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COLONIC AND INTESTINAL TISSUE-RESIDENT NK CELLS ACTIVITY IN RELATION TO METABOLIC PROFILE IN A MICE MODEL OF INFLAMMATORY BOWEL DISEASE

J. Amer [1], A. Salhab [1], A. Rabaiaa [1], M. Shoman [1], O. Maqbol [1]
[1]*An Najah National University*

BACKGROUND-AIM

Inflammatory Bowel Disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract that significantly affects patients' quality of life. Natural Killer (NK) cells are vital components of the immune system; however, their role in IBD is still unclear. This study aimed to investigate the involvement of colonic and intestinal tissue-resident NK (trNK) cells in the progression of metabolic dysregulation and immune suppression using a dextran sulfate sodium (DSS)-induced colitis mouse model.

METHODS

Twenty 12-week-old male C57BL/6J mice were divided into a control group and a DSS-induced colitis group. Body weight, food and water intake, and IBD characteristics (colon length and mass, mucin, occludin, and claudin) were assessed. Metabolic alterations of fasting blood sugar, insulin, cholesterol, and triglycerides were assessed through ELISA. Immune profiling included flow cytometry to evaluate colonic and intestinal trNK cell populations, their activation markers of CD107a and IFN-γ, and metabolic checkpoints such as insulin receptor expression.

RESULTS

Colon length was significantly reduced, while colon weight increased markedly in the IBD group compared to naïve mice, indicating inflammation-induced structural changes. Markers of epithelial barrier integrity [Occludin and claudin-1] were significantly decreased, whereas mucin expression was elevated, reflecting disrupted barrier function and compensatory response to inflammation. Metabolic findings revealed significant dysregulation in glucose, insulin, and lipid metabolism, consistent with the inflammatory state. Colonic and intestinal trNK cell activity was significantly decreased, with a more pronounced reduction observed in colonic trNK cells, as evidenced by a 2-fold reduction in CD107a expression and a 3-fold reduction in IFN-γ levels, indicating impaired activation and functionality of trNK cells. This activity reduction was associated with a decrease in insulin receptor expression in colonic trNK cells, whereas no such reduction was observed in intestinal trNK cells.

CONCLUSIONS

Data underscore the potential therapeutic value of targeting metabolic pathways and immune cell function to alleviate immune-related complications in IBD.

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THE ROLE OF LIVER NK CELLS FOLLOWING TESTOSTERONE ADMINISTRATION IN CHANGING METABOLIC PROFILE OF MICE MODEL OF LIVER FIBROSIS

J. Amer [1], A. Salhab [2], M. Takhman [3], A.G. Qeshawi [3], M.N. Sbeih [3], I.S. Snoubar [3]
[1]*Department of Allied Sciences, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine*
[2]*Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine.*
[3]*Department of Medicine, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine*

BACKGROUND-AIM

To investigate the role of liver tissue resident NK (trNK) cells in metabolic profile changes in an animal mice model of liver fibrosis following testosterone administrations.

METHODS

This study involved 36 naïve C57BL/6J mice (22 ± 2 g, 12 weeks old). Liver injury was induced with intraperitoneal (i.p.) injections of carbon tetrachloride (CCl4; 0.5 μl/g, diluted 1:9 in corn oil) twice weekly for 2 or 4 weeks, modeling acute and chronic injury, respectively. Testosterone (4 mg/kg, Merck; purity ≥98%) was administered i.p. twice weekly during the injury period: 1 week for acute and 2 weeks for chronic models. Mice were sacrificed 2 days after the final CCl4 injection, following anesthesia with 5% isoflurane and cervical dislocation.
The study groups were:
Naïve mice (control).
Testosterone-treated mice.
Acute liver injury (CCl4-only).
Acute liver injury with testosterone.
Chronic liver injury (CCl4-only).
Chronic liver injury with testosterone.
Each group included six mice, with three experimental repeats, totaling 108 mice.

RESULTS

Testosterone treatment significantly mitigated metabolic dysfunction by reducing fasting blood glucose, insulin, cholesterol, and triglycerides by 30-55%. These findings highlight testosterone's ability to restore metabolic balance. Furthermore, testosterone enhanced NK cell recruitment and functionality, as evidenced by increased NK cell percentages, IFN-γ production, IL-2 secretion, and granzyme B levels. The hormone also restored insulin receptor expression on tissue-resident NK cells and improved metabolic functionality via mTOR pathway activation, indicating improved immune and metabolic responses.

CONCLUSIONS

Testosterone's anti-inflammatory properties and its modulation of NK cells underline its potential as a therapeutic agent in liver fibrosis associated with metabolic syndrome. These findings contribute to the understanding of testosterone's multifaceted role in liver disease and suggest new avenues for targeted therapies.

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DICARBOXYLIC ACIDURIA. CLINICAL CHARACTERIZATION OF PATIENT PRESENT TO A BIOCHEMICAL GENETICS LABORATORY OF A LOW -MIDDLE INCOME COUNTRY.

H. Azeem [1], H. Majid [1], S. Ahmed [1], S. Abdul Mateen [1], A. Habib Khan [1]
[1] *Aga Khan University Hospital Karachi, Pakistan.*

BACKGROUND-AIM

The objective of this study was to evaluate the prevalence of dicarboxylic aciduria and to examine the range of clinical and biochemical features in cases referred to a specialized Biochemical Genetics Laboratory in Pakistan.

METHODS

This study was conducted at Biochemical Genetics Laboratory (BGL) of AKU, reviewing urine organic acid (UOA) analyses performed from January 2013 to 2022. Patient diagnosis with Dicarboxylic aciduria based on the presence of DCA markers (Adipic acid, Suberic acid, sebacic acid) detected in Gas Chromatography Mass Spectroscopy in UOA were included in the analysis. Demographic details, clinical history, and biochemical findings were collected and analyzed. Neurological deficits were characterized by the presence of certain symptoms, lethargy, fever, vomiting, developmental delay, septicemia, hypotonia, poor sucking, seizures, mental retardation, involuntary movements, coma, jaundice.

RESULTS

The study analyzed 112 patients diagnosed with dicarboxylic aciduria based on plasma amino acids (PAA) and urine organic acids. The median age of the patients was 454 days (Q3-Q1). Of these patients, 55.35% (n=62) were male and 44.64% (n=50) were female, with average ages of 475 days for males and 428 days for females. Consanguinity was noted in 66.96% (n=75) of the patients.
The most frequently observed clinical features included lethargy (57.14%, n=63), fever (41.96%, n=47), vomiting (33.92%, n= 38), developmental delay (32.13%, n=36), septicemia (30.35%, n=34), hypotonia (29.46%, n=33), poor sucking (28.57%, n=32), seizures (25%, n=28), and failure to thrive (25%, n=28). Other features were mental retardation (13.39%, n=15), involuntary movements (14.28%, n=16), coma (12.5%, n= 14), and jaundice (11.6%, n=13).

CONCLUSIONS

The analysis of 112 dicarboxylic aciduria cases at a single center reveals an unexpectedly high incidence of this rare condition within the country. Identifying dicarboxylic aciduria via high screening using urine organic acid helps detect inherited metabolic disorder preventing complication through timely targeted management. This finding also underscores the urgent need for increased awareness among healthcare provides to facilitate early diagnosis and optimal management.

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PREVALENCE AND PREDICTORS OF METABOLIC SYNDROME IN EASTERN INDIA

I. Basu [3], S. Gupta [2], S. Paul [1]
[1]*JIS University, Kolkata*
[2]*Midnapore Medical College and Hospital*
[3]*Ruby General Hospital, Kolkata*

BACKGROUND-AIM

The sedentary life style and food habits has led to increased incidence of non communicable diseases like Diabetes Mellitus (DM), Hypertension, Obesity & now they are collectively known as Metabolic Syndrome (MS). The global prevalence of MS is 25% & thus the diagnosis becomes important to prevent the development of complications like stroke & heart diseases. India is the second largest country in the world for the number of people with DM, after China. Advent of molecular testing implicates a genetic predisposition of the MS. Studies have suggested the genes associated with insulin resistance being instrumental in diagnosing the syndrome so far giving an overview in the main metabolic pathway. In view increasing trend of MS we aim to look at the disease prevalence in India with following objectives to assess the relationship between MS, other health conditions & biochemical parameters & to identify the TCFL2 gene polymorphism & associated biochemical parameters in MS patients.

METHODS

This clinico-epidemiological study involved 378 known cases of MS & measurement of their their anthropometric data & biochemical parameters of Liver Function test, Lipid Profile, Hormone function tests, Vitamins, Inflammatory marker levels. The prevalence is compared with NFHS (National Family Health Survey) 5 data available. The TCF7L2 (Transcription factor 7- like 2) gene polymorphism was studied for its association with MS & any particular allele (C/T) being implicated.

RESULTS

The prevalence of the MS was noted to be 23.3% in the study. Triglycerides levels are more in males than females of MS. Total Cholesterol & its components were more in females than males of MS. A positive correlation between Bilirubin & Liver Enzymes, Weight & Uric acid, Micro albumin urine & Plasma glucose, Glycated hemoglobin. The T allele was observed in 79.9% cases (p=0.0234) & homozygous T allele is found in 66.7% cases (p=0.0488). There was significant association between inflammatory markers (hs-CRP, AFP) & TCF7L2 gene polymorphism, suggesting effects of TCF7L2 on development of MS.

CONCLUSIONS

A proper genetic and biochemical testing enhances the chances of diagnosis even at an earlier stage leading to chances of primordial prevention and this can led to decrease in disease burden reducing the morbidity level in the country.

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PREDICTIVE VALUE OF MICRORNAS AND CYTOKINES IN THE DEVELOPMENT OF TYPE 2 DIABETES MELLITUS AND HYPERTENSION IN A SOUTH AFRICAN MIXED ANCESTRY POPULATION.

D.J. Botes [4], C.J. Weale [4], D.M. Matshazi [4], L.M. Graham [4], S.F. Davids [4], R.T. Erasmus [2], A.P. Kengne [3], A.P. Kengne [1], G.M. Davison [4], T.E. Matsha [4], T.E. Matsha [5]

[1]*Department of Medicine, University of Cape Town, Cape Town, South Africa*

[2]*Division of Chemical Pathology, Faculty of Health Sciences, National Health Laboratory Service (NHLS) and University of Stellenbosch, Cape Town, South Africa*

[3]*Non-Communicable Diseases Research Unit, South African Medical Research Council, Cape Town, South Africa*

[4]*SAMRC/CPUT Cardiometabolic Health Research Unit, Department of Biomedical Sciences, Faculty of Health and Wellness Science, Cape Peninsula University of Technology, Cape Town, South Africa*

[5]*Sefako Makgatho Health Sciences University (SMU), Pretoria, South Africa*

BACKGROUND-AIM

The rising prevalence of type 2 diabetes mellitus (T2DM) and hypertension, coupled with inadequacies of current screening methods in African settings, have prompted efforts to identify more effective, alternative markers. Consequently, there has been growing interest in exploring population-specific biomarkers such as microRNAs (miRNAs), which have been touted as potential early indicators for the development of T2DM and hypertension. As such, our study aimed to investigate the relationship between serum extracellular vesicle - derived miRNA expressions (miR-182-5p, miR-92a-3p and miR-29a-3p) with T2DM and hypertension.

METHODS

This case control study involved 150 participants, grouped as: normoglycaemia and normotensive (n=27); normoglycaemia and prehypertension (n=27); prediabetes and normotensive (n=27); prediabetes and prehypertension (n=27); screen-detected T2DM and normotensive (n=15); and screen-detected T2DM and screen-detected hypertension (n=27). MicroRNA expression was assessed using SYBR Green-based reverse transcription qPCR (RT-qPCR), with data normalised to an endogenous control (miR-16-5p).

