Clin Chem Lab Med 2017; 55, Special Suppl, pp S1 – S1121, June 2017 • Copyright © by Walter de Gruyter • Berlin • Boston

Rare Diseases

Cod: W292

SCREENING OF ACTIVITY A-GALACTOSIDASE IN POPULATION OF PATIENTS WITH CHRONIC RENAL UNKNOWN ETIOLOGY. IDENTIFICATION OF A FAMILY WITH FABRY DISEASE

J. Bobillo Lobato [1], S.I. Villanueva Heráiz [1], E. Lepe Balsalobre [1], I. Peral Camacho [1], M.d.M. Viloria Peñas [1], A. Moro Ortiz [1]
[1]*Unit of Clinical Management of Laboratory. University Hospital Nuestra Señora de Valme. Southern Sanitary Area, Seville.*
(Spain)
joaquin.bobillo.lobato.sspa@juntadeandalucia.es

INTRODUCTION

Fabry disease is a lysosomal disease caused by deficiency of the enzyme α-galactosidase (α-GAL) activity which causes a storage globotriaosylceramide (Gb3), among other glycosphingolipids.

It is a recessive disorder caused by mutations in the GLA gene on the X chromosome (Xq22.1), resulting enzyme deficiency.

Determining α-GAL activity by dried blood spot (DBS) is a useful and effective analytical method for the diagnosis of lysosomal disease that causes serious kidney and cardiac abnormalities in patients undiagnosed. Thus, it has been shown in several studies that there is a high prevalence of Fabry disease in the population of patients with chronic renal failure of unknown etiology.

In a screening carried out in the population of patients with chronic renal failure of unknown etiology, it was located at a carrier family of Fabry disease.

MATERIALS AND METHODS

Enzymatic determinations were performed in DBS (Chamoles et al) by spectrofluorimetry using as substrate 4-methylumbelliferyl-α-D-galactopyranoside. DBS samples are a good method for initial screening, since they have sufficient sensitivity and specificity.

All measurements were performed in duplicate, using a target for each of the samples. The activities were analyzed against a calibration curve with 4-methyl-umbelliferone.

RESULTS

As results of the study, 8 individuals possible affects of the disease were identified in a family of 14 members: 3 men who already had symptoms and 5 women without clinical manifestations.
It has conducted a genetic studies to male 10 years old, identified a polymorphism of the gene GLA described as nonpathological. The rest of the family is expected to expand the studies and establish a diagnosis.

CONCLUSIONS

The screening of the α-GAL activity by DBS in populations where there is a high prevalence of Fabry disease is especially useful for diagnosing individuals with the disease and from them identify family members who may be affected.

Clin Chem Lab Med 2017; 55, Special Suppl, pp S1 – S1121, June 2017 • Copyright © by Walter de Gruyter • Berlin • Boston

Rare Diseases

Cod: W293

DIAGNOSIS OF POMPE DISEASE BY CLINIC LABORATORY

J. Bobillo Lobato [1], M.J. Vélez González [1], E.M. García Agudo [1], M.d.M. Viloria Peñas [1], I. Peral Camacho [1], A. Moro Ortiz [1]
[1]*Unit of Clinical Management of Laboratory. University Hospital Nuestra Señora de Valme. Southern Sanitary Area, Seville.*

(Spain)
joaquin.bobillo.lobato.sspa@juntadeandalucia.es

INTRODUCTION
Pompe disease is a metabolic, neuromuscular, progressive and rare disease with high morbidity and mortality. The base is the acid alpha-glucosidase deficiency (GAA) which hydrolyzes glycogen. The affected are unable to degrade the polymer accumulating in lysosomes.
The objective is to present the diagnostic algorithm for the clinical laboratory developed and evaluated by our group.

MATERIALS AND METHODS
Population of 505 patients with symptoms compatible from all over Spain.
The main symptoms of our patients undergoing diagnostic tests were: myopathy, myalgia, muscle weakness, exercise intolerance, girdle dystrophy, cardiomyopathy (in childhood cases), respiratory failure and hyperCK.
Enzymatic determinations (DBS/lymphocytes) were performed by spectrofluorimetry using as substrate 4-methylumbelliferyl-α-D- glucopyranoside. Analysis versus calibration curve with 4-methyl-umbelliferone.
GLC4 determination by HPLC with UV detection in Agilent 1100 HPLC system. Whit N-butyl-4-aminobenzoate as derivatizing agent and cellopentose as internal standard.

RESULTS
The result of the study, the experience of our service and the logical sequence of tests (by sensitivity/specificity, efficiency, costs ...) eventually led to propose the following algorithm:
Starting from a patient with clinical susceptible, shall first place to make the determination of GAA activity in DBS. In the case of finding alteration results it requests new sample, this whole blood, accompanied by urine (24 hours). In the urine sample concentration GLC4 will be quantified (increased levels of tetrasaccharide are compatible with glycogenosis). With whole blood sample, proceed to study of intra-lymphocytaire activity (gold-standard technique). If the results are positive, it has been the diagnosis of Pompe disease.
Of the 505 patients starting DBS we obtained 530 samples of which 42 were pathological. Of these, were made 35 study of intra-lymphocytaire activity, 16 whit results below normal.
These samples corresponded to 16 patients who were eventually diagnosed with Pompe disease.
In all pompe patients the levels of GLC4 were high, with values between 1.05 and 65.63 mmol/mol creatinine.
Of these 16 patients, 12 had the adult form of the disease, 2 infant-juvenile form and 2 severe infantile form.

CONCLUSSION
Thanks to the diagnosis made by our laboratory could to provide treatment for many patients.

Clin Chem Lab Med 2017; 55, Special Suppl, pp S1 – S1121, June 2017 • Copyright © by Walter de Gruyter • Berlin • Boston

Rare Diseases

Cod: W294

DIAGNOSIS OF A CASE OF GANGLIOSIDOSIS GM1

J. Bobillo Lobato [1], E.M. García Agudo [1], S.I. Villanueva Heráiz [1], M.d.M. Viloria Peñas [1], I. Peral Camacho [1], A. Moro Ortiz [1]
[1]*Unit of Clinical Management of Laboratory. University Hospital Nuestra Señora de Valme. Southern Sanitary Area, Seville.*

(Spain)
joaquin.bobillo.lobato.sspa@juntadeandalucia.es

INTRODUCTION
GM1 gangliosidosis is a lysosomal storage disorder, rare, biochemically characterized by deficient activity of beta-galactosidase and clinically for a wide range of neurovisceral, ophthalmic and dysmorphic features. The disorder is pan-ethnic with an estimated prevalence of 1:100,000-200,000.
We report a case of GM1 gangliosidosis due to the rarity of the disease and the urgent need to raise awareness of this type of diseases of low prevalence, since early diagnosis is essential.

EXHIBITION CASE
10 month old infant was referred to our hospital for severe psychomotor retardation. On examination good general condition is observed, although dismorfologías appreciated.
The first warning signs of additional tests were: red cherry stain, supports a storage disease, blood smear with numerous vacuolated lymphocytes, Brain MRI with impaired myelination suggesting the existence of metabolic disease, considering (for signal appearance and distribution of lesions) diagnostics of gangliosidoses vs disorders hypomyelination / delayed myelination.
The determination of beta galactosidase activity in DBS (0.4 mmol/L/hour; VN: 10-40.10.4 mmol/L/hour) and leukocytes (8.9 nmol/h/mg protein, 7.5% of normal activity) by service Clinical Biochemistry our hospital allowed a definitive diagnosis of GM1 gangliosidosis.

COMMENTS AND DISCUSSION
This paper presents the case of a little common disease and aims to highlight the importance of early diagnosis as well as the importance of following these patients in order to establish processes for concomitant diseases prevention and proper establishment of palliative care.

Clin Chem Lab Med 2017; 55, Special Suppl, pp S1 – S1121, June 2017 • Copyright © by Walter de Gruyter • Berlin • Boston

Rare Diseases

Cod: W295

LABORATORY DETECTION OF MACRO-COMPLEXES IN ROUTINE CLINICAL PRACTICE

J. Komrskova [1], K. Bartosova [1], Z. Kubicek [1], J. Franekova [2], A. Jabor [2]

[1]*Department of Laboratory Methods, Institute for Clinical and Experimental Medicine, Prague, Czech Republic*

[2]*Department of Laboratory Methods, Institute for Clinical and Experimental Medicine/Third Faculty of Medicine, Charles University, Prague, Czech Republic*

(Czech Republic)
komj@ikem.cz

BACKGROUND
Protein and enzyme macro-complexes are produced by self-polymerization or by association with plasma components, mainly immunoglobulins. Reduced clearance, prolonged half time, elevated serum concentrations or activities without clinical symptoms result in diagnostic confusion and errors. The aim of our study was to detect macro-complexes in sera of patients with isolated asymptomatic elevation of serum AST, CK, GGT, amylase (AMS), lipase (LPS), troponin T (hs-cTnT) or prolactin (PRL).

METHODS
Sera of tested patients were precipitated with polyethylene-glycol in volume ratio of 1:1. After centrifugation, concentrationsof components in the supernatant were determined (AST, CK, GGT, AMS, LPS: Architect ci16200, Abbott; hs-cTnT, PRL: Cobas 6000, Roche). These values were compared to those of the patient sera, which were diluted with NaCl 9 g/L in a volume ratio of 1:1. Results were expressed as a percentage of PEG-precitable activity (%PPA). Patient serum and serum from control persons were analysed in parallel. Reference ranges of %PPA (AST, CK, GGT, AMS, LPS) were taken from Davidson and Watson (PEG precipitation method verified by isoenzyme electrophoresis method), reference range %PPA for prolactin was 0-40 %. Ranges of %PPA are missing for hs-cTnT in the literature.

RESULTS
During a period of 16 months we investigated 36 asymptomatic patients with suspected macro-complexes: macro-CK (19 pts), macro-AST (13), macro-AMS (2), macro-LPS (2), macro-GGT (1), macro-hsTnT (1) and macro-PRL (1). We confirmed 4 cases of macro-AST and 3-cases of macro-CK activity, with %PPA for macro-AST of 92, 79, 96, and 60 % (cut-off for confirmation at least 53 %) and 42, 52, and 42 % for macro-CK (cut-off at least 37 %). % PPA of prolactin in patient and control serum was similar (27 and 28 %, reference range 0-40 %). Repeated measurements of patient and control samples revealed no significant changes in case of suspected macro-troponin.

CONCLUSIONS
Simple and inexpensive detection of macro-complexes with polyethylene-glycol precipitation can aid in the differential diagnosis to prevent diagnostic errors.

Clin Chem Lab Med 2017; 55, Special Suppl, pp S1 – S1121, June 2017 • Copyright © by Walter de Gruyter • Berlin • Boston

Rare Diseases

Cod: W296

EFFECTS OF CARBOHYDRATE NUTRIENTS ON OXIDATIVE STRESS AND ARGININOSUCCINATE SYNTHETASE

H. Lin [1], J. Liao [1], S. Shiesh [1]

[1]*Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, Tainan*

(Taiwan, Republic of China)
jessielinsy@gmail.com

Argininosuccinate synthetase (ASS), catalyzing the synthesis of argininosuccinate from aspartate and citrulline, plays an important role in urea cycle and the synthesis of nitric oxide and arginine. Citrullinemia is an autosomal recessive urea cycle disorder, caused by defective ASS or citrin. Carbohydrate uptake exacerbates the symptoms of type II citrullinemia and leads to early death. Nutrient stress such as galactose or low glucose was shown to enhance oxidative metabolism and mitochondrial dysfunction, indicating carbohydrates may play an important role in the pathogenesis of citrin deficiency. Therefore, this study was aimed to investigate the effects of carbohydrate nutrients on oxidative stress, mitochondrial membrane potential (MMP) and cytosolic ASS levels in hepatocytes, and to investigate whether antioxidants would alleviate these changes. AML12 hepatocytes were cultured in DMEM/F12 medium with different carbohydrate nutrients, including 18 mM glucose, 13.5 mM glucose, 9 mM glucose, 9 mM glucose plus 9 mM galactose and 9 mM glucose plus 9 mM fructose. Oxidative stress and MMP#were assessed by MitoSOX Red mitochondrial superoxide indicator and JC-1dye respectively, using flow cytometry. Mitochondrial superoxide production was increased in AML12 cells treated with 9 mM and 13.5 mM glucose. Decreased MMP and ASS protein levels were found in AML12 cells cultured with low concentration of glucose. Fructose and galactose treatments also lead to increased mitochondrial superoxide production, decreased MMP and lowered ASS protein levels in AML12 cells. Cells cultured in glucose–insufficiency conditions had increased mitochondrial oxidative stress and decreased ASS levels in hepatocytes with disrupted MMP. Cells cultured in 13.5 mM glucose-containing medium, the treatment of N-acetylcysteine or pyruvate alleviated oxidative stress and MMP in hepatocytes cultured. In conclusion, carbohydrate nutrients are related to oxidative stress and ASS expression in vitro.

Clin Chem Lab Med 2017; 55, Special Suppl, pp S1 – S1121, June 2017 • Copyright © by Walter de Gruyter • Berlin • Boston

Rare Diseases

Cod: W297

CLINICAL AND GENETIC EVALUATION OF 9 NEW OSTEOPOROSIS-PSEUDOGLIOMA (OPPG) CASES ASSOCIATED WITH A LRP5 MUTATION. RESPONSE TO TREATMENT

A. Papadopoulou [1], I. Papadopoulos [1], E. Bountouvi [1], E. Gole [1], A. Dinopoulos [1], V. Papaevangelou [1], A. Attilakos [1]

[1] *3rd Department of Pediatrics, Medical School, University General Hospital "ATTIKON", Athens, Greece*

(Greece)
a_papado@yahoo.com

Background
Osteoporosis-pseudoglioma syndrome (OPPG) is a rare autosomal recessive disorder characterized by impaired bone accrual and defective regression of fetal ocular fibrovascular system (pseudoglioma). Loss of function mutations in the LRP5 gene, either in homozygous or compound heterozygous state, are established as the genetic defect of the disease. Here, we report the clinical and genetic investigation of 9 new OPPG cases.

Methods
Nine patients and 40 members of their families were admitted at the 3rd Department of Pediatrics at Attikon University General Hospital. The clinical examination of the patients included the evaluation of somatometric and ophtalmological features, musculoskeletal and psychomotor development. Bone mineral density was measured in all patients. Biochemical analysis included calcium homeostasis, lipid profile, fasting glucose levels, glycosylated hemoglobin and serum seroronin. DNA was isolated and genotyping of the entire LRP5 gene was performed. Total RNA was extracted from peripheral blood of two patients.

Results
All participants belonged to a Roma tribe, residing in Athens. The age of each patient at first evaluation varied from 6 months to 9 years. Mutational analysis of the LRP5 gene led to the identification of the c.2409_2503+79del mutation (G804_G835delfsX49) expected to lead to a truncated protein (853 aminoacids) compared to the normal protein of 1615 aa. The mean duration of patients' follow up was 2,86 years. All patients had congenital ocular impairment while 6 of them presented with severe osteoporosis. Carriers of the OPPG syndrome had normal vision, with no history of fractures. Cognitive function and mental development were notably impaired in patients. Biochemical analysis for calcium homeostasis, lipid profile, fasting glucose levels were within normal range. 25(OH)D levels were low, while serum serotonin was elevated in all affected probands. All patients were daily treated with 800 IU Vitamin D3 and oral calcium.

Conclusions
Molecular analysis of the LRP5 gene in the extended kindred revealed 9 homozygous OPPG patients and a high prevalence of carriers (60%) of the mutation c.2409_2503+79del mutation (G804_G835delfsX49). Bisphosphonate treatment, administrated to three patients, lead to pain recession and BMD improvement, though two of them did sustain fracture under therapy.