusion.

at least to 65-70 mg/dL.

Since the patient's 4-year-old sister did not show glycemia under 75 mg/dL at harvest, genetic testing was not indicated (8, 9).

Conflict of interest

The authors declare that they have no conflict of interest.

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