RESULTS

Target miRNAs showed no significant expression differences between the normal and hypertensive groups ($p \geq 0.293$). However, adjusted Spearman correlations revealed significant positive associations between miR-92a-3p expression and diastolic blood pressure (DBP) across all groups ($r = 0.191$, $p = 0.023$), as well as between miR-29a-3p and DBP in hypertensives only ($r = 0.272$, $p = 0.024$). Furthermore, miR-29a-3p was positively correlated with fasting and 2-hour blood glucose ($r \geq 0.181$, $p \leq 0.042$), and negatively with 2-hour insulin ($r = -0.189$, $p = 0.038$), across all subgroups. Additionally, receiver operating characteristic (ROC) curve analysis revealed the superior discriminatory power of miR-92a-3p in distinguishing prediabetes (AUC = 0.537), in comparison to other conventional markers such as fasting glucose and HbA1c (AUC ≤ 0.513).

CONCLUSIONS

This study demonstrated the superior diagnostic capability of miR-92a-3p for prediabetes, in comparison to established tests. As such, these findings warrant further explorations.

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ASSESSING SIRTUIN 1 AND MIR-30A-5P ACROSS ADIPOSITY PARAMETERS IN A MIXED-ANCESTRY POPULATION FROM SOUTH AFRICA

B. Gaxamba [1], S. Raghubeer [1], G.M. Davison [1], T.E. Matsha [2], S.F. Davids [1]

[1]*South African Medical Research Council/ Cape Peninsula University of Technology, Dept of Biomedical Science*

[2]*South African Medical Research Council/ Cape Peninsula University of Technology, Dept of Biomedical Science. 2. Sefako Makgatho Health Sciences University*

BACKGROUND-AIM

The steady increase in obesity and metabolic disorders is a global challenge that necessitates understanding the molecular mechanisms underlying adiposity. This study investigated the regulatory relationship between miR-30a-5p, sirtuin 1 (SIRT1), and adiposity in a mixed-ancestry population from Bellville South and Belhar, Cape Town, South Africa.

METHODS

Two adiposity parameters were investigated in 300 adult participants, namely body mass index (BMI) and waist circumference (WC). SIRT1 concentration was measured using serum samples and a protein specific enzyme-linked immunosorbent assay (ELISA). SIRT1 and miR-30a-5p expression were measured using real-time quantitative polymerase chain reaction (RT-qPCR). Statistical analyses, including Spearman's correlation and logistic regression, assessed relationships and differences across BMI and WC categories, while receiver operating characteristic (ROC) curves evaluated the potential of miR-30a-5p and SIRT1 in predicting elevated BMI and high WC.

RESULTS

A total of 150 females and 150 males were selected for this study with an average age of 50 years. miR-30a-5p expression was significantly higher in overweight and obese participants compared to normal-weight individuals. SIRT1 protein concentration decreased with obesity, consistent with miR-30a-5p-mediated repression, while SIRT1 gene expression was increased in obese and high WC groups, suggesting post-transcriptional regulation of SIRT1 by miR-30a-5p.

CONCLUSIONS

These findings contribute to understanding the relationship between miR-30a-5p and SIRT1 in obesity and their potential as biomarkers and therapeutic targets for metabolic regulation. Insights into metabolic regulatory mechanisms contribute to the identification of therapeutic pathways in populations with high obesity prevalence, such as South Africa's mixed-ancestry community.

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EFFECT OF EXERCISE ON SPEXIN LEVELS IN DIABETIC RATS

M. Katar [2], F. Gevrek [1], E. Ulutanrıverdi [2]
[1]*Tokat Gaziosmanpasa University Faculty of Medicine Department of Histology and Embriology*
[2]*Tokat Gaziosmanpasa University Faculty of Medicine Department of Medical Biochemistry*

BACKGROUND-AIM

Diabetes is a disease that has reached serious levels today due to its frequency and acute and chronic complications. Exercise is very important in controlling diabetes and managing risk factors.In studies, spexin levels have been associated with diabetes, and this has encouraged increased research on the effect of exercise on spexin levels. This study aimed to evaluate spexin levels in diabetic rats. In our study, we aimed to investigate the relationship between spexin levels and biochemical parameters between groups.

METHODS

Before starting the study, 21 male Wistar albino rats were divided into 3 groups according to the random procedures. 7 of the rats were determined as the control group, 7 as the sedentary diabetes group and 7 as the exercise diabetes group. The Control Group was fed with normal water and feed for 4 weeks. Sedentary Diabetes Group was fed with normal water and feed for 4 weeks. Exercise Diabetes Group was fed with normal water and feed for 4 weeks. Exercise protocol was applied. Chronic running exercises were applied for 30 minutes/day. Spexin concentrations were measured by ELISA method. SPSS 26.0 program was used in all statistical analyses.

RESULTS

Glucose levels were found to be significantly higher in sedentary diabetic rats and exercise diabetic rats compared to the control group ($p<0.005$). The decrease in Total Cholesterol levels was found to be significant in the control group and sedentary diabetic rats ($p<0.005$). A decrease in LDL levels was found in all exercise groups. The groups showed decreased LDL levels compared to the control group results in the healing effect of exercise on the impaired lipid profile seen with diabetes. Exercise may be considered to have an anti-hyperlipidemic effect.

CONCLUSIONS

Based on all the results we obtained, it is controversial whether exercise has a reducing effect on serum spexin levels in diabetics, but it significantly reduces glucose and cholesterol levels and in this way, it can contribute to pharmacological agents in the treatment of Diabetes and preventing its complications.

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CLINICAL MANIFESTATION AND DIAGNOSTIC CHALLENGES OF CANAVAN DISEASE IN PAKISTAN

N. Khan [1], S. Abdul Mateen [1], h. Majid [1], A. Jamil [1], S. Ahmed [1], A. Habib [1]
[1]*Aga Khan University*

BACKGROUND-AIM

Canavan disease is a neurological disorder in which the brain degenerates into spongy tissue full of small fluid-filled spaces. It is caused by a mutation in the ASPA gene which makes an enzyme called aspartoacylase. This study was done to determine the prevalence of specific Canavan disease through Urine Organic Acid (UOA) analysis in Pakistani population

METHODS

A cross-sectional study was conducted by the Departments of Chemical Pathology at Biochemical Genetics Laboratory, Aga Khan University Hospital. Patient samples included from 2013 till Dec 2024. The UOA analysis was done using ethyl acetate extraction method, derivatized by BSTFA (N, O-bis(trimethylsilyl)trifluoroacetamide), using helium gas as mobile phase in GCMS, patient's chromatogram analyzed against specific Libraries such as ORGACIDS, ORGANIS, ACID97 on scan mode. Those positive for Canavan's based on the presence of N-acetylaspartate peak identified on UOA were included in final analysis. Analysis was done by Microsoft Excel 2021.

RESULTS

A total of 22622 urine samples were analyzed over a Eleven-year period, out of these 0.15% (n=34) patients were diagnosed with Canavan Disease. The median age of Canavan diagnosis was 270 days, with 47% (n=16) being female. Common symptoms included fever in 36% (n=12), involuntary movement in 27% (n=9), seizures in 24% (n=8), development delay in 82% (n=28), and mental retardation in 51% (n=17) respectively. Consanguinity was observed in 70% of cases (n=24), with the regional distribution across provinces as follows: Sindh 47% (n=16), Punjab 35% (n=12), KPK 18% (n=6), and Baluchistan 0% (n=0). Elevated levels of N-acetyl aspartate were detected on the UOA chromatogram in all patients. Radiological findings were identified in 18% of cases (n=6).

CONCLUSIONS

This study shows a high prevalence of Canavan disease on UOA analysis. Majority affected patients were female and presented with non-specific signs and symptoms. Mostly were from Sindh province. There is a need to conduct genealogical or ancestral studies to understand the lineage of these patients. Early recognition is critical given the severe neurological manifestations observed in affected patients.

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METABOLIC AND MOLECULAR GENETIC MARKERS OF EPILEPSY IN CHILDREN WITH OBESITY

I. Mamedov [2], O. Perevezentsev [2], I. Zolkina [1]
[1]*LLC Clinic of New Medical Technologies "ArchiMed", Moscow, Russia*
[2]*V.F. Voyno-Yasenetsky Scientific and Practical Center of Specialized Medical Care for Children*

BACKGROUND-AIM

Epilepsy is one of the important problems of modern neurology. Various epileptic syndromes can be comorbid with other pathologies, in particular, with excess weight. Currently, various aspects of the etiopathogenesis of epileptic seizures against the background of obesity are actively studied. The aim of the work is to identify the main metabolic and molecular genetic changes in epilepsy in children associated with obesity in order to develop a personalized approach to the diagnosis of this pathological condition

METHODS

The study included 24 patients aged from 1 year to 17 years (mean age 11.7 years): 16 patients with various epileptic syndromes against the background of obesity and 8 patients of the comparison group. The study of the profile of 60 organic acids in urine was carried out by HPLC-MS. Whole exome sequencing was performed in 5 patients using a Helicon G50 genetic analyzer.

RESULTS

In the group of patients with epilepsy against the background of obesity, the upper reference limit of a number of markers of the Krebs cycle (fumaric and 2-ketoglutaric acids), bacterial dysbiosis and lactic acid levels were exceeded. Also, in the sample of patients, in contrast to the comparison group, an increase in the levels of a number of markers of mitochondrial dysfunction was found. Whole exome sequencing of 5 patients with epilepsy against the background of obesity did not reveal known pathological mutations.

CONCLUSIONS

Thus, we have identified a number of metabolic markers associated with epilepsy in children against the background of obesity. Molecular genetic testing of a number of patients did not reveal significant pathological mutations. The results of the study confirm the importance of a personalized approach to the diagnosis and treatment of this pathological condition and further study of metabolomic and molecular genetic markers of this pathological condition.

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OPTIMIZING PLASMA AMINO ACID (PAA) TESTING USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) WITH FLUORESCENCE DETECTION (FLD): ENHANCING THROUGHPUT, SENSITIVITY AND PRECISION

H. Naeem [1], S. Abdul Mateen [1], H. Majid [1], S. Ahmed [1], A. Khan [1], A. Shah [1]
[1]*Aga Khan University*

BACKGROUND-AIM

PAA are analyzed using ion exchange chromatography, a reliable albeit time-intensive method.HPLC has emerged as a less time-consuming method for achieving high sensitivity and specificity in amino acid quantification.To optimize quantification of plasma amino acid on HPLC coupled with FLD.

METHODS

Cross-sectional study according to College of American Pathology standards was performed to analyze PAA at BGL, AKU on HPLC analyzer coupled with FLD. Optimization, include mobile phase composition having sodium diphosphate buffer and organic buffer in combination of diluted methanol with acetonitrile, Zorbax AAA column through C18 symmetry column (150 × 4.6 mm, particle size 3.5 μm). Method optimization and validation studies involved the use of the ERNDIM specimens, CAP proficiency test material, and known PAA previously analyzed on a BIOCHROM analyzer. Precision studies, accuracy assessments, linearity, determination of the analytical measurement range (AMR), evaluation of carryover, and comparison of two methods on HPLC-FLD were conducted. The EP-evaluator version 10 was used for statistical analysis.

RESULTS

Precision studies on Clincheck Controls (n=20) demonstrated acceptable CVs of arginine L1 and L2 respectively, (3.4%, 1.0%), asparagine (4.7%, 1.9%),aspartate(8.9%,7.8%), citrulline(6.1%,3.6%), cysteine(5.7%,4.7%), glutamate(6.9%,3.4%), glutamine(1.0%,3.4%), glycine(1.8%,2.9%), histidine(2.8%,2.0%), hydroxyproline(2.5%,6.9%),Isoleucine(5.1%,1.7%),leucine(1.2%,0.5%), lysine(3.7%,1.1%),methionine(3.0%,2.0%),ornithine(4.3%,4.4%),phenylalanine(3.8%,1.1%), proline(6.8%,7.5%),serine(4.3%,5.2%), threonine(2.9%, 3.4%), tyrosine(3.4%, 1.1%), valine(3.4%, 4.6%).The linearity study confirmed the AMR, by analyzing samples of low, medium, and high concentrations ranging from 0.5 to 2500 μmol/L. No peak in-between injections were observed indicating a successful study and no carryover in PAA assay. In the method comparison analysis (n=20 proficiency samples) a slope of 1.0 was observed, with an intercept of -1.0 μmol/L.

CONCLUSIONS

HPLC assay successfully validated reports of a rapid assay (twenty-six minutes) amino acid quantification methodology using HPLC-FLD to identifying aminoacidopaties in high-risk symptomatic children in AKU, Pakistan.

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IMPACT OF DIETARY INTERVENTION ON GROWTH DIFFERENTIATION FACTOR 15 (GDF-15) CONCENTRATION IN OBESE AND OVERWEIGHT PATIENTS

M. Paskudzka [1], M. Rosiak [3], K. Wiśniewska [2], K. Okręglicka [2], O. Ciepiela [1]
[1]*Department of Laboratory Medicine, Medical University of Warsaw, Warsaw, Poland*
[2]*Department of Social Medicine and Public Health, Faculty of Medicine, Medical University of Warsaw, Warsaw, Poland*
[3]*Students Scientific Group of Laboratory Medicine, Medical University of Warsaw, Warsaw, Poland*

BACKGROUND-AIM

It is estimated that 43% of adults are overweight and 16% are living with obesity. Dietary intervention may be an effective strategy for obesity management. Growth differentiation factor 15 (GDF-15) is a distinct member of the transforming growth factor β (TGF-β) superfamily. Serum GDF-15 is elevated in humans experiencing a broad spectrum of cellular stress, that may be associated with reduced food intake associated with amino acid deprivation and increased energy expenditure. On the other hand, GDF-15 is suggested to exert anti-inflammatory effect. In this study, we investigated the impact of five different plant-based dietary models on GDF-15 concentration.

METHODS

The study group consisted of 44 overweight or obese patients aged >18 and ≤60 years (BMI >30 kg/m² or BMI 25-29.9 kg/m² with waist circumference ≥88 cm in women, ≥94 cm in men).Patients were randomly assigned to one of five groups of diet: (1) Mediterranean, (2) "diet of the future" proposed in the EAT-Lancet Commission report, (3) lacto-ovo-vegetarian, (4) vegan, and (5) control group on standard balanced diet. GDF-15 concentration in serum was assessed before and after 12 weeks of dietary intervention using electrochemiluminescence immunoassay on Cobas e801, Roche. Statistical analysis was performed using GraphPad Prism.

RESULTS

The concentration of GDF-15 was 606.79±214.26 pg/ml and 648.86±242.73 pg/ml before and after 12 weeks of diet, respectively (p=0.0097). However, the analysis of individual groups revealed that only vegan diet caused significant increase in GDF-15 (556,72±122,97 pg/ml vs 660,55±156,93 pg/ml, p=0.002). Interestingly, all dietary interventions resulted with similar decrease of BMI (32.20±3.98 before and 30.61±4.04 after 12 weeks of diet, p<0.0001), regardless of the diet introduced. Moreover, the increase in GDF-15 concentration was correlated with a decrease of BMI (r=0.31, p=0.0478).

CONCLUSIONS

The study shows that implementing a vegan diet leads to the highest increase in GDF-15 concentration in obese and overweight patients, what may be associated with reduced supply of amino acids. However, it should be emphasized that all dietary interventions undertook in obese subjects contribute to improvement of anti-inflammatory response by increase of GDF-15.

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THE ROLE OF CHEMICAL PATHOLOGY LABORATORY IN DIAGNOSING MONOGENIC CONDITIONS RELATED TO PAEDIATRIC UROLITHIASIS : INSIGHTS FROM A TERTIARY CARE HOSPITAL SPECIALIZED FOR CHILDREN

K.D. Rammuthupura [3], N. De Silva [3], M. Vidanapathirana [3], S. Jayasena [3], M. Fernando [3], D. Warawita [3], V. Gunasekara [3], S. Chandrarathne [4], L. Fairbanks [5], M. Hemachandra [6], A. Bertoli [2], B. Stiburkova [1], E. Jasinge [3]
[1] *Institute of inherited metabolic disorders, Czech Republic*
[2] *Centogene, Rostock, Germany*
[3] *Lady Ridgeway Hospital for children,Sri Lanka*
[4] *National Hospital, Sri Lanka*
[5] *Purine research laboratory, UK*
[6] *Sirimawo bandaranayake specialized hospital for children, Peradeniya*

BACKGROUND-AIM

Inborn metabolic conditions related to urolithiasis are a very rare entity which may present early with characteristic renal and extrarenal manifestations. Early diagnosis and management of these patients are important to prevent further metabolic complications.

METHODS

Four patients with urolithiasis referred to the Department of Chemical Pathology, Lady Ridgeway Hospital for further evaluation, who were found to have an inherited metabolic disorder were selected.

RESULTS

Patient 1
A nine-year old boy, investigated for recurrent abdominal pain, hematuria with a kidney stone was biochemically suspected of having renal hypouricemia due to hypouricemic hyperuricosuria. Genetic analysis established the diagnosis of renal hypouricemia type 1.
Patient 2
An eight-year-old boy presented with global developmental delay, spasticity, dystonia, self-mutilation, and renal calculi. Hyperuricemic hyperuricosuria confirmed the clinical diagnosis of Lesch Nyhan syndrome. Reduced Hypoxanthine-guanine phosphoribosyltransferase enzyme levels supported the diagnosis.
Patient 3
An eighteen-month old boy presented with recurrent hematuria and a bladder stone. Hypouricosuric hypouricemia along with xanthine crystals, high urinary xanthine and hypoxanthine levels confirmed the diagnosis of xanthine oxidase deficiency.
Patient 4
A 13-year old boy with a history of recurrent renal stones had normal serum and urine biochemistry. 100% dihydroxyadenine in stone analysis suggestive of adenosine phosphoribosyl transferase enzyme deficiency was genetically confirmed.

CONCLUSIONS

Performing biochemical investigations and stone analysis, the chemical pathology laboratory plays a key role in diagnosing monogenic conditions related to urolithiasis.

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ESTABLISHING A RARE REGISTRY FOR INHERITED METABOLIC DISORDERS AT THE BIOCHEMICAL GENETIC LAB IN PAKISTAN: A DECADE OF DATA

S. Abdul Mateen [1], H. Majid [1], S. Ahmed [1], A. Khan [1], A. Shah [1]
[1]*Aga Khan University*

BACKGROUND-AIM

Patient registries help address research challenges posed by rare diseases with small patient populations. The Rare Registry aims to gather epidemiological data on these disorders from Biochemical Genetics Laboratory (BGL). Its objectives include comparing disease burdens internationally and identifying risk factors and clinical spectrums.

METHODS

The registry documents Inherited Metabolic Disorder (IMDs) prevalence from Jan 2013 to Sept 2023, gathering demographic, geographical, clinical, biochemical, Plasma amino acid (PAA), Urine Organic Acid (UOA) and risk factor information. Samples were collected from various regions of Pakistan, utilizing the extensive Clinical Laboratories network of AKU, which spans over 300 sample collection stations across all 94 districts in the country. Data entry follows a strict protocol, ensuring confidentiality through coding and password protection. Standardization and quality control measures, including monthly evaluations and periodic audits, are enforced to maintain data accuracy and completeness. Key performance indicators are calculated regularly, including disease-specific metrics.

RESULTS

Since its inception, the Rare Registry has screened 38,711 patients suspected of metabolic disorders, identifying 2,677 (6.91%) positive cases. This Rare Registry not only quantifies the prevalence but also provides a detailed categorization of 51 distinct metabolic diseases conditions. Among these, 15 were classified as aminoacidopathies, while 36 were characterized as organic acidurias/conditions. The most prevalent conditions included Methylmalonic aciduria, accounting for 14% (n=377) of cases, followed by Multiple Carboxylase Deficiency at 7.65% (n=205), PKU in 5.31% (n=142), Non-Ketotic Hyperglycinemia at 4.44% (n=119), Propionic aciduria at 3.85% (n=103), and MSUD at 3.81% (n=102).

CONCLUSIONS

The establishment of the first Rare Registry for IMD's marks a significant milestone in addressing the high burden of rare diseases prevalent in the country.

Metabolic diseases

P1778

PLASMA BIOTINIDASE ACTIVITY AS CONFIRMATION OF PATHOGENICITY OF GENETIC TESTING

M.J. Aguilar Castillo [3], J. Sanchez Cortes [1], A. Perojil Jimenez [3], J. Luaces Puente [2], M. Garcia De Burgos [3], R. Yahyaoui Macias [3]
[1]*CLINICAL LABORATORY DEPARTMENT / HOSPITAL UNIVERSITARI SANT JOAN DE REUS*
[2]*LABORATORIO CLINICO / COMPLEJO ASISTENCIAL DE SEGOVIA*
[3]*SERVICIO DE ANÁLISIS CLÍNICOS /HOSPITAL REGIONAL UNIVERSITARIO DE MÁLAGA*

BACKGROUND-AIM

Biotinidase deficiency is an autosomal recessive metabolic disorder that causes alterations in biotin metabolism due to deficiency in multiple carboxylases, enzymes involved in gluconeogenesis, fatty acid synthesis and aminoacid catabolism. The clinical symptoms are nonspecific, with predominating dermatological and neurological syntoms.
Its specific diagnosis results from measuring biotinidase activity and genetic testing. Treatment involves biotin supplementation, although it does not reverse the neurological damage that has already occurred. Therefore, this disease is included in some neonatal screening programs.

METHODS

We present the case of two brothers: One of them was detected by neonatal screening. His sister was detected as a result of this event.
In the case of the child, the activity was decreased in Dried blood spot (DBS) at neonatal screening (0.2 nmol pABA/min.mL (4.9-8.3). He has been taking biotin since 2.5 months old with normal psychomotor development so far.
A genetic study was performed in which the presence of heterozygosity of the pathogenic variants c.98_104delinsTCC (p.Cys33Phefs*36) and c.1330G>C (p.Asp444His) in the BTD gene was identified.

RESULTS

Plasma BIOTINIDASE ACTIVITY is requested: (QUANTIFICATION BY SPECTROPHOTOMETRY) Result: 1.3 nmoles PABA/min/ml serum (VR10.6 ± 3.3). This corresponds to 20% of the mean of the controls. (Normal 70 − 100% Carrier (heterozygous) ≈ 50% Partial deficiency 10 − 30% Profound deficiency < 10%). This result confirms the pathogenicity of these variants.
In the case of the sister, it was detected at 2 years of age and she has been on replacement therapy since then with Biotin 5 mg since then and she has a language disorder (receptive-expressive: phonological predominance). Enzyme activity studied in DBS have also been carried out with normal results and genetic studies have detected the same variants. The determination in plasma has shown decreased values. 2.1 nmoles PABA/min/ml serum (VR:10.6 ± 3.3) confirming the pathogenicity of these variants.

CONCLUSIONS

Plasma biotinidase activity confirms the pathogenicity of these variants. Differences in clinical manifestations between these siblings shows the importance of newborn screening programs.

Metabolic diseases

P1779

A CASE REPORT OF A FEMALE WITH MCAD DEFICIENCY DETECTED THROUGH NEWBORN SCREENING

M.I. Cabrera González [2], M.J. Aguilar Castillo [2], S. Franco Freire [1], M. García De Burgos [1], R. Yahyaoui Macías [2]
[1]*Regional Hospital of Málaga. Genetics Department*
[2]*Regional Hospital of Málaga. Metabolic Laboratory Department*

BACKGROUND-AIM

Medium-chain acyl-CoA dehydrogenase deficiency (MCAD) constitutes a rare autosomal recessive disorder with an estimated prevalence of 1 in 14,600 newborns worldwide. It is caused by variants in the ACADM gene, resulting in a dysfunction of the MCAD enzyme involved in mitochondrial fatty acid β-oxidation. Currently, approximately 400 mutations are known. The inability to provide energy to tissues during fasting results in a wide spectrum of symptoms. The main symptoms are hypoketotic hypoglycemia with vomiting, progressing to seizures, and coma, which are typical manifestations. Newborn screening (NBS) has enabled the early identification of asymptomatic individuals, which improves the prognosis of patients with MCAD.

METHODS

In Oriental Andalusia's NBS program, medium-chain acylcarnitines are detected by FIA-MS/MS in dried blood spots (DBS). In positive cases, genetic analysis is also performed.

RESULTS

A newborn female showed high levels of medium-chain acylcarnitines (μM): C6 0,413 (NV 0-0,15), C8 0,74 (NV 0-0,17); C10 0,502 (NV 0-0,2); C10:1 0,174 (NV 0-0,14); C12/C8 0,25 (NV 0,4-16). Biochemical analyses in plasma confirmed the elevation by MS/MS (C6: 0,21 (NV 0-0,13) ; C8: 0,4 (NV 0,01-0,25); C10:1 0,27 (NV 0,02-0,24); C18:1 0,26 (NV 0,04-0,19); C0: 68,01 (NV 21,5-64,58). Two pathogenic variants in heterocygosity in the ACADM gene were found, c.683C>A, p.Thr228Asn and c.985 A>G, p.Lys329Glu.

CONCLUSIONS

NBS for MCAD deficiency is crucial for early identification and intervention, preventing severe health consequences and ensuring a better prognosis for affected newborns. Confirmation of the diagnosis enables continuous follow-up, including monitoring by a dietitian and carnitine level controls, and facilitates early genetic counselling.

Metabolic diseases

P1780

A 5 YEARS OLD BOY WITH ABNORMAL MOVEMENTS AND INCREASED URINARY EXCRETION OF SEVERAL NEUTRAL AMINO ACIDS

A. Desmons [1], D. Doummar [2], C. Ravelli [2], A. Lamazière [1]
[1]*Metabolomic clinic department, Saint Antoine Hospital, APHP, Paris*
[2]*Pediatric Neurology Department , Trousseau Hospital, APHP, Paris*

BACKGROUND-AIM

A 5 years old boy was addressed in neuropediatric department for abnormal movements. The treatments given were ineffective so metabolic tests including amino acids profiles were performed using a liquid chromatography in tandem with mass spectrometry method.

METHODS

It is a quantitative method developed in our laboratory using a mixed mode stationary phase and a binary gradient of elution for the rapid separation and quantification of 52 AAs in plasma, urines without derivatization or ion pairing reagent addition.

RESULTS

Plasma amino acids were normal. Urinary profile revealed some quantitative abnormalities: increase of several neutral AAs such as: histidine (381 μmol/mmol creatinine), threonine (92 μmol/mmol creatinine), tyrosine (57 μmol/mmol creatinine), alanine (115 μmol/mmol creatinine), serine was normal (20 μmol/mmol creatinine) and tryptophan slightly increase: 35 μmol/mmol creatinine. The mono amino-dicarboxylic amides were increased: asparagine (104 μmol/mmol creatinine) and glutamine (170 μmol/mmol creatinine).
To evidence the diagnosis a genome analysis was performed and a homozygous variant of uncertain significance in SLC6A19 gene predicted to be pathogenic was identified. The diagnosis of Hartnup disease was mentioned. In our case, the clinical presentation was incomplete: the patient did not present classical symptoms such as pellagra-like dermatitis and cerebellar ataxia and the excretion of amino acids was incomplete. AAs analysis in 24-hour urine sample was performed for the follow-up: histidine (266 μmol/mmol creatinine), threonine (63 μmol/mmol creatinine), tyrosine (36 μmol/mmol creatinine), alanine (70 μmol/mmol creatinine), serine (177 μmol/mmol creatinine) and tryptophan was normal: 19 μmol/mmol creatinine.
The mono amino-dicarboxylic amides were increased: asparagine (62 μmol/mmol creatinine) and glutamine (127 μmol/mmol creatinine). A decrease of urinary amino profile was evidenced but no neurological improvement was evidenced until now.
These incomplete profiles let us to think that it could be an intermittent form of the disease.

CONCLUSIONS

This case of abnormal AAs profile but not characteristic of one pathology highlights the contribution of genome analysis and the necessity to repeat metabolic assays on different samples.

Metabolic diseases

P1781

GENOTYPIC VARIABILITY AND ENZYMATIC PROFILES IN PATIENTS WITH FABRY DISEASE

I. Ghoufa [1], S. Mrad [1], S. Boughzala [1], M. Khalili [1], S. Boughzala [1], Y. Jouini [1], M. Ennouri [1], B. Charfeddine [1], J. Ben Abdallah [1], S. Ferchichi [1]

[1]*Laboratory of Biochemistry,Farhat Hached University Hospital,Sousse,Tunisia*

BACKGROUND-AIM

Fabry disease is a rare hereditary disorder linked to the X chromosome. In Tunisia, this condition appears to be more common than initially assumed, with a steady increase in the number of suspected and confirmed cases over the years. This study aims to investigate the correlation between genotype and enzymatic activity in a Tunisian population affected by Fabry disease.

METHODS

This is a retrospective descriptive study conducted over six years, from 2018 to January 2024. All requests sent to our laboratory,the only center for biological screening of Fabry disease in Tunisia,for suspected cases of the disease were included. Diagnosis was confirmed by measuring alpha-galactosidase A activity in men and by lyso-globotriaosylceramide quantification and molecular analysis in women.

RESULTS

During the study period, 32 analysis requests were reviewed, with 6 confirmed cases: one in 2018 and five in 2022, four of which belonged to the same family. All six cases involved adults aged over 18 years. Among them, 4 (67%) were male and 2 (33%) were female.
In our study, the three male patients were all homozygous (60%) and exhibited reduced enzymatic activity. Among the two female patients, one was heterozygous with reduced alpha-galactosidase A activity, while the other was heterozygous but had normal enzymatic activity.
In 100% of the male patients, enzymatic activity was below 3%.
Additionally, Lyso-Globotriaosylceramide levels were elevated in all patients.

CONCLUSIONS

This study reveals the heterogeneity in genotypic and enzymatic profiles among patients with Fabry disease, emphasizing the importance of detailed genetic and enzymatic analyses for accurate diagnosis and effective family screening.

Metabolic diseases

P1782

METHYLMALONIC ACIDEMIA; CASE REPORT

X. Galiana Vallés [1], A. Sierra Rivera [1], C. Hernández Hernández [1], C. Pascual Escriva [1], A. Comes Raga [1], J. Ferriz Vivancos [1], M. Morales Garces [1], A. Domenech Peris [1], Á. Férez Martí [1], L. Sahuquillo Frías [1]
[1]*Consorcio Hospital General Universitario de Valencia*

BACKGROUND-AIM

Methylmalonic acidemia (MMA) is a group of inborn errors of branched amino acid metabolism characterized by accumulation of methylmalonic acid and caused by the inability to convert L-methylmalonyl-CoA to succinyl-CoA in the propionate pathway.
The incidence in Western populations ranges from 1:48000 to 1:61000. Signs and symptoms may include vomiting, hypotonia, hepatomegaly. Without treatment it can result in coma or death of the patient.

METHODS

15-day-old infant admitted for digestive intolerance, with vomiting and poor sucking, and hypotonia. At birth, he presented neonatal respiratory distress, hypoglycemia 26mg/dL [74-100]. No family history of matabolopathies.
Blood gases, complete blood count, complete biochemistry and metabolic study, amino acids in serum and urine, acylcarnitines and organic acids were requested due to suspicion of metabolopathy.

RESULTS

The analytical findings showed lactate 6.32mmol/L (0.3-1.6), hemoglobin 11.2g/dL [15-24], leukocytes 2.91x10^3/L [9.1-34], neutrophils 0.08x10^3/L [6-23.5], platelets 62x10^3/L [150-350], vitamin B12 250pg/mL [180-914] and homocysteine 84.79mol/L (5.08-15.39). The amino acid study measured by HPLC-MS/MS: methionine 4umol/L (14-30). Organic acids by GC-MS/MS: methylmalonic acid 1546mmol/mol creatinine [0-11], 3-hydroxypropionic acid 302mmol/mol creatinine [0-18] and lactic acid 8893mmol/mol creatinine [1-121]. Acylcarnitines by MS/MS: propionylcarnitine/acetylcarnitine (C3/C2) 1.2umol/L [0.29 (p99.75)], propionylcarnitine (C3) 7.68 umol/L [0.37-3.73] and C3/Methionine 1.96 umol/L [0.45 (p99.75)].
MMA with homocystinuria type cblC was confirmed by genetic study. The patient showed good clinical and analytical response to treatment, with progressive normalization of analytical values.

CONCLUSIONS

Currently, diagnosis begins before the expression of symptoms thanks to the inclusion of this disease in the neonatal screening program.
The screening consists of the analysis of the acylcarnitine profile in a paper-dried blood sample. In the event of a positive result for increased C3), C3/C2 or C3/Methionine confirmed in a second sample, a complete metabolic study is initiated. If the suspicion is reinforced, a genetic study is performed to rule out or confirm the diagnosis.

Metabolic diseases

P1783

PATIENT WITH OSTEOARTHRITIS IN MULTIPLE JOINTS AND DARK URINE.

Y. Gamarra Morales [1], M. López Melchor [1], P. Ruiz Ruiz [1], M.C. Mérida Rodríguez [1], M.J. García López [1]
[1]*Hospital Comarcal Santa Ana de Motril*

BACKGROUND-AIM

A 71-year-old male patient came to Cardiology for a check-up for follow-up of moderate aortic stenosis. His family history includes a brother diagnosed with alkaptonuria, a granddaughter with glycogenosis and two healthy children. As personal history he has suffered from arterial hypertension for 8-9 years; ex-smoker for more than 30 years; left knee surgery for ligament injury, bilateral inguinal herniorrhaphy, umbilical herniorrhaphy, right knee ligament, menicectomy. Bilateral hip prosthesis due to osteoarthritis: left coxarthrosis associated with ochronosis. Transurethral resection of the prostate due to benign prostatic nodular hyperplasia (2020). Studied in Sept 2023 in cardiology after referral for study of asymptomatic murmur. Aortic stenosis classified as moderate by echocardiogram. Left ventricle with concentric hypertrophy and preserved LVEF. Treatment: enalapril 20/hydrochlorothiazide 6 mg, condrosan, naproxen if pain.

METHODS

The authors analysed the case and assessed other possible differential diagnoses: acute intermittent porphyria, rheumatoid arthritis, ankylosing spondylitis and osteoarthritis.
A 24-hour urine homogentisic acid test was requested and sent to an external laboratory (Reference) where the test was performed by High Performance Liquid Chromatology.
As for the laboratory parameters that were altered, this patient had total bilirubin at 1.73 mg/dL, potassium at 5.2 mEq/L and LDL at 123 mg/dL.

RESULTS

The patient currently has joint pathology in multiple locations. He has reported dark urine since childhood. Skin pigmentation (ochronotic) in the ear and sclera. Usual lumbar pain. Arthrosis in multiple joints: hip and knees, with prosthesis.
The 24-hour urine homogentisic acid result was 3311.5 mg/24 hours. The urine of this sample was dark.
To confirm the diagnosis, a genetic study should be carried out. In this case it was not necessary as there was a family history of the diagnosis.

CONCLUSIONS

The role of the laboratory in the diagnosis of alkaptonuria in patients with dark urine and radiographic evidence of osteoarthritis is fundamental, as a homogentisic acid determination can easily diagnose the disease. There is a group of patients who are affected but have no significant symptoms and are not aware of their disease until adulthood.

Metabolic diseases

P1784

INHERITED METABOLIC DISORDERS (IMDS) BURDEN BASED ON NEWBORN SCREENING

A. Jamil [1], L. Jafri [1], S. Ahmed [1], A.H. Khan [1], N.A. Khan [1], H. Majid [1]
[1]*The Aga khan University Hospital*

BACKGROUND-AIM

In Pakistan, diagnosing rare hereditary metabolic diseases has been challenging due to limited resources and expertise. This study aims to perform newborn screening (NBS) for hereditary metabolic disorders, focusing on 12 genetic metabolic disorders, using LC-MS/MS to assess amino acids and acylcarnitine levels.

METHODS

Newborn blood samples were collected between 33-72 hours after birth using heel prick, with five drops placed on filter paper to create dry blood spots (DBS). Samples were air-dried, stored, and analyzed using LC-MS/MS to detect metabolic disorders of Maple Syrup Urine Disease, Citrullinemia Type I & II, Argininemia, Homocystinuria, Methylmalonic Aciduria, Propionic acidemia, Glutaric Aciduria Type I, Isovaleric Acidaemia, Argininosuccinic aciduria and Cobalamin intracellular defects (Cobalamin-A, Cobalamin-B, Cobalamin-C, Cobalamin-D, Cobalamin-F, TC-II). Amino acids and acylcarnitine were extracted and analyzed with assay kits and tandem mass spectrometry (Xevo TQ Detector, Waters Corporation).

RESULTS

Between August 2023 and December 2024, 6812 newborn DBS samples were analyzed. A small percentage (4%) had a history of blood transfusion, and 34% were on total parenteral nutrition. Gestational ages ranged from 25 to 40 weeks (average 35 weeks), with birth weights between 0.5-4.0 kg (average 2.5 kg). Screening identified 261 high results, 57% (n=150) were male. Among positives C3-Carnitine 42% (n=110), C5-Carnitine 17% (n=45), C5DC-Carnitine 6% (n=15), methionine 16% (n=41), leucine 14% (n=36), arginine 3% (n=9) and citrulline 2% (n=6). Patients were immediately referred for confirmatory tests by the physician if the results exceed cutoffs for organic acids by gas chromatography-mass spectrometry (GC-MS) and amino acid analysis by HPLC. Two true positive cases were identified, Homocystinuria and citrullinemia were confirmed.

CONCLUSIONS

The study screened over 6000 newborns, identifying metabolic abnormalities in a small percentage. The confirmed incidence of metabolic disorders was 1:6812. The results emphasize the need for establishing independent cut-off values for neonatal screening, which would improve diagnostic efficiency for hereditary metabolic diseases in Pakistan.

Metabolic diseases

P1785

ASSOCIATION OF PLASMA AMINO ACIDS WITH RISK FACTORS FOR METABOLIC SYNDROME IN MEXICAN POPULATION

E. Lares-Villaseñor [1], M. Guevara-Cruz [3], S. Salazar-García [1], O. Granados-Portillo [3], I. Medina-Vera [3], L.E. González-Salazar [3], P.E. Cossío-Torres [2], D.P. Portales-Pérez [1], A. Rodríguez-Rodríguez [1], P. Hernández-Morales [1], B.B. Jurado-Manzano [1], L.G. Noriega-López [3], J.M. Vargas-Morales [1]

[1]*Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, San Luis Potosí*

[2]*Facultad de Medicina, Universidad Autónoma de San Luis Potosí, San Luis Potosí*

[3]*Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán, Ciudad de México.*

BACKGROUND-AIM

Metabolic syndrome (MetS) encompasses biochemical, physiological, and anthropometric alterations associated with insulin resistance (IR) or obesity, increasing the risk of type 2 diabetes. Notably, alterations in plasma amino acid concentrations have been linked to MetS risk factors. However, available data are limited, particularly regarding the Mexican population. Our aim was to evaluate the association between plasma amino acids and MetS risk factors in the Mexican population.

METHODS

A cross-sectional study was conducted on subjects over 18 years of age with one or more MetS risk factors. Anthropometric and clinical variables, including blood pressure, were measured. Glucose, total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were enzymatically measured using an automated clinical biochemistry analyzer. Insulin levels were determined by immunoassay. The Dysfunctional Adiposity Index (DAI) was calculated as follows:
For males: [WC (Waist circumference)/[22.79+[2.68*BMI (Body mass index)]]]*[Triglycerides (mmol/L)/1.37]*[1.19/HDL-C (mmol/L)]. For females: [WC/[24.02+[2.37*BMI]]]*[Triglycerides (mmol/L)/1.32]*[1.43/HDL-C (mmol/L)]. Plasma amino acids, including alanine, arginine, aspartic acid, glutamic acid, asparagine, glycine, glutamine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, tryptophan, and valine, were quantified using high-performance liquid chromatography. Spearman correlation analysis was performed to assess associations. Data were analyzed using SPSS v.20.

RESULTS

Ninety-eight subjects (63 females and 35 males) were evaluated, all presenting with obesity, hypertriglyceridemia, and IR. Most amino acid levels were higher in males, except for glycine and serine. Aspartate, glutamate, serine, alanine, tyrosine, valine, tryptophan, isoleucine, leucine, lysine, and proline were positively correlated with one or more MetS risk factors, including triglycerides, DAI, IR, weight, and WC, in both sexes ($p < 0.05$).

CONCLUSIONS

Plasma amino acids are positively associated with MetS risk factors in Mexican population, underscoring their potential as biomarkers for cardiometabolic disease risk.

Metabolic diseases

P1786

CALCITE UROLITHIASIS IN AN ANORECTIC FEMALE

P. Neyer [2], A. Papagiannakis [1], D. Wicki [1], M. Kwiatkowski [1], S. Wyler [1], L. Bernasconi [2]
[1]*Department of Urology, Cantonal Hospital Aarau, Aarau, CH*
[2]*Institute of Laboratory Medicine, Cantonal Hospital Aarau, Aarau, CH*

BACKGROUND-AIM

Kidney stones are a common condition with a multifactorial etiology, including genetic, metabolic, and environmental factors. Rare cases of calcite stones have been reported in patients with specific predispositions or severe metabolic imbalance. Anorexia nervosa, a psychiatric disorder characterized by severe nutritional deficiencies, is known to lead to a range of metabolic and renal abnormalities, including hypercalciuria and altered urine pH. The presented case highlights the unique challenges of managing urolithiasis in a vulnerable group, where profound metabolic disturbances and low body weight may influence the composition of kidney stones.

METHODS

The composition of a spontaneous passage calculus was confirmed by the congruent spectra in an endoscopically removed specimen. Both yielded excellent correlations with reference spectra.

RESULTS

A 21-year-old cachectic (BMI 12.9) female with anorexia presented to our emergency department with right-sided colicky pain and gross hematuria, had no history of kidney stones. Urine pH was 9 and the dipstick showed hemat- and leukocyturia. Non-contrast CT showed bilateral nephrolithiasis, ureterolithiasis of the right distal ureter and right-sided hydronephrosis. Stones had 930 HU. To manage the obstruction, an emergency placement of a JJ–stent was performed. Fifteen days later, the patient underwent endoscopic stone removal. Intraoperatively, we identified a singular calculus of the lower renal calyx. Infrared spectroscopy confirmed the composition of calcite. Postoperative follow-up was uneventful.

CONCLUSIONS

Stone analysis is standard workup with urolithiasis. Calcite is a crystal polymorph of calcium carbonate and, if identified as component of urinary calculi, is often considered an artifact. It is reported as a true component with a frequency of .01% to .25%, and usually mixed with other components. In literature, a few cases report pure calcite calculi in humans. Severe malnutrition is identified as a predisposing factor along with laxative-abuse. Limited studies have demonstrated that anorexia nervosa increases the risk of nephrolithiasis because of low urinary output. The underlying cause remains unclear. Stone composition of pure calcite is exceptionally rare and underscores the metabolic complexity associated with severe malnutrition.

Metabolic diseases

P1787

THE POTENTIAL ASSOCIATION OF SERUM BRANCHED SHORT CHAIN FATTY ACIDS WITH METABOLIC DYSFUNCTION-ASSOCIATED FATTY LIVER DISEASE IN CENTRAL OBESITY

M.N. Rahman [3], M. Fattah [3], M.I. Barliana [1], A. Diantini [2]
[1]*Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, Indonesia*
[2]*Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, Indonesia*
[3]*Prodia Clinical Laboratory, Jakarta, Indonesia*

BACKGROUND-AIM

Obesity is a complex disease caused by excessive fat accumulation which can cause many metabolic diseases. Obesity prevalence has increased in recent decades and reaching epidemic proportions. Abdominal obesity also known as central obesity that have stronger association with metabolic disease and might predispose or worsen the progression of fatty liver disease. Metabolic dysfunction-associated fatty liver disease (MAFLD) refers to fatty liver in obesity and/or type 2 diabetes mellitus. Short Chain Fatty Acids (SCFAs) are the primary end product of carbohydrate fermentation by gut microbiota. SCFAs have an important role in the interaction between gut and organs. Among SCFAs, branched SCFAs mainly isobutyric and isovaleric, produced in less amount than three major SCFAs. Their influence on metabolic diseases are insufficiently known. The aim of this study was to evaluate the association of serum branched SCFA with central obesity and MAFLD.

METHODS

Subjects in this study were 150 man and woman with central obesity measured by waist circumference >90 cm for man and >80 cm for woman. Serum SGOT and SGPT were measured with routine clinical chemistry method. Serum isobutyric and isovaleric were measured using Gas Chromatography-Mass Spectrometry (GCMS).

RESULTS

There were positive correlation between waist circumference with SGOT (R=0.334, p<0.05), SGPT (R=0.476, p<0.05), and SGPT/SGOT Ratio (R=0.482, p<0.05). There were no correlation between isobutyric with waist circumference (R=0.075, p=0.365) and SGOT (R=0.139, p=0.090) but there were positive correlation with SGPT (R=0.288, p<0.05) and SGPT/SGOT Ratio (R=0.336, p<0.05). There were positive correlation between isovaleric with waist circumference (R=0.276, p<0.05), SGPT (R=0.212, p<0.05), and SGPT/SGOT Ratio (R=0.232, p<0.05) but there was no correlation with SGOT (R=0.122, p=0.138). SGPT/SGOT Ratio recently explored as marker to indicate fatty liver in obesity. Subjects with SGPT/SGOT ratio >1 showed significantly higher waist circumference compared to SGPT/SGOT ratio <1 (100.57 ± 9.34 vs 92.69 ± 10.15 cm, p<0.05) and also the same way with isobutyric (19.64 ± 6.80 umol/L vs 15.73 ± 5.21 umol/L, p<0.05).

CONCLUSIONS

It was clear enough to conclude that branched SCFAs showed a potential feature as a biomarker for MAFLD in central obesity.

Metabolic diseases

P1788

CLINICAL AND BIOCHEMICAL MANIFESTATIONS OF PATIENT'S WITH ISOVALERIC ACIDEMIA (IVA): A DECADE LONG EXPERIENCE FROM A LARGE TERTIARY CARE CENTER OF A LOW-MIDDLE INCOME COUNTRY

S. Abdul Mateen [1], H. Majid [1], S. Ahmed [1], A. Khan [1], A. Shah [1]
[1]*Aga Khan University*

BACKGROUND-AIM

IVA is characterized by a deficiency of enzyme isovaleryl-CoA dehydrogenase, leading to accumulation of isovaleric acid in the body. Our objective is evaluation of patients with IVA presenting at the BGL, AKU.

METHODS

A cross-sectional study was performed at BGL, Section of Clinical Chemistry, AKU including urine organic acid (UOA) data from Jan 2013-Dec 2023. The UOA analysis was done using ethyl acetate extraction method, derivatized by BSTFA (N, O-bis(trimethylsilyl)trifluoroacetamide), using helium gas as mobile phase in GCMS, patient's chromatogram analyzed against specific Libraries such as ORGACIDS, ORGANIS, ACID97 on scan mode. The clinical history was collected, IVA was labeled if chromatogram has peaks of 3-hydroxyisovaleric acid and Isovalerylglycine.

RESULTS

A total of 19518 urine samples were analyzed over a ten-year period, out of these 0.15% (n=29) patients were diagnosed with IVA. The median age of IVA diagnosis was 12 months (0.5-69), and 52% (n=15) males. The median blood pH, Lactate, ammonia and plasma glucose were 7.37 (7.30-7.46), 1 mmol/L (0.73-12.69), 241 ug/dl (111-304), and 95 mg/dl (61-134) respectively.
Most common clinical manifestation was lethargy 72% (n=21), followed by poor sucking 66% (n=19), vomiting 48% (n=14), septicemia 41% (n=12), hypotonia 34% (n=10), fever 28% (n=8), Developmental delay 24% (n=7), failure to thrive 21% (n=6), Mental retardation 17% (n=5), coma 14% (n=4), jaundice 10% (n=3), seizures 7% (n=2). The peaks along with marked excretion, of 3-hydroxyisovaleric acid and Isovalerylglycine were noted on the UOA chromatogram in all patients.

CONCLUSIONS

IVA remains relatively rare but clinically impactful, highlighting its significance in pediatric care in Pakistan. The diagnosis of IVA is often delayed due to its nonspecific symptoms, emphasizing the need of implementing screening programs and enhancing diagnostic capabilities to facilitate prompt management of this challenging metabolic disorder.

Metabolic diseases

P1789

A LATE ONSET L-2-HYDROXYGLURATIC ACIDURIA

I. Bilandžija Kuš [1], A. Škaričić [1], I. Križić [1], D. Šimić [1], L. Vinković [1], K. Fumić [1]
[1]*University Hospital Centre Zagreb*

BACKGROUND-AIM

L-2-hydroxyglutaric aciduria (L2HGA; OMIM #236792) is a rare autosomal recessive neurodegenerative disorder with estimated birth prevalence of less than 1:1 000 000. L2HGA is caused by mutations in L2HGDH gene, responsible for encoding mitochondrial 2-hydroxyglutarate dehydrogenase. Clinical picture is highly heterogeneous, but the most common symptoms include developmental delay, speech difficulties, epilepsy and macrocephaly, and usually appear within the first year of life. High levels of 2-hydroxyglutaric acid (2-HG) in urine, cerebrospinal fluid and plasma are the biochemical hallmark of 2-hydroxyglutaric aciduria (2-HGA). 2-HGA is a mixture of two isomers, D-2-hydroxyglutaric acid and L-2-hydroxyglutaric acid, causing two different diseases. Identification of specific enantiomers plays a decisive role in the diagnosis of these diseases. Treatment for L2HGA is only supportive, with riboflavin and L-carnitine

METHODS

Urine and plasma samples from 16-year-old male were admitted in metabolic laboratory for organic (OA) and amino acids (AA) analyses. Patient had a mild mental, motor and speech delay, learning difficulties and an episode of epileptic seizure. Brain MRI showed pathological changes in white matter. OA in urine were measured with in-house method performed by gas chromatography-mass spectrometry analysis. AA in plasma were measured using liquid chromatography coupled with tandem mass spectrometry

RESULTS

OA analysis in urine detected a massive amount of 2-HG excretion. AA analysis in plasma was unremarkable, with mild elevation of a large number of AA. Homozygous pathogenic mutation of L2HGDH gene confirmed the L2HGA diagnosis.

CONCLUSIONS

The heterogeneous clinical picture and rather low incidence make the diagnosis of L2HGA difficult. Different analytical methods for the separation and identification of 2-HG enantiomeres in urine are essential for correct diagnosis. Since there is no other therapy available other than supportive care, early recognition of the disease, with constant patient monitoring, may be beneficial to delay disease progression.

Metabolic diseases

P1790

ELEVATED INCIDENCE OF PEROXISOMAL DISEASES IN SOUTHERN SPAIN. NEWBORN SCREENING FOR X-LINKED ADRENOLEUKODYSTROPHY: EXCEPTIONAL PILOT STUDY USING HOME-MADE LC-MS/MS METHOD

M.I. Cabrera González [1], Y. De Diego Otero [1], R. Calvo Medina [2], R. Yahyaoui Macías [2]
[1]*Regional Hospital of Málaga. Metabolic Laboratory Department*
[2]*Regional Hospital of Málaga. Neuropediatric Department*

BACKGROUND-AIM

X-linked adrenoleukodystrophy (XALD) is the most common leukodystrophy and peroxisomal disease, with an estimated prevalence 1:10,000 live births. In 2013, New York became the first state to introduce newborn screening (NBS) for X-ALD. Our laboratory has started, for the first time in Europe, a universal prospective NBS pilot study for XALD from July 2022 using LC-MS/MS as a first and second tier.

METHODS

From July 2022 to January 2025, C24:0-LPC and C26:0-LPC levels were measured in DBS by an inhouse LC-MS/MS in both the first and second tier in 75,500 dot blood spots (DBS) samples. Also, biochemical and genetic analysis were carried out.

RESULTS

A total of 15 newborns were positive, being 9 males and 6 females. The first one ([1] patient), expressed variants in the HSD17B gene c.1369 A>T (p.Asn457Tyr) pathogenic heterozygous and c.1681G>C (p.Ala561Pro) likely pathogenic. The [2] patient, also for HSD17B4 gene, c.742C>T (p.Arg248Cys) homozygous including other mutation in the ACADM gene c.985A>G (p.Lys329Glu) causing comorbidity MCAD deficiency. The [3] patient, homozygous for the PEX6 gene c.2111 C>T (p.Ala704Val), VUS variant. These three patients died before six months of life. The rest of newborns were asymptomatics. Seven of them showed ABCD1 mutations. [4] patient c.761 C>T (p.Thr254Met) heterozygous pathogenic; [5] patient c.1900 G>A (p.A634T) hemizygous likely pathogenic; [6] patient c.1747 G>A (p.V583M) hemizygous likely pathogenic; [7] patient c.1415_1416delAG (p.Gln427fs) heterozygous pathogenic; [8] patient c.2111 C>T (p.Ala704Val) hemizygous VUS; [9] patient c.893G>A p.Gly298Asp heterozygous likely pathogenic; [10] patient c.872A>G p.(Glu291Gly) heterozygous pathogenic. The other five newborns are still under study.

CONCLUSIONS

Neonatal screening for XALD is an effective tool for early detection of the disorder. Our study is the first universal neonatal screening performed in Europe. The prevalence of peroxisomal disorders could be higher than estimated in our population (rate of NBS positive result detection 1/5,033). NBS for XALD allows identification of other peroxisomal diseases characterized by the increase of C26:0-LPC.

Metabolic diseases

P1791

WHEN METABOLIC DISORDERS MISLEAD: A DIAGNOSTIC JOURNEY THROUGH MEVALONIC ACIDURIA IN A PEDIATRIC PATIENT.

N. De Silva [1], K. Ranmuthupura [1], D. Perera [2], E. Jasinge [1]
[1]*Department of Chemical pathology, Lady Ridgeway Hospital for children, Colombo 08*
[2]*Pediatric unit, Lady Ridgeway Hospital for children, Colombo 08*

BACKGROUND-AIM

Mevalonic aciduria (MVA) is a rare, severe metabolic disorder caused by mutations in the mevalonate kinase (MVK) gene, leading to impaired cholesterol biosynthesis. As one of the phenotypes within the spectrum of mevalonate kinase deficiency (MVD), MVA is characterized by significant clinical heterogeneity, with manifestations ranging from febrile crises, developmental delays, and dysmorphisms, to hepatosplenomegaly, neurological deficits, and skin involvement. The diagnostic challenge arises from the wide overlap of clinical symptoms with other metabolic, infectious, and storage diseases.

METHODS

We present a challenging case of a 1-month-old female infant, born to consanguineous parents and part of a twin pregnancy, in which the other twin was miscarried early on. On her first day of life, she showed direct hyperbilirubinemia, hepatosplenomegaly, hypoglycemia, and an atypical purpuric rash, which were initially treated as neonatal sepsis. At three months of age, she presented again with a febrile illness, along with failure to thrive, hypotonia, and a cardiac lesion. By the age of three, she developed persistent cholestasis and pneumonia, which raised further concerns and led to an extensive diagnostic workup. Investigations into possible diagnoses, including various storage diseases, cystic fibrosis, familial hemophagocytic lymphohistiocytosis, and progressive familial intrahepatic cholestasis, yielded inconclusive results. Additionally, her surviving elder sibling was being evaluated for hepatosplenomegaly.

RESULTS

Despite negative findings on plasma amino acid, urine organic acids and enzyme assays, genetic testing revealed a homozygous pathogenic mutation in MVK, confirming the diagnosis of MVA.

CONCLUSIONS

Urine organic acid analysis, commonly used to identify elevated mevalonic acid excretion in suspected MVD cases, did not conclusively support the diagnosis in this instance. While urine organic acid testing can guide subsequent genetic and enzyme assays, its diagnostic specificity is limited and should not be relied upon as a stand-alone diagnostic criterion. This case underscores the importance of integrating clinical suspicion with genetic and biochemical analysis to diagnose MVA early in complex scenarios.

Metabolic diseases

P1792

MYOADENYLATE DEAMINASE DEFICIENCY AS A KEY DIAGNOSIS IN A METABOLIC MYOPATHY

M. Del Rio Rico [2], B. Lara Del Rio [2], L. Suarez Garzon [1], C. Espinar Barranco [2]
[1]*Hospital comarcal de Melilla*
[2]*Hospital Universitario virgen de las nieves*

BACKGROUND-AIM

Metabolic myopathies are a group of disorders that disrupt muscle metabolism, impairing energy production and causing weakness, cramps, and exercise intolerance. Among them, myoadenylate deaminase (MAD) deficiency is a genetic condition that affects adenosine monophosphate (AMP) metabolism, which is crucial during muscle exertion.The ischemia test with lactate and ammonia measurement is essential for diagnosing metabolic myopathies. During anaerobic exercise, increased lactate reflects normal glycolysis, while rising ammonia levels evaluate nucleotide metabolism, vital for energy regeneration. This analysis, combined with genetic studies, enables the identification of specific conditions and guides treatment, which is primarily symptomatic and preventive in this case.

METHODS

A 41-year-old man with recurrent episodes of finger stiffness in his hands, asthenia, and lower limb weakness following moderate activities. Initial examination revealed no structural or inflammatory abnormalities. Metabolic evaluation included an ischemia test, showing results indicative of a metabolic myopathy: lactate levels rose normally (from 1.2 mmol/L to 4.7 mmol/L), but ammonia showed minimal changes (from 36 μmol/L to 44 μmol/L). Additionally, creatine kinase (CK) levels were elevated (4,500 U/L)

RESULTS

Suspecting MAD deficiency, genetic studies confirmed a mutation in the AMPD1 gene, establishing a definitive diagnosis. The patient was advised to avoid prolonged exertion, stay hydrated, and consider supplements like ribose in severe cases. With these measures, the prognosis is favorable, though exercise tolerance remains limited.

CONCLUSIONS

MAD deficiency directly affects the muscle because this enzyme is essential in muscle energy metabolism. The enzyme converts AMP into IMP and ammonia, releasing the energy the muscle needs to continue functioning. In its absence, AMP accumulates and insufficient energy is produced, leading to muscle fatigue, weakness, and difficulty maintaining physical effort. This case underscores the indispensable role of the laboratory in diagnosing rare metabolic disorders. The ischemia test, by evaluating key metabolic pathways through lactate and ammonia, identified a specific alteration, while genetic studies confirmed the diagnosis. A multidisciplinary approach was vital in solving this case.

Metabolic diseases

P1793

STUDY OF N-GLYCOSYLATION AND COAGULATION DURING PREGNANCY IN A PATIENT WITH MPI-CDG TREATED WITH MANNOSE SUPPLEMENTATION

E. Lebredonchel [1], A. Bruneel [1], A. Raynor [1], K. Peoc'H [1], T. Dupré [1], S. Vuillaumier-Barrot [1], D. Borgel [2], L. Martzolff [4], C. Douillard [5], D. Desprez [3], P. De Lonlay [6]
[1]*Hopital Bichat, APHP, Paris, France*
[2]*Laboratoire d'hématologie, Hôpital Necker, Paris, France*
[3]*Laboratoire d'Hématologie, Hôpitaux Universitaires de Strasbourg, France*
[4]*Médecine Interne, Hôpitaux Civils de Colmar, France*
[5]*Service d'endocrinologie, diabétologie, maladies métaboliques et nutrition, CHU de Lille, France*
[6]*Service de pédiatrie, Hôpital Necker, Paris, France*

BACKGROUND-AIM

Congenital Disorders of Glycosylation (CDG) are a rapidly expanding group of rare inherited metabolic disorders, with over 160 distinct subtypes characterized to date. Among these, MPI-CDG (CDG-Ib) is caused by a deficiency in mannose phosphate isomerase (MPI) due to mutations in the MPI gene. This enzymatic defect disrupts the biosynthesis of GDP-mannose and lipid-linked oligosaccharide precursors—essential intermediates in the synthesis of N-glycans—thereby impairing the N-glycosylation process. Oral D-mannose supplementation has been widely implemented for MPI-CDG patients and is known to greatly improve clinical manifestations. Historically, mannose supplementation was discontinued during pregnancy in MPI-CDG patients. However, recent evidence suggests that treatment interruption can have detrimental effects on hemostasis.
For the first time, this study reports the biological follow-up of an MPI-CDG patient who continued oral D-mannose supplementation throughout her pregnancy.

METHODS

Serum and plasma samples were collected from the MPI-CDG patient to assess the glycosylation and coagulation status at multiple time points during pregnancy. Pre-pregnancy samples served as controls. The study was conducted in accordance with the ethical policies of the institution.

RESULTS

Early in pregnancy, the patient exhibited improvements in glycosylation, evidenced by an increase in 5- and 4-sialotransferrin fractions, alongside a reduction in 0-, 2-, and 3-sialotransferrin fractions. These changes were accompanied by a decrease in transaminase levels, normalization of platelet counts, and improved percentages of antithrombin and coagulation factor XI in correlation with the improvement in glycosylation.

CONCLUSIONS

The continuation of oral D-mannose supplementation during pregnancy demonstrated a significant beneficial impact on glycosylation, hemostasis, and liver function tests in the MPI-CDG patient. These findings highlight the importance and the safety of sustained mannose therapy in managing MPI-CDG during pregnancy.

Metabolic diseases

P1794

COMPARISON OF TWO MEASUREMENT PROCEDURES OF AMINO ACIDS IN CEREBROSPINAL FLUID

L. Peris Serra [1], C. Carnicer Caceres [1], N. Prades Gimeno [1], C. Perez Martinez [1], R. Ferrer Costa [1], J.A. Arranz Amo [1]

[1]*Inborn Errors of Metabolism Laboratory .Clinical Biochemistry Department. Vall d'Hebron University Hospital. Clinical Biochemistry, drug delivery and therapy Research Group. Vall d'Hebron Research Institute (VHIR). Vall d'Hebron Barcelona Hospital Campus.*

BACKGROUND-AIM

The analysis of amino acids in cerebrospinal fluid (CSF) is essential for the diagnosis of metabolic, neurological and neurotransmission disorders. On the basis of the recent implementation of the plasma amino acid quantification method by high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS), the aim of this study is to compare the current measurement procedure of amino acid quantification in CSF by liquid chromatography coupled to ultraviolet-visible spectrophotometric detector (uHPLC-UV) with that by (HPLC-MS/MS), to assess the interchangeability of results, as part of measurement procedure implementation process.

METHODS

Two 50 µl aliquots of twenty-one CSF samples from the emergency laboratory were preserved and the amino acid profile were quantified in parallel by both the Acquity® reverse uHPLC-UV method (Waters, Milford, MA) and the Chromsystems (Gräfelfing, Germany) normal phase HPLC-MS/MS method, Nexera X2 chromatograph coupled to LCMS-8050® (Shimadzu, Kyoto, Japan). For those amino acids of potential clinical interest, Bland Altman and Passing Bablok were applied using Med Calc 11 software.

RESULTS

The Bland Altman and Passing Bablok analyses revealed systematic errors in the evaluated amino acids. Both analyses found that the concentrations of several amino acids, including alanine, γ-aminobutiric acid, glycine, serine, taurine and valine, showed proportional systematic errors, while proline exhibited constant and proportional systematic errors. Consequently, they could not be considered interchangeable. By contrast, arginine, asparagine, phenylalanine, glutamine, isoleucine, leucine, lysine, ornithine and proline exhibited constant and/or proportional systematic errors in just one of the two studies.

CONCLUSIONS

The different specificity of the procedures evaluated could contribute to the differences observed, in particular the presence of constant systematic errors in several amino acids. Although difficulties in obtaining sufficient CSF volumes for the recommended verification procedures justified the adaptation of the experimental protocol, it would be desirable to increase the sample to ensure study robustness. In any case, it would be appropriate to re-estimate the reference values for all amino acids with the new measurement procedure.

Metabolic diseases

P1795

BROMHIDROSIS CAUSED BY PRIMARY TRIMETYLAMINURIA: A CASE REPORT.

A. Perojil Jiménez [1], C. Tapia Córdoba [1], M.J. Aguilar Castillo [1], M. García De Burgos [1], R. Yahyaoui Macías [1]
[1]*Hospital Universitario Regional de Málaga. Málaga. Spain*

BACKGROUND-AIM

Trimethylaminuria (TMAU), also known as fish odour syndrome is a rare and underdiagnosed metabolic disorder due to the defect in the hepatic oxidation of trimethylamine (TMA) to trimethylamine N-oxide (TMAO). TMA results from the intestinal metabolism of foods rich in choline (eggs, meat, fish, legumes, nuts and fast food) and TMAO (marine fish and crustaceans). Under normal conditions, 95% of TMA undergoes hepatic N-oxidation mediated by the enzyme flavin monooxygenase 3 (FMO3), which transforms it into TMAO, an odourless compound that is eliminated in urine.
In addition, this enzyme is involved in nitrogen metabolism and in the detoxification of other endogenous amines, tyramine, nicotine and drugs such as tricyclic antidepressants. Patients with TMAU do not degrade TMA into TMAO and there is a massive excretion of unoxidized TMA in urine, sweat, exhaled air and other bodily secretions, giving off a strong odour (bromhidrosis) reminiscent of rotten fish. This causes a high impact on the patient's personal and social life, inducing psychosocial disorders, anxiety, depressive syndromes and even suicide attempts.

METHODS

We present a case of TMAU in a 9-year-old boy, who, according to his mother, gave off a fishy odour when he ate certain foods.

RESULTS

Due to the high suspicion of this disease, a biochemical study was performed, the results of which were TMA: 5.85 mmol/mol creat (0.08-1.51); TMAO: 199.63 mmol/mol creat (15.40-186.90); TMA/TMAO: 0.029 (0.002-0.043), being compatible with a slightly increased excretion of TMA and therefore with a mild variant of TMAU.
A sequencing study of the FMO3 gene (Chr: 1q) of the patient and his parents confirmed the diagnosis by showing one pathogenic variant c.151A>G p.(Arg51Gly) and two polymorphisms c.[472G>A; 923A>G] p.[Glu158Lys; Glu308Gly]. The trans configuration of these findings would be compatible with a FMO3 deficiency causing the patient's mild TMAU symptoms.

CONCLUSIONS

Bad odour derived from TMAU can cause social rejection, so a quick diagnosis would allow for greater control over the disease. Controlled restriction of foods rich in choline and/or TMAO can reduce the odour as well as improve mood. Thus, any clinical suspicion must be confirmed by analysis of urinary TMA excretion and the study of the FMO3 gene.

Metabolic diseases

P1796

COEXISTENCE OF MCADD AND DBP DEFICIENCY: IMPORTANCE OF NEONATAL SCREENING AND GENETIC DIAGNOSIS

M. Palmero Llopis [1], C. Tapia Córdoba [2], A. Perojil Jiménez [2], M.J. Aguilar Castillo [2]
[1]*Clinical Laboratory Department, Hospital La Línea de la Concepción, Cádiz (Spain)*
[2]*Clinical Laboratory Department, Hospital Regional Universitario Carlos Haya, Málaga (Spain)*

BACKGROUND-AIM

Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCADD) and D-Bifunctional Protein (DBP) deficiency are autosomal recessive inherited metabolic diseases.
MCADD compromises the oxidation of medium-chain fatty acids in the mitochondria, causing hypoglycemia and lethargy during periods of fasting. On the other hand, DBP deficiency affects fatty acid beta-oxidation in peroxisomes, with severe manifestations such as neonatal hypotonia, seizures and developmental delay.

METHODS

This case describes a 37+4 weeks term neonate who was admitted for hypotonia, hypoglycemia and infectious risk after presenting with limb weakness. Metabolic support was started with supplemented parenteral nutrition, achieving stabilization of blood glucose levels.
She underwent neonatal screening for endocrine-metabolic diseases in dried blood by means of the heel prick test. Given the clinical suspicion and altered screening results, a genetic analysis using next-generation sequencing (NGS) was requested.

RESULTS

The results of the neonatal screening were a significant increase in C6 (Hexanoylcarnitine) and C8 (Octanoylcarnitine).
The genetic study identified two pathogenic variants in homozygosis:
- The variant c.742C>T (p.Arg248Cys) in the HSD17B4 gene confirms DBP deficiency, associated with very long chain fatty acid (VLCFA) accumulation and severe neurological damage.
- The second variant, c.985A>G (p.Lys329Glu) in the ACADM gene, is responsible for MCADD, characterized by episodes of hypoglycemia.
Clinically, the patient presented hypotonia, hyporeactivity, and spasticity, with altered electroencephalogram (EEG) and magnetic resonance imaging (MRI) findings compatible with metabolic disease.

CONCLUSIONS

This case highlights the coexistence of MCADD and DBP deficiency in a newborn with severe neurological involvement, together with the importance of early identification by neonatal screening, which is essential to prevent complications and guide appropriate treatment. Genetic diagnosis confirmed the underlying cause, allowing for improved clinical management and follow-up.

Metabolic diseases

P1797

A RARE METABOLIC PUZZLE: DIAGNOSING FANCONI-BICKEL SYNDROME IN AN INFANT WITH FAILURE TO THRIVE

N. De Silva [1], K. Ranmuthupura [1], D. Perera [2], E. Jasinge [1]
[1]*Department of Chemical pathology, Lady Ridgeway Hospital for children, Colombo 08*
[2]*Pediatric unit, Lady Ridgeway Hospital for children, Colombo 08*

BACKGROUND-AIM

Fanconi bickel syndrome (FBS) is a rare metabolic disorder, involving mutations of SLC2A2 gene encoding for facilitative monosaccharides transporter, GLUT2. The disease is characterized by hepatomegaly & renomegaly, disordered glucose metabolism, hyperlipidemia, renal Fanconi syndrome, short stature, failure to thrive & delayed puberty

METHODS

A ten-month-old baby girl, born to non-consanguineous parents was admitted for evaluation of failure to thrive noticed at the age of one month. On examination, she had a doll-like face & mild genu varum deformity. Serum studies revealed hypophosphatemia with normocalcaemia, hyperphosphatasia, hypouricemia, mildly elevated liver transaminases, hypertriglyceridemia & normal renal functions. Urine investigation depicted evidence of proximal renal tubular dysfunction (glycosuria, proteinuria, phosphaturia, hyperuricosuria and natriuresis). A skeletal survey showed features suggestive of rickets & the USS abdomen confirmed moderate hepatomegaly.

RESULTS

The diagnosis of FBS was considered & mutational analysis revealed a homozygous variant in the SLC2A2 gene. She was managed with vitamin D, phosphate supplementation & dietary advice. Due to the timely initiation of treatments, not only her growth parameters & hepatomegaly but also the biochemical abnormalities were improved during follow-up.

CONCLUSIONS

As having heterogeneous phenotypic presentations, FBS can be confused with other metabolic conditions causing Fanconi syndrome. FBS & glycogen storage disease (GSD) shares common clinical findings such as the doll-like face, hepatomegaly, renomegaly, short stature & fasting hypoglycemia. But, the evidence of renal Fanconi syndrome will distinguish FBS from all other types of GSD, except the GSD-type I. However the absence of hyperuricemia, hyperlactatemia and high creatinine kinase along with the absence of myopathic (skeletal or cardiac) symptoms in our patient helped to distinguish GSD-type I from FBS. The x-linked recessive Lowe syndrome & Dent disease were also unlikely due to the female phenotype. Proper ocular examination helped to distinguish most of the differential diagnoses such as Wilson disease, galactosemia, Lowe syndrome and cystinosis.

Metabolic diseases

P1798

DIAGNOSIS OF PROPIONIC ACIDEMIA IN A PEDIATRIC PATIENT WITH METABOLIC DECOMPENSATION

M. Del Rio Rico [2], L. Suarez Garzon [1], B. Lara Del Rio [2], C. Espinar Barranco [2]
[1]*Hospital comarcal de Melilla*
[2]*Hospital Universitario Virgen de las Nieves*

BACKGROUND-AIM

Propionic acidemia (PA) is an inherited metabolic disorder caused by deficiency of propionyl-CoA carboxylase, key in the metabolism of branched amino acids and fatty acids. This results in toxic accumulation of metabolites such as propionate, causing multisystemic manifestations. It is a rare and potentially fatal disease without timely diagnosis and treatment. Its management includes diet, pharmacotherapy and acute metabolic support. We present the case of an eight-month-old infant with metabolic decompensation secondary to AP, highlighting the essential role of the clinical laboratory in its diagnosis and management.

METHODS

An 8-month-old infant, initially diagnosed with cow's milk protein intolerance due to recurrent vomiting, came to the emergency department for cyclic vomiting and neurological deterioration. She was admitted to intensive care for metabolic decompensation, lethargy and loss of motor skills. He presented hyperammonemia (935 µmol/L), which suggested urea cycle dysfunction, explaining his neurological symptoms. Metabolic studies were performed in crisis and intravenous sodium benzoate was started to reduce ammonium. Blood gases showed metabolic acidosis with pH 7.26, bicarbonate 16.2 mEq/L and elevated GAP anion, indicating accumulation of organic acids. In addition, elevated glycine levels were detected in blood, urine and cerebrospinal fluid, suggesting organic acidemia.

RESULTS

The laboratory completed amino acid and organic acid analyses, confirming typical AP metabolites such as propionylcarnitine (C3 carnitine).
Carnitine was administered to improve fatty acid transport and eliminate toxic metabolites, along with dietary restrictions. The patient was discharged with home follow-up.

CONCLUSIONS

The clinical laboratory was crucial to identify alterations such as hyperammonemia, elevated glycine and C3 carnitine, confirming the diagnosis of AP and adjusting treatment. Its interpretation and a detailed report guided the medical management. Currently, the patient follows a multidisciplinary management adjusted to her clinical and analytical evolution. This case highlights the fundamental role of the laboratory in the diagnosis and management of metabolic diseases, being an essential pillar for diagnosis and optimal patient care.

Metabolic diseases

P1799

HYPERORNITHINEMIA AND GYRATE ATROPHY OF THE CHOROID

C. Espinar Barranco [2], B. Lara Del Río [2], L. Suárez Garzón [1], J.M. Romero Noguera [2], S. Pardo Moreno [2]
[1]*Hospital Universitario de Melilla*
[2]*Hospital Universitario Virgen de las Nieves*

BACKGROUND-AIM

A 4-year-old patient was brought to an ophthalmology consultation due to reported vision loss at school and occasional alternating exophoria. Initial ophthalmological examination revealed circumferential atrophy of the retinal pigment epithelium in the mid-peripheral fundus, with extensive spicular depigmentation and normal maculae. Retinal evaluation showed extensive patches in the mid-periphery of both eyes, suggestive of gyrate atrophy.

METHODS

After the clinical findings, the patient was referred for a genetic study in which a change in the ornithine aminotransferase gene was detected, in apparent homozygosity, classified as a variant of uncertain clinical significance.
At the same time, an amino acid metabolic study was performed in plasma and urine samples.

RESULTS

The blood amino acid profile showed a strong increase in the concentration of ornithine (932 umol/L), and the urinary amino acid profile showed a very marked increase in ornithine (10651 umol/g), accompanied by elevation of other dibasic amino acids such as lysine (4156 umol/g), arginine (617 umol/g) and cystine (717 umol/g), which share the same transporter as ornithine for its tubular reuptake.
These analytical determinations unequivocally allowed the diagnosis of gyrate atrophy.

CONCLUSIONS

Retinal dystrophies are a series of hereditary diseases that lead to degeneration of the photoreceptor cells and cause vision loss. They are rare and congenital diseases, almost always bilateral, affecting both eyes more or less symmetrically.
There are several types of retinal dystrophies, the most frequent being retinitis pigmentosa, whose symptoms are indistinguishable from those of patients with gyrate atrophy.
Since the result of the genetic study was inconclusive, the determination of amino acids in blood and urine was essential in the detection of hyperornithinemia to establish the diagnosis, treatment and follow-up of the disease.

Metabolic diseases

P1800

HYPERAMMONEMIC ENCEPHALOPATHY IN AN EPILEPTIC PATIENT TREATED WITH VALPROIC ACID. A CASE REPORT.

I. Osuna Benavente [1], F.d.B. Tellez Hernández [1], A.I. Allo Lois [1], M. Jiménez Barragán [1], A. León-Justel [1]
[1]*Clinical Biochemistry Department, Virgen Macarena University Hospital, Seville*

BACKGROUND-AIM

Valproic acid (VPA) is a broad-spectrum anticonvulsant used in the treatment of epilepsy. Among the rare complications of VPA treatment, hyperammonemic encephalopathy can occur following acute overdose or chronic use of VPA.

METHODS

A 39-year-old female patient with a history of juvenile myoclonic epilepsy, who had been treated with topiramate and valproic acid, seizure-free for 10 years. On arrival at the emergency department, the patient's mother reported a five-day history of self-limited seizures, with minimal postictal state, which had become increasingly frequent and had been accompanied by marked interictal somnolence. VPA dosage was reduced five months prior to the onset of the present seizures, due to the previously well-controlled epilepsy.

RESULTS

The reduction in VPA dosage was proposed as a potential trigger for the seizures, leading to valproic acid infusion. Despite it, the patient continued to experience multiple seizures. Given the suspicion that the underlying cause of the increased seizure activity might have been misidentified, blood ammonia levels were then measured, revealing a value of 213.0 µg/dL (18.7–86.9 µg/dL). The final diagnosis was VPA-induced hyperammonemic encephalopathy.

CONCLUSIONS

Although hyperammonemic encephalopathy is a uncommon complication, early recognition is crucial, as it can lead to loss of consciousness (often misinterpreted as a consequence of increased seizure activity in epileptic patients), coma, and increased seizure frequency.

Metabolic diseases

P1801

PEDIATRIC PATIENT WITH GLYCEROL KINASE DEFICIENCY: A CASE REPORT.

A. Perojil Jiménez [1], C. Tapia Córdoba [1], M. Palmero Llopis [1], M.J. Aguilar Castillo [1], M. García De Burgos [1], R. Yahyaoui Macías [1]

[1]*Hospital Universitario Regional de Málaga. Málaga. Spain*

BACKGROUND-AIM

Glycerol Kinase deficiency (GKD) is a rare genetic X-linked recessive inheritance due to a mutation or deletion in GK gene on chromosome Xp21.3. It is characterized by the presence in patients with isolated and persistent hypertriglyceridemia, without serum turbidity and without the presence of chylomicrons or increased VLDL. In addition, a lack of response to triglyceride-lowering therapy is observed, and in some cases the association with alterations in glucose metabolism or insulin resistance.
Thus, these levels are falsely elevated (pseudo-hypertriglyceridemia) as a result of the laboratory technique used to determine triglycerides, which is based on determining glycerol levels, which are elevated in serum due to the enzyme deficiency.
Clinical expression varies from asymptomatic form with isolated hyperglycerolemia to severe/fatal form with Congenital Adrenal Hyperplasia or Duchenne Muscular Dystrophy.

METHODS

Our case focuses on a 14-year-old boy who presented focal epilepsy, marked thinness and persistent triglyceride levels higher than 500 mg/dL without response to rosuvastatin.

RESULTS

Due to these clinical signs, a genetic analysis was performed by massive sequencing directed at genes involved in hypertriglyceridemia including screening for lipoprotein lipase deficiency. The results identified the homozygous presence of a pathogenic delection in the GK gene (chrX:30671645-30746866) which can justify the clinical phenotype of the patient. The protein encoded by this gene is key in regulating glycerol uptake and metabolism. Pathogenic variants in this gene are associated with GKD (OMIM: 307030). Furthermore, subsequent measurement of serum glycerol by GC-MS confirmed the diagnosis.

CONCLUSIONS

Glicerol kinase deficiency (GKD) is a rare genetic disorder that it is usually diagnosed by occasional analytical findings and should be suspected in patients with adequate weight and a healthy lifestyle who have maintained serum/urine triglyceride concentrations without any other altered lipid profile magnitude and who do not respond to lipid-lowering drugs.
A rapid interpretation of these results by the analyst is crucial to detect this disease and improve the patient's life.

Metabolic diseases

P1802

INTRONIC VARIANTS AND THEIR ROLE IN SPLICING DEFECTS: A CASE OF PHENYLKETONURIA

J. Sánchez Cortés [3], M.J. Aguilar Castillo [2], A. Perojil Jiménez [2], J. Luaces Puente [1], M. García De Burgos [2], R. Yahyaoui Macías [2]

[1]*Clinical Laboratory Department / Complejo Asistencial de Segovia*
[2]*Clinical Laboratory Department / Hospital Regional Universitario de Málaga*
[3]*Clinical Laboratory Department / Hospital Universitari Sant Joan de Reus*

BACKGROUND-AIM

Phenylketonuria (PKU) is a genetic disorder that leads to elevated levels of phenylalanine in blood. Phenylalanine is an amino acid found in various foods, including meat, eggs, nuts, as well as certain artificial sweeteners. The enzyme phenylalanine hydroxylase plays a crucial role in converting phenylalanine into tyrosine (Tyr).

METHODS

We present the case of a newborn at 39 weeks of gestation referred to the pediatric gastroenterology unit due to suspected hyperphenylalaninemia detected through neonatal screening program. Phenylalanine levels in a dried blood sample taken at 48 hours of life were 604 µmol/L (normal range: 54–116 µmol/L), with a Phe/Tyr ratio of 11.2 (normal range: 0.25–1.8). These results were confirmed in a follow-up plasma sample 15 days later, showing a phenylalanine level of 2724 µmol/L (normal range: 31–77).
These findings are consistent with a potential diagnosis of classic PKU. To further investigate, a clinical exome analysis was performed, targeting six genes associated with hyperphenylalaninemia: GCH1, PAH, PCBD1, PTS, QDPR, and SPR.

RESULTS

The c.932T>C (p.Leu311Pro) variant, classified as pathogenic, was identified in the PAH gene (NM_000277.3:c.932T>C). As PKU follows an autosomal recessive inheritance pattern, the presence of two pathogenic variants is required for the disease to manifest.
Subsequently, a multiplex ligation-dependent probe amplification analysis was performed to check for deletions/duplications linked to PKU, but none were detected in the analyzed gene. Given the strong diagnostic suