Inherited disorders, metabolic disorders, rare diseases

M411

RECURRENT SEIZURES IN A CHILD DUE TO HYPOKETOTIC HYPOGLYCAEMIA

B. Chale-matsau[1], T. Pillay[1]
[1]*Department of Chemical Pathology, Faculty of Health Sciences, School of Medicine, University of Pretoria & National Health Laboratory Services, Tshwane Academic Division, Pretoria, South Africa*

BACKGROUND-AIM

IEMs are individually rare, but collectively common. The age of presentation is variable, and it is usually patients presenting at post neonatal ages that tend to pose a diagnostic dilemma. Fatty acid oxidation disorders are inherited in an autosomal recessive manner, usually manifesting in a previously healthy infant when the transition is made from regular frequent feeding to prolonged fasting during sleep. The aim of this presentation is to demonstrate an unusual presentation of an infant with an inorn error of metabolism.

METHODS

We report a case of an 11 year old child, who as an infant developed recurrent early morning seizures from the age of 5 months and these were associated with hypoketotic hypoglycaemia. Hypoketotic hypoglycaemia is pathognomonic of a fatty acid oxidation disorder. As the infant had a significant family history of epilepsy, the child had since been managed on antiepileptic treatment which included sodium valproate.At age 11 the child was referred to a tertiary centre for investigation. The child also had features of developmental delay.

RESULTS

The presenting blood tests revealed significant hyperammonaemia213 (RI: 40 - 80 µmol/L), mildly elevated lactate 2.8 (RI: 0.5 - 2.2 mmol/L) with a normal anion gap. Serial glucose monitoring using a point of care instrument (Accuchek) revealed recurrent hypoglycaemic episodes. Hyperinsulinism was excluded (Insulin 1.4; RI: 1.9 - 23 mIU/L). A provisional diagnosis of IEM was made, and a metabolic profile screen ordered. Urine organic acid screening did not show organic aciduria and there was no evidence of an amino acidopathy. The patient was managed on protein restricted diet, phenobarbital and L-Carnitine therapy. Regular dextrose water was given through NG tube to prevent hypoglycaemia. The patient improved significantly on this treatment with a declining trend in ammonia levels.

CONCLUSION

This case report highlights the risk of misdiagnosis in patients with IEM as a result of the variable presenting features of these syndromes.

Inherited disorders, metabolic disorders, rare diseases

M412

A FAMILY WITH HIGH LEVELS OF PHENYLALANINE

T. Enkhjargal[1]
[1]*National Center for Public Health, Ulaanbaatar, Mongolia*

BACKGROUND-AIM

It is reported that 1% of the Mongolian population suffer from oligophrenia. One of the causes of the disease might be an inherited disorder of amino acid metabolism. The obligatory system of screening analyses for amino acid disturbances in newborns is implemented in most countries. In order to decide whether there is a necessity to introduce this system in Mongolia, assessment of the status of inherited disorders of amino acid metabolism in the population is needed.

METHODS

Screening analysis of 6,416 urine samples of healthy individuals and the population at risk was carried out using the paper chromatography method. Urine and blood samples of the patients with potential disturbances of amino acid metabolism, as well as samples of their parents and siblings, were quantified using the high performance liquid chromatography system.

RESULTS

Numbers of disturbances of amino acid metabolism of benign and transitory character were detected. The disturbances were mostly caused by medications, diet and changes in the transport mechanism of amino acids in the kidneys.
A very high urinary concentration of phenylalanine (1,985.25 μmol/L) was detected in a 15-year-old girl diagnosed with a mental disability. Her blood level of Phe was also increased (221.42 μmol/L). The girl was born full term after an uncomplicated pregnancy and attended a special school for children with mental disabilities. Her mother also showed low mental development with the elevated levels of Phe in her urine (2,227.29 μmol/L) and blood (154.23 μmol/L). High concentrations of Phe were detected in urine samples of the patient's two younger brothers (1,608.61 μmol/L and 2,136.79 μmol/L), two younger sisters (1,789.45 μmol/L and 1,815.67 μmol/L) and an older brother (2,124.92 μmol/L) who all had mental disabilities.

CONCLUSION

The fact that it was too late to start appropriate treatment for the girl and her siblings, urges the necessity of preventive screening analyses of newborns for timely identification and treatment of affected individuals.

Inherited disorders, metabolic disorders, rare diseases

M413

THE P.I244T MUTATION ASSOCIATED WITH PRIMARY HYPEROXALURIA TYPE1: A TUNISIAN EXPERIENCE

s. Mdimegh[1], a. Omezzine [1], i. Mbarek[1], s. Mabrouk[3], d. Zellama[2], a. Moussa[1], o. Achour[1], n. Zayani[1], n. Ben Rjeb[1], a. Achour[2], s. Abroug[3], a. Bouslama [1]
[1]*Biochemistry Departement, Sahloul University Hospital, LR12SP11*
[2]*Nephrology Department, Sahloul University Hospital*
[3]*Pediatric Department, Sahloul University Hospital*

BACKGROUND-AIM

Primary Hyperoxaluria Type I (PH1) is an autosomal recessive metabolic disorder caused by inherited mutations in the AGXT gene encoding liver peroxisomal alanine:glyoxylate aminotransferase (AGT) which is deficient or mistargeted to mitochondria. PH1 shows considerable phenotypic and genotypic heterogeneity. The incidence and severity of PH1 varies in different geographic regions. It is much prevalent in Mediterranean countries. In Tunisia, the I244T mutation is considered as the first cause of PH1.it is present with a 31% frequency .This aim of this study was to analyze the clinical features in PH 1 patients, who have detected the p.I244T mutations, and to establish a possible association between genotype and phenotype

METHODS

We present a retrospective study of 51 Patients who have been diagnosed with PH1, carriers of I244T AGXT mutations. Ten patients, sibling of confirmed PH1 patients were included in the analysis. The clinical data were compiled and genetic testing was done by determining AGXT haplotype (Minor or Major) and I244T mutation analysis using PCR/ RFLP.

RESULTS

The I244T mutation was found in 51 patients, co segregates with the Minor allele. At diagnosis, 20% of these patients were asymptomatic vs.80% of index patients. 40% of them were diagnosed at an adult age. The I244T mutation has been associated with various renal symptoms (45%). Renal manifestations were 35% urolithiasis, 25% nephrocalocinosis and 15% both. Onset of symptoms occurred early with age at onset of symptoms was 3, 25 years (range 0, 1-33 years). The median age of disease detection was 13 years (range 0, 2-50 years). ESRD was reached in 50% homozygous patients. Renal function was preserved over time in all ten patients identified by family screening. For the relationships between a genotype and clinical phenotype, we marked differences in age at diagnosis in carriers the I244T mutation were very higher, also for age at onset of symptoms. We showed that the majority of patient carrying this variation were in infantile form, and with a variable progression to kidney failure. Systemic oxalosis was extremely severe in most patients.

CONCLUSION

In our study, molecular analysis showed an extreme phenotypic heterogeneity ranged from ESRD in infancy to late onset form with occasional stones diagnosed in adulthood to normal renal function in asymptomatic ones. Others genetic and /or environmental factors play a role in determining the ultimate phenotype.

Inherited disorders, metabolic disorders, rare diseases

M414

MUTATION ANALYSIS OF THE FGF23 GENE IN SOUTH AFRICAN PATIENTS WITH HEREDITARY HYPOPHOSPHATAEMIC RICKETS

E. Pretorius[1], N. Oosthuizen[2], J. Pettifor[4], K. Thandrayen[4], G. Van Biljon[3], C. Van Niekerk[2]

[1]*Department of Chemical Pathology, University of Pretoria, Pretoria*

[2]*Department of Chemical Pathology, University of Pretoria/National Health Laboratory Services, Tshwane Academic Division, Pretoria*

[3]*Department of Paediatrics, University of Pretoria/Steve Biko Academic Hospital, Pretoria*

[4]*Mineral Metabolism Research Unit, Department of Paediatrics, University of the Witwatersrand/Chris Hani Baragwanath Hospital, Johannesburg*

BACKGROUND-AIM

Inherited hypophosphataemic rickets (HR) includes X-linked, autosomal dominant (ADHR), autosomal recessive and hypercalciuric forms. Dysregulation of fibroblast growth factor 23 (FGF23) is central to disease pathogenesis in all except the hypercalciuric variant. ADHR is unique in that it shows variable penetrance, delayed onset and a waxing/waning clinical course that has recently been linked to iron homeostasis. ADHR is commonly caused by four mutations in the FGF23 gene, each leading to substitution of either tryptophan or glutamine for arginine at position 176 or 179. These changes at the consensus cleavage site impart resistance to cleavage, resulting in elevated FGF23 levels. The aim of our study was to determine the frequency and types of FGF23 gene mutations in South African patients with HR.

METHODS

DNA was extracted from whole blood of 76 patients (including familial and sporadic cases) and 97 controls. All three exons of FGF23 and flanking intronic regions were amplified before screening by high-resolution melting curve analysis. Amplicons identified as variants were sequenced.

RESULTS

Although no variations were detected in the first and second exons, sequencing revealed variations in the third exon in 12 patients. Only one of these patients harboured the typical R179Q mutation. This patient also had a cited synonymous polymorphism, c.423G>A or T (p.A141A), which was present in five other patients. One patient had a novel missense variation, c.550G>A (p.D184N), the clinical significance of which has yet to be established. The remaining five patients had a cited polymorphism c.716C>T (p.T239M). Studies have reported higher urinary phosphate excretion, and lower serum phosphate and parathyroid hormone levels in subjects with T239M compared to the wild-type, suggesting that it is a functional allelic variant.

CONCLUSION

In this first mutational analysis of the FGF23 gene in a South African cohort of HR patients, ADHR was rare, in keeping with findings from international studies. One known mutation (R179Q) and one novel possible mutation (D184N) was identified. In patients with the T239M functional allelic variant, further investigation of other HR genes and iron status may be informative.

Inherited disorders, metabolic disorders, rare diseases

M415

A CASE OF IGG-λ/IGA-κ BICLONAL GAMMOPATHY WITH ABNORMAL FLC RATIO IN A PATIENT WITH POEMS SYNDROME

K.E. Song[2], J.Y. Ham[2], G.Y. Ha[1]
[1]*Department of Laboratory Medicine, Dongguk University Gyeongju Hospital*
[2]*Department of Laboratory Medicine, Kyungpook National University Medical Center*

BACKGROUND-AIM

POEMS syndrome is a rare multisystem disorder that includes polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes. Among them, polyneuropathy and monoclonal plasma cell-proliferative disorder are two mandatory major criteria of POEMS syndrome. Nearly all patients of POEMS syndrome present λ-restricted monoclonal gammopathy. Authors have experienced a case of POEMS syndrome with IgG-λ/IgA-κ biclonal gammopathy with dominant κ free light chain and abnormal sFLC-R.

METHODS

A 56-year-old man who was suspected POEMS syndrome, was admitted to the department of internal medicine for further evaluation of B-cell proliferative disease.

RESULTS

Four years ago, he had an operation of wide wedge resection of right middle lobe of lung and mediastinal lymph node dissection due to Castleman's disease. Since then, he has complained sustained tingling sensation on both feet and disturbance of gait, in association with slurred speech and impairment of his vision. Nerve conduction studies and electrophysiological investigation resulted diffuse peripheral sensori-motor polyneuropathy with demyelinating features. He had hyperlipidemia with cholesterol of 235 mg/dL and triglyceride of 694 mg/dl, hypothyroidism with free T4 of 1.4 ng/L and TSH of 6.0 mIU/L. on his laboratory findings. He also had hepatosplenomegaly and hypertrichosis. Autoimmune laboratory results were negative and CSF protein was increased. Serum protein electrophoresis seemed normal except a very weak band at the end of gamma region, and urine protein electrophoresis had no abnormal findings. Serum immunofixation electrophoresis confirmed IgG-λ and IgA-κ biclonal gammopathy. Serum IgA quantitation result was increased (630 mg/dL), and IgG, IgM, IgD levels were within reference limit. Both serum FLC κ and λ values were increased (κ: 288 mg/dL, λ: 50 mg/dL) and κ/λ ratio was out of normal (5.76). Unfortunately in this case, serum VEGF was not checked. He has taken medications with physiotherapy, but his neurological symptoms had gradually worsened.

CONCLUSION

The finding of IgG-λ/IgA-κ biclonal gammopathy in our case was very rare, and that the patient had an abnormal sFLC ratio with dominant κ clonality was a more interesting feature.

Inherited disorders, metabolic disorders, rare diseases

M416

CASE OF A PATIENT WITH MT-TS1 NONSYNDROMIC HEARING LOSS AND AXONAL NEUROPATHY

M. Spodenkiewicz[3], D. Ploton[2], R. Garnotel[1], A. Doe De Maindreville[4]
[1]*Laboratoire de Biologie et Recherche Pédiatriques, Hôpital Maison Blanche, CHU Reims*
[2]*Laboratoire d'anatomopathologie, Hôpital Maison Blanche, CHU Reims*
[3]*Service de Génétique, Hôpital Maison Blanche, CHU de Reims*
[4]*Service de Neurologie, Hôpital Maison Blanche, CHU Reims*

BACKGROUND-AIM

We report the case of a 46-year-old man with nonsyndromic mitochondrial hearing loss and deafness. At the origin of this condition often lie pathogenic variants of mitochondrial DNA involving the MT-TS1 gene. It is characterised by childhood-onset sensorineural hearing loss.

METHODS

Our patient has had bilateral fasciculations and dysesthesia in the calf for 6 months, with no triggering factors. He has presented congenital sensorineural deafness.

RESULTS

High levels of lactic acid, high ratio of lactic acid to pyruvic acid were found during the redox cycling evaluation. The electromyogram test detects a bilateral peripheral neuropathy of the external popliteal nerves. The muscular biopsy showed non-specific abnormalities on the mitochondrial structures but cytosolic phospholipidic inclusions with electron microscopy. Molecular analysis by direct PCR revealed a homoplasmic m.7445A>G mutation.

CONCLUSION

Peripheral nervous system neuropathies are frequent features of mitochondriopathy. However, in the case of m.7445A>G mutation it is not possible to determine whether the axonal neuropathy is related to that mutation. Our patient could be possibly carrier of more than one metabolic disorder?

Inherited disorders, metabolic disorders, rare diseases

M417

EVALUATION OF A NEW COMMERCIAL SOLUTION FOR NEW BORN SCREENING AND COMPARISONS WITH ESTABLISHED METHODS

D. Blake[2], A. Paccou[1]
[1]*ABSCIEX, France*
[2]*ABSCIEX, Warrington, UK*

BACKGROUND-AIM

LC-MS/MS is a powerful tool for the study of metabolic disorders. The simultaneous analysis of amino acid and acylcarnitine panels can provide information on over 40 metabolic disorders such as Phenylketonuria (PKU) and Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCAD). We present here a discussion of the results obtained using a new commercially available kit for the LC-MS/MS analysis of these compounds, with performance comparison and evaluation with existing commercial kits on the market today.

METHODS

Dried Blood Spot (DBS) samples were analysed using an established commercially available method. In summary the method consisted of an addition of two extraction solutions (containing internal standards) to a 3mm punched DBS, a simple mix and equilibration, followed by direct injection of the supernatant onto the LC-MS/MS System.
The exact same samples were analysed using the proposed new commercial kit, briefly comprising of the addition of a single extraction solution (containing internal standards), a brief mix and injection of the supernatant

RESULTS

Samples were analysed as above and mean percentage bias values between kits were generated for analytes in question. The established kit data was used as a baseline and results generated by the new commercial kit were compared to this data.
Examples of percentage difference in calculated concentration for selected individual compounds:
Alanine 15.9 C3 15.2
Arginine 10.2 C4 7.4
Leucine 3.7 C5 2.7
Tyrosine 3.1 C14 9.7

CONCLUSION

It has been shown that for the majority of compounds analysed differences in calculated concentration between kits evaluated is <16%. For many analytes this bias is <10%.
The new commercial kit offers therefore a high performance and simple to use alternative to established kit methods currently utilized.

Inherited disorders, metabolic disorders, rare diseases

M418

TRIPLEX TANDEM MASS SPECTROMETRY ASSAYS FOR SCREENING OF 3 LYSOSOMAL STORAGE DISORDERS IN A KOREAN POPULATION

S.E. Cho[1], J.R. Kwak[1], H. Lee[1], D.H. Seo[1], J. Song[2]
[1]*Labgenomics Clinical Laboratories, Korea*
[2]*Seoul National University Bundang Hospital, Department of Laboratory Medicine, Seoul National University College of Medicine, Korea*

BACKGROUND-AIM

We evaluated the performance of triplex tandem mass spectrometry (MS/MS) assays using dried blood spots for screening of 3 lysosomal storage disorders (LSDs), namely, Pompe, Fabry, and Gaucher diseases.

METHODS

Chromatographic separation was completed using mobile phase involving water-formic acid and acetonitrile-formic acid over 2.3 min of run time on a column with Acquity UPLC CSH C18 column (Waters, USA). The detection of column effluent was performed using TQD triple quadrupole mass spectrometer (Waters, USA) in the multiple-reaction-monitoring mode. We evaluated the precisions of 3 enzyme assays (acid alpha glucosidase, acid alpha galactosidase, acid beta glucocerebrosidase) at four activity levels (base, low, medium, and high). We evaluated the linearity, limit of detection, recovery, carryover, and ion suppression. We analyzed the 3 enzyme activities in 376 anonymous newborn dried blood spots (DBS). Control materials were provided from Centers for Disease Control and Prevention (CDC).

RESULTS

Intra- and inter-assay precisions were between 0 % and 14.1 %, between 0 % and 18.9 %, respectively, for 3 enzyme activities. The linearity of each enzyme activity was good (R2=0.9952, 0.9982, 0.9974, respectively). The lower limit of detection was 0.79 umol/h/L, 0.39 umol/h/L, 0.22 umol/h/L, respectively. The recovery was 102.65 %, 101.52 %, 103.50 %, respectively. Carryover was 0 %, -0.14 %, 0.39 %, respectively. There was no ion suppression. Data from 376 anonymous newborn DBS showed an approximate bell-shaped distribution of enzymatic activities (median values were 16.02 umol/h/L, 6.61 umol/h/L, 26.82 umol/h/L, respectively).

CONCLUSION

The performance of triplex tandem mass spectrometry assays for screening of 3 lysosomal storage disorders using dried blood spots was generally acceptable in a Korean population.

Inherited disorders, metabolic disorders, rare diseases

M419

EVALUATION OF URINE PERFORMANCE ON THE VITROS® CHLORIDE MICROSLIDE ASSAY

S. Danielson[1], T. Grupp[1], J. Ramerman[1]
[1]*Ortho Clinical Diagnostics, Rochester, NY 14626*

BACKGROUND-AIM

VITROS Chemistry Products Chloride (Cl-) Slides quantitatively measure chloride (Cl-) concentration in serum and plasma using VITROS 250/350/950/5,1FS/4600 and VITROS 5600 Integrated System. The VITROS Cl- slide is a multilayered, analytical element coated on a polyester support that utilizes direct potentiometry for measurement of chloride ions.1 Chloride is an essential electrolyte, and testing in urine is conducted to determine if there is an electrolyte imbalance. Testing is especially important in cases of persistent metabolic alkalosis where measured urine chloride levels are low.

METHODS

We evaluated the accuracy of 81 patient urine samples (11 – 195 mmol/L) and 7 commercial Urine linearity fluids (1 – 316 mmol/L) diluted 1:1 with the VITROS Calibrator Kit 2, Level 1 on the VITROS 5,1 System compared to two commercial methods: titration using a Corning 926S Chloridometer and indirect potentiometry with the Chloride assay on the Siemen's ADVIA 1800 Chemistry System.

RESULTS

The VITROS Chloride assay showed excellent correlation with both methods. VITROS 5,1= 0.989*Corning 926S + 3.08; (r) = 0.999 and VITROS 5,1 = 1.001* ADVIA 1800 + 1.68; (r) = 0.997. Accuracy was also evaluated for 100 low chloride urine patient samples (5 – 50 mmol/L) run undiluted on the VITROS 5,1FS analyzer compared to the Siemen's ADVIA 1800 assay. The VITROS Chloride assay showed comparable correlation to the ADVIA 1800 assay as was observed in the previous assessment; VITROS 5,1 = 1.053* ADVIA 1800 – 4.03; (r) = 0.987. A 5-day precision study conducted on the VITROS 350 and 5600 with undiluted and diluted samples showed excellent precision with undiluted samples on both chemistry systems. Mean Chloride concentrations of 3.70 mmol/L, 9.99 mmol/L, 32.5 mmol/L, 97.1 mmol/L and 315.4 mmol/L resulted in within-laboratory percent coefficient of variation (%CV) of 2.0% 0.81 %, 0.60%, 0.42%, and 0.67% respectively on the VITROS 5600 system.

CONCLUSION

The VITROS Chloride assay has exhibited good correlation with urine across a broad measuring range compared to commercial titration and indirect potentiometry methods. In addition excellent precision has been observed on the VTIROS 350, 5,1, and 5600 systems with undiluted urine specimens.
1 The VITROS Cl- slide is not currently approved for use with urine.

Inherited disorders, metabolic disorders, rare diseases

M420

QUANTITATIVE ANALYSIS OF PLASMA CHOLESTANE-3BETA,5ALPHA,6BETA-TRIOL AND 7-KETOCHOLESTEROL BY MASS SPECTROMETRY–LIQUID CHROMATOGRAPHY FOR THE DIAGNOSIS OF NIEMANN-PIC TYPE C

A. Mate De Gerando[2], Y. Najar[1], S. Lam[4], D. Bonnefont-rousselot[4], C. Jardel[4], F. Lamari[3]
[1]*Department of Neurology, Pitié-Salpëtrière University Hospital - Assistance Publique-Hôpitaux de Paris*
[2]*Metabolic Biochemistry, Pitié-Salpêtrière University Hospital - Assistance Publique-Hôpitaux de Paris*
[3]*Neurometabolic Unit-Pitié-Salpëtrière University Hospital - Assistance Publique-Hôpitaux de Paris*
[4]*Pitié-Salpëtrière University Hospital - Assistance Publique-Hôpitaux de Paris*

BACKGROUND-AIM

Niemann-Pick type C (NPC) is a rare inherited error of metabolism (IEM) in which the intracellular trafficking of cholesterol, is altered, leading to the accumulation of unesterified cholesterol in the late endosome/lysosome. Until recently, the diagnosis still based on the filipin test requiring the invasive skin biopsy and cultured fibroblasts. Recently, two oxysterols, cholestane-3ß,5α,6ß-triol (3ß,5α,6ß-Triol) and 7-Ketocholesterol (7-KC), have been reported as a sensitive and specific markers for the diagnosis of NPC.

METHODS

In the present study we described a simple, sensitive, and specific liquid chromatography-electrospray tandem mass spectrometry (LC-MS/MS) method for the determination of 3ß,5α,6ß-Triol and 7-KC in human plasma. In order to enhance the spectrometric detection, 3ß,5α,6ß-Triol and 7-KC were first converted into the corresponding picolinyl-esters derivatives.

RESULTS

The percent recovery of spiked plasma was close to 99% and the method is linear in the range 15 to 2000 ng/ml, which is completely adequate to the patho-physiological interval of values. Intra-assay imprecision is 5.4% for 3ß,5α,6ß-Triol 3.2% for 7 KC. The inter-assay imprecision is 7.7% for 3ß,5α,6ß-Triol and 13.5% for 7KC. The method was used to measure unesterified 3ß,5α,6ß-Triol and 7-KC in plasma from 8 NPC and 18 controls subjects. The results confirms an increased 3ß,5α,6ß-Triol l and 7-KC in NPC subjects (3ß,5α,6ß-Triol = 447.9 + 235 nmol/, $p<0.0001$; and 7-KC = 554.2 + 365.8 nmol/l, $p<0.0001$) compared to control subjects (3ß,5α,6ß-Triol = 18.9 + 9.4 nomol/l, $p>0.0001$; 7-KC = 12.7 + 11.1 noml/l, $p<0.0001$).

CONCLUSION

In conclusion, LC-MS/MS is a simple and rapid technique for the quantification of triol and 7KC in human plasma and a sensitive and specific method for NPC screening.

Inherited disorders, metabolic disorders, rare diseases

M421

REFERENCE INTERVALS FOR URINARY PORPHYRINS AND PORPHOBILINOGEN DERIVED FROM AN AMERICAN LABORATORY DATABASE

H.N. Signorelli[2], L. Mccord[1], E.L. Frank[2]
[1]*Analytic Biochemistry Laboratory, ARUP Laboratories, Salt Lake City, UT, USA*
[2]*Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT, USA*

BACKGROUND-AIM

The porphyrias, a group of rare diseases associated with defects in heme biosynthesis, can be evaluated by measuring porphyrin compounds in body fluids. To aid in the diagnosis and management of porphyria, we determined reference intervals (RIs) for urinary porphobilinogen (PBG) and porphyrins by analyzing results from our laboratory database.

METHODS

Results for consecutive assays performed between 01 December 2010 and 30 November 2014 were extracted from the database. PBG, uroporphyrin (Uro), heptacarboxylate porphyrin (Hepta), coproporphyrin I (Copro I), and coproporphyrin III (Copro III) concentrations from a single submission for patients of known age and gender were grouped by collection type (random or timed). Outliers were removed and RIs were calculated for adults and children using an indirect Hoffmann method. For each dataset, the cumulative frequency distribution was determined, regression over the linear portion of the distribution was performed, and reference limits were calculated from the regression equation at 2.5% and 97.5%. RIs were expressed as a ratio to creatinine (CRT) for random samples and as excretion per day (d) for 24 hour (h) collections.

RESULTS

For the four year period, 21,081 assays met study criteria. Samples were from adults (9711 F, 9380 M) aged 18 to 97 years at the time of testing and children (1058 F, 932 M) younger than 18 years of age. Values from 12,034 random urine specimens (10, 571 adult; 1463 pediatric) and 9047 timed (24h) collections (8520 adult; 527 pediatric) were assessed. Random and 24h PBG RIs were determined for adults: 0.4-1.2 mmol/mol CRT, 3.0-7.6 μmol/d; and children: 0.1-0.7 mmol/mol CRT; 3.1-4.7 μmol/d. Random porphyrin RIs were (adult) Uro <2.1, Hepta <0.7, Copro I <5.0, Copro III <12.2 μmol/mol CRT and (pediatric) Uro <1.8, Hepta <0.4, Copro I <4.3, Copro III <13.2 μmol/mol CRT. Porphyrin excretion RIs were (adult female) Uro <19.0, Hepta <5.9, Copro I 6.8-42.1, Copro III 6.4-121.3 nmol/d; (adult male) Uro <25.9, Hepta <8.0, Copro I 7.0-84.5, Copro III 6.0-166.6 nmol/d; and (pediatric) Uro <13.0, Hepta <3.8, Copro I 5.1-31.7, Copro III 13.0-90.2 nmol/d.

CONCLUSION

Urinary PBG and porphyrin RIs calculated from stored laboratory data were comparable to published values and consistent with RIs in current use at our institution.

Inherited disorders, metabolic disorders, rare diseases

M422

GLUCOCEREBROSIDASE ACTIVITY IN CD19+ LEUKOCYTE IN GAUCHER DISEASE AS A TARGET FOR TREATMENT STRATEGY

M. Dondurmaci[1], S. Kalkan Ucar[2], M. Coker[2], E. Canda[2], m. Kose[2]
[1]*DEPARTMENT OF MEDICAL BIOCHEMISTRY, EGE UNIVERSITY FACULTY OF MEDICINE IZMIR*
[2]*DEPARTMENT OF PEDIATRIC METABOLISM, EGE UNIVERSITY FACULTY OF MEDICINE IZMIR*

BACKGROUND-AIM

Gaucher disease (GD) is an autosomal recessive lysosomal storage disease caused by insufficient glucocerebrosidase activity and the resultant accumulation of glucosylceramide particularly in white blood cells, most often macrophages. Recently it has been shown that Gaucher patients have higher risk for Multiple myeloma and B cell lymphoma compared to healthy subjects. Our previous data suggested an increase in percent of CD19 antigen presenting leucocytes of patients with Gaucher disease. B-lymphocyte antigen CD19 (also known as CD19) is expressed on B cells from earliest recognizable B-lineage cells during development to B-cell blasts and this protein has been used to diagnose cancers that arise from this type of cell - notably B-cell lymphomas. We determined the glucocerebrosidase activities in leucocytes having different specific cell surface antigens (CD33, CD19, CD14 and CD8) in order to investigate any relationship between the clinical symptoms of Gaucher disease and the enzyme activities.

METHODS

We collected blood samples from 11 Gaucher type I patients, four males and seven females, age range, 3–27 years. 20 age matched healthy controls were included. The leukocytes were seperated manually by the use of a Ficoll gradient and the leukocytes were stained with dyes for different specific cell surface antigens (CD33, CD19, CD14 and CD8) and sorted out by flow cytometry. Glucocerebrosidase enzyme activity were determined fluorometrically in these leucocytes.

RESULTS

Our data show that percent of CD19 (+) leukocytes in GD patients were significantly higher compared to the control group ($p<0.01$). The glucocerebrosidase activities in leucocytes (positive for CD33, CD14 and CD8) samples of Gaucher patients were slightly lower than healthy controls, CD19(+) leucocytes have one third of enzyme activity compared to those of controls (9047 nmol/h/mg protein versus 3493 nmol(h/mg protein).

CONCLUSION

In conclusion we suggest that the number of CD19 antigen presenting cell and the enzyme activity in this type of leucocytes might be useful as a marker in diagnosing and monitoring of Gacuher disease and also as a target of innovative treatment strategies.

Inherited disorders, metabolic disorders, rare diseases

M423

A CASE OF KEARN-SAYRE SYNDROME WITH SEVERE CEREBRAL FOLATE DEFICIENCY

M. Spodenkiewicz[2], L. Daelmann[3], R. Garnotel [1], A. Tourbah[3]
[1]*Laboratoire de Biologie et Recherche Pédiatriques, Hôpital Maison Blanche, CHU Reims*
[2]*Service de Génétique, Hôpital Maison Blanche, CHU de Reims*
[3]*Service de Neurologie, Hôpital Maison Blanche, CHU Reims*

BACKGROUND-AIM

We report a case of a 43-year-old man with Kearn-Sayre syndrome (KSS). KSS is a mitochondrial DNA deletion syndrome. In some patients with KSS, an energetic defect associated with the accumulation of mutated mitochondrial DNA copies in the plexus choroid cells impairs its ability to transport 5-methyltetrahydrofolate (5MTHF) from the blood to the CSF, thus leading to a severe decrease of 5MTHF in the CSF.

METHODS

The patient has presented a deterioration of walking and cerebellar syndrome with dysathria for 6 years. He had an evolutive atrophic retinitis pigmentosa, bilateral ophtalomoplegia and ptosis since the age of 18, associated with presbycusis during last 2 years.

RESULTS

The vitaminic blood assessment found normal levels of acid folinic in the blood but a severe deficiency of 5MTHF in the CSF (5MTHF: 0 nmol/L - reference value: 200-1000 nmol/L) accompanied with a high CSF protein content (1447 mg/L – reference value: 150-450 mg/L). The electrocardiogram reveals a right bundle branch block with left anterior hemiblock. Magnetic resonance imaging (MRI) of the brain showed periventricular and cerebellar leukoencephalopathy.
Analysis of mtDNA on long PCR showed a unique band < 13kB (nucleotids 3214F-16146B) and a unique band <15 kB (nucleotids 15698F-14861B).

CONCLUSION

The patient was treated with folinic acid 90 mg per day (for one year) and slightly improved his walking performance. This strengthens the hypothesis that the treatement of KSS with (high-dose) folinic acid seems to be advisable for the therapy of KSS with decresed 5MTFHR CSF levels.

Inherited disorders, metabolic disorders, rare diseases

M424

THE FIRST SCREENING RESULTS OF SIX LYSOSOMAL STORAGE DISORDERS BY USING A HPLC-MS/MS MULTIPLEX ASSAY IN TURKEY

H. Akbas[1], E. Soyucen[1], G. Yucel[1]
[1]*University of Akdeniz*

BACKGROUND-AIM

Mass spectrometry has been used for the diagnose of lysosomal storage disorders (LSD) such as Pompe, Fabry, Gaucher, Krabbe, Niemann-Pick A/B and mucopolysaccharidosis I in dried blood spots (DBS). Diminished enzyme activities can be simultaneously evaluated by MS/MS determination of the products obtained after incubation with specific substrates. In this study we aimed to investigate a HPLC-MS/MS method for multiplex screening of LSDs in dried blood spots in Turkey.

METHODS

Dried blood spots (3.2-mm) were incubated for 20 h with cocktails containing substrates and internal standards. We determined the resulting product and internal standard using LC-MS/MS (Shimadzu 8030 Triple Quadrupole Liquid Chromatograph Mass Spectrometer, Shimadzu Scientific Instruments, Japan). The method did not require offline sample preparation such as liquid-liquid and solid-phase extraction. Between- and within-run imprecision, carryover, limits of detection and quantification were determined. We also analyzed CDC QC samples and 10 samples from patients with known LSDs.

RESULTS

A total of 450 dried blood samples were analyzed for the lysosomal α-glucosidase, β-glucocerebrosidase, α-galactosidase, acid sphingomyelinase, galactocerebrosidase, and α-L-iduronidase activities. Affected patient's enzyme activities were found as significantly lower. Carryover were not observed, whereas between -run and within-run imprecision were <10%.

CONCLUSION

Our data shown that the mass spectrometric techniques can be easily used for the screening of lysosomal storage diseases which presents remarkable technical advantages compared with traditional methods. This method allows to significant decreases in sample preparation and analytical times and reagent costs. The screening for several LSDs simultaneously is appropriate for use in high-throughput screening laboratories.

Inherited disorders, metabolic disorders, rare diseases

M425

SCREENING FOR ALPHA-1 ANTITRYPSIN DEFICIENCY USING DRIED BLOOD SPOTS

C.C. Colette[1], Z. Farid[2], O. Marie-françoise[2], B. Malika[2]
[1]*Laboratoire d'immunologie Centre Hospitalo Universitaire Lyon Sud*
[2]*Laboratoire de biochimie et biologie moléculaire Centre Hospitalier Regional Universitaire Lille*

BACKGROUND-AIM

Alpha- 1 Antitrypsin (A1AT) deficiency is a genetic disorder resulting in low levels of serum A1AT. It is associated with lung deteriorations and/or liver injuries and significantly underdiagnosed. Facilitating an earlier diagnosis of this deficiency might allow a better management of the lung disease. We have thus developed and standardized within three French laboratories a method for quantifying and phenotyping A1AT extracted from dried blood spots (DBS).

METHODS

DBS were prepared by spotting EDTA-anticoagulated whole blood on a filter paper which was air dried and stored until use. Paper disks were punched from the blood spots and eluted with water for the measurement of A1AT levels and glycine 1M pH 7.4 buffer, for the phenotyping. Automated immunonephelemetric and immunoturbidimetric techniques were set up for the quantitation of low levels of A1AT. Phenotyping was performed on ready-to-use agarose gels (Hydragel 18 A1AT Isofocusing; Sebia) run on a semi-automated system (Hydrasys System™; Sebia) with a specific programme devoted to diluted samples designed by the manufacturer (Sebia).
All the results obtained with DBS within each laboratory were compared (1) to the results obtained with the corresponding plasma and (2) to the results obtained in the other laboratories. The correlation between those results was studied with linear regression analysis using Statview™ and Excel™ (Microsoft) softwares.

RESULTS

90 DBS issued from 90 patients were studied. The correlation coefficients between the concentration of A1AT in DBS and in plasma were 0.965, 0.970 and 0.953 within the 3 laboratories. The regression lines issued from the comparison between the laboratories appear to merge as one single line. So, for a target value of 0.500 g/L, the results obtained were between 0.50 and 0.54 g/L. A 100% of concordance was obtained for the interpretation of the phenotypes.

CONCLUSION

This study shows that the results obtained with DBS are highly correlated with those obtained with venous blood samples. It becomes then possible to undertake a large scale screening program of A1AT deficiency relying on a kit designed to perform a capillary blood sampling on filter paper.

Inherited disorders, metabolic disorders, rare diseases

M426

CLINICAL AND GENETIC CHARACTERIZATION OF A COHORT OF PATIENTS AFFECTED BY LAMINOPATHIES: A 5 YEARS STUDY.

C. Di Resta[6], S. Sala[2], P. Della Bella[2], S.C. Previtali[4], G. Zerbini[3], D. Lazarevic[1], M. Ferrari[6], S. Benedetti[5]
[1]*Center for Translational Genomics and Bioinformatics, San Raffaele Scientific Institute*
[2]*Department of Arrhythmology, San Raffaele Scientific Institute*
[3]*Department of Cardiovascular and Metabolic Sciences, Complications of Diabetes Unit, San Raffaele Scientific Institute*
[4]*Department of Neurology, San Raffaele Scientific Institute*
[5]*Laboratory of Clinical Molecular Biology and Cytogenetics, San Raffaele Scientific Institute*
[6]*Università Vita-Salute San Raffaele*

BACKGROUND-AIM

LMNA gene encodes for the nuclear proteins lamin A/C, that play an important role in nuclear assembly and chromatin organization. Genetic variants on this gene have been associated with several rare disorders, involving neurological and cardiac pathologies with a high risk of sudden death. To date the only treatment is the implant of a cardioverter defibrillator (ICD) to prevent the occurrence of fatal ventricular tachycardia. Moreover there are no clear guidelines for the management of asymptomatic patients because of the variable progression of disease.

METHODS

In order to study the natural history of cardiopathy and define a risk stratification protocol for ICD implant, at San Raffaele Hospital (Milan) the genetists designed a clinical protocol in collaboration with neurology and arrhythmology teams. It includes extensive cardiological examination and strict follow up of patients bearing LMNA gene mutations. To date, we have enrolled 25 patients, including familial cases, affected by laminopathies and followed for 5 years

RESULTS

We detected 17 LMNA mutations using Sanger Sequencing and 8 were novel. Most of them are missense and 3 are deletions. 10 of them were localized in the rod domain, 3 were in the Ig fold domain of the protein, 2 in the C-terminus and 1 in the N-terminus. Mutations in the rod domain and in the Ig-fold C-terminal domain may alter the surface of the lamin and the epitope for interaction with specific ligands. Age at onset of cardiac or neurological deficit was markedly different, although not significant, in patients harboring mutations in lamin rod domain vs Ig fold (14.3 vs 31.8, p=0.09). 60% of patients developed cardiac symptoms during follow up; 3 patients required cardiac transplantation and one deceased for heart failure.

CONCLUSION

We plan to extend the evaluation of possible genotype-phenotype correlations also to improve risk stratification and management of asymptomatic patients. Moreover we are performing exome sequencing studies to identify also possible modifier genes associated with intrafamilial phenotype variability. In order to increase patient number we are now collecting data from other Italian centers.

Inherited disorders, metabolic disorders, rare diseases

M427

MOLECULAR CHARACTERIZATION OF NEW ANTITHROMBIN MUTATIONS

R. Gindele[2], Á. Udvari[2], M. Speker[2], Z. Oláh[3], A. Selmeczi[3], Á. Schlammadinger[3], H. Bárdos[1], I. Komáromi[2], A. Fekete[2], G. Haramura[2], L. Muszbek[2], Z. Bereczky[2]

[1]*Department of Preventive Medicine, University of Debrecen, Debrecen, Hungary*
[2]*Division of Clinical Laboratory Science, Department of Laboratory Medicine Faculty of Medicine, University of Debrecen, Hungary*
[3]*Institute of Internal Medicine, University of Debrecen, Debrecen, Hungary*

BACKGROUND-AIM

Antithrombin (AT) deficiency is a rare but major risk factor in venous thrombosis. It is classified as type I (quantitative) and type II (qualitative) deficiency. More than 230 mutations have been described in the gene encoding AT.
Our aim was to describe the mutation spectrum of AT deficiency in the Hungarian population and to characterize three novel (p.Leu205Pro, p.Asn450Ile, p.Gly456delins-Ala_Thr) mutations causing type I AT deficiency at molecular level.

METHODS

Wild type and mutant plasmids were transfected to HEK293 cells and the expressed AT proteins were investigated in the cell media and cell lysates by ELISA and Western blotting (WB) technique. Intracellular localization of the different mutants were examined by immunofluorescent staining detected by confocal laser scanning microscopy. Structural alterations were investigated by molecular modeling.

RESULTS

AT with p.Leu205Pro mutation was detected intracellularly in the same level as wild type, however only a tiny amount of mutant AT was secreted into the medium. This mutant showed significant co-localization with the 26S proteasome. In silico experiments using 4 μs molecular dynamics simulation suggested major structural alteration.
The level of p.Asn450Ile and p.Gly456delins mutants were strongly reduced in the cell lysates and no AT was detected in the cell media.

CONCLUSION

The p.Leu205Pro mutation leads to impaired folding and secretion defect; the mutant AT retains in the 26S proteasome and subsequently suffers intracellular degradation.
The p.Asn450Ile and p.Gly456delins mutants result reduced protein synthesis.
There are different mechanisms which are able to cause AT deficiency.

Inherited disorders, metabolic disorders, rare diseases

M428

MISDIAGNOSIS OF CONGENITAL ERYTHROPOIETIC PORPHYRIA (CEP) DUE TO METHODOLOGICAL INSUFFICIENCY: A CASE REPORT OF TWO BROTHERS WITH CEP

M. Uyanik[2], E. Sertoglu[1], V. Brancaleoni[3], S. Tapan[4], I. Kurt [4], M.D. Cappellini[3]
[1]*Ankara Mevki Military Hospital, Anittepe Dispensary, Biochemistry Laboratory, Ankara, Turkey*
[2]*Corlu Military Hospital, Biochemistry Laboratory, Tekirdag, Turkey*
[3]*Fondazione IRCCS "Cà-Granda" Ospedale Maggiore Policlinico, U.O. di Medicina Interna, Milano, Italy*
[4]*Gulhane School of Medicine, Department of Medical Biochemistry, Ankara, Turkey*

BACKGROUND-AIM

The porphyrias are a group of rare, mainly inherited, disorders of heme biosynthesis, characterized by the accumulation and excessive excretion of heme precursors Here, we report two brothers, who were previously misdiagnosed as Porphyria Cutanea Tarda (PCT) relying upon the results of solvent extraction method (SEM), but after correctly diagnosed as Congenital Erythropoietic Porphyria (CEP) due to the results of high pressure liquid chromatography (HPLC) method.

METHODS

Here we report two brothers aged 37 and 40 years with photosensitive skin lesions. The older brother had previously been diagnosed as PCT by the sum of signs, symptoms and results of porphyrin fractionation analysis by SEM, at an age of 30, and was treated accordingly (phlebotomy plus chloroquine medication). Two years later, the younger one also had the same diagnosis and then treated likewise. When patients applied to our department, we performed faecal and urinary free porphyrin fractionation analyses by thin layer chromatography (TLC) and HPLC methods, along with the other porphyrin analyses. Afterwards, the definite diagnosis was validated by DNA mutation analysis of the Uroporphyrinogen III synthase (UROS) gene.

RESULTS

After analyzing the patients' samples with HPLC method, porphyrin results showed characteristic CEP patterns with high concentrations of uroporphyrin I and coproporphyrin I in urine and a high excretion of coproporphyrin I in feces. The molecular analysis of exon 9 of the UROS gene revealed the presence of the familiar missense mutation c.562G>A (p.G188R) in homozigosity (already reported before by Fortian et al. previously, as the mutation responsible for clinical and biochemical manifestation of CEP).

CONCLUSION

This kind of later onset and/or mild phenotype cases may be confused with another sort of porphyrias. Our cases clearly show that complete separation of the type I and III porphyrin isomers by HPLC is crucial for the differential diagnosis of CEP and PCT. In addition, SEM can yield misleading information and is insufficient for the definitive diagnosis of porphyrias. Thus, using SEM cautiously for only tentative diagnosis would be more appropriate due to its insufficiency in the isomer separation.

Inherited disorders, metabolic disorders, rare diseases

M429

HYDROXYMETHYLBILANE SYNTHASE GENE MUTATION ANALYSIS IN ACUTE INTERMITTENT PORPHYRIA PATIENTS

M. Uyanik[2], I. Kurt[4], V. Brancaleoni[3], E. Sertoglu[1], S. Tapan[4], M.D. Cappellini[3]
[1]*Ankara Mevki Military Hospital, Anittepe Dispensary, Biochemistry Laboratory, Ankara, Turkey*
[2]*Corlu Military Hospital, Biochemistry Laboratory, Tekirdag, Turkey*
[3]*Fondazione IRCCS "Cà-Granda" Ospedale Maggiore Policlinico, U.O. di Medicina Interna, Milano, Italy*
[4]*Gulhane School of Medicine, Department of Medical Biochemistry, Ankara, Turkey*

BACKGROUND-AIM

The porphyrias are group of diseases, each caused by inherited deficiencies (primary) or acquired inhibition (secondary) of enzymes in the heme biosynthesis process. Acute intermittent porphyria (AIP) is an autosomal dominant inherited disease caused by a decreased activity of hydroxymethylbilane synthase (HMBS), which is a result of mutation in HMBS gene. It is the most common type of acute hepatic porphyria in the world. Although it's a rare disease, if the attack goes untreated or unrecognized, it may be fatal. The key point for AIP is to avoid precipitating factors to prevent attacks. In this study, we aimed to identify HMBS gene mutations in all clinically and/or biochemically diagnosed AIP patients and scan their first-degree relatives to provide an early diagnosis of presymptomatic AIP carriers in Turkey.

METHODS

A total of 28 individual, 13 clinically and/or biochemically diagnosed as AIP and 15 symptom-free relatives were included in the current study. We performed biochemical and molecular analysis for all individuals.

RESULTS

Molecular analyses of the HMBS gene in 13 AIP patients revealed 4 previously reported mutations (89T>G, 517C>T, 1028T>C, 913-2A>G). Molecular genetic examination of 15 relatives of AIP patients from 4 families revealed 5 latent AIP gene carriers.

CONCLUSION

Molecular investigations on the family members should be applied not only for more accurate diagnosis, but also for understanding the molecular genetic heterogeneity in Turkish population.
Although this study does not add a novel mutation to those that have been previously reported, it emphasizes that molecular analysis would be very useful not only for the identification of asymptomatic gene carriers in the family but also for the detection of ancestral founders in porphyria families. Since the sudden manifestation of the disease maybe prevented by early diagnosis, identification of AIP gene carriers is the best preventive measure.

Inherited disorders, metabolic disorders, rare diseases

M430

POINT OF CARE (POCT) IN THE MANAGEMENT OF METABOLIC DISORDERS. NEAR PATIENT LIPID TESTING

L. Rossi[3], L. Della Bartola[2], G. Pellegrini[1], O. Giampietro[2], E. Matteucci[2]
[1]*Clinical Analysis Laboratory, University Hospital of Pisa*
[2]*Department of Clinical and Experimental Medicine, Pisa University, Italy*
[3]*Training Area, Direction of Technical Professions Health, University Hospital of Pisa*

BACKGROUND-AIM

POCT provides immediate results for clinical decision-making; however, quality assessment is a necessary condition to ensure system performance requirements. CardioChek PA (CCPA), is a portable whole blood analyser for rapid lipid measurement. Aim of the study was to evaluate the accuracy and precision of CCPA compared with conventional laboratory in healthy subjects and patients with dyslipidemia.

METHODS

Several CardioChek PA Analyzers (CCPA, PTS, Indianapolis, USA), which employ light reflectance and PTS PANELS Lipid Panel test strips to measure total cholesterol, HDL cholesterol and triglycerides in whole blood, were repeatedly evaluated on consecutive days together with designed quality control kit (ChekMate) and PTS Panel Quality Control materials. First, fasting venous samples were analysed on CCPA and results compared with the clinical laboratory assay of plasma lipids (COBAS 6000, Roche Diagnostics, Milano, Italy). Second, fasting finger-stick samples were analyzed on CCPA and compared with laboratory venous results. Precision was calculated by performing 10-20 replicates of the three fresh venous blood samples with different levels of cholesterol and triglycerides on the same instrument.

RESULTS

From 2010 to 2014, six CCPA instruments and six PTS PANELS Lipid Panel test strip lots were evaluated by use of venous blood samples (n=784 samples). The regression analysis showed a significant correlation between plasma lipids determined by laboratory analysis and CCPA (R value 0.97-1.0, $p < 0.001$). Results obtained using capillary blood (n=153 samples): 1) paired t test did not show any significant difference between laboratory and CCPA determinations of plasma lipids; 2) the R value was 0.95-0.99, $p < 0.001$; 3) overall intra-assay CV for total cholesterol, HDL cholesterol, and triglycerides were in the ranges of 1.3-2,9%, 2.3-5.6%, and 2.3-4.3%, respectively.

CONCLUSION

POCT devices need continuous quality management, including both quality control and quality assurance. External quality surveillance may provide information useful to ensure system performance. As a result, CCPA seems to be adequate for use in screening programmes aimed at metabolic control and early detection of lipid disorders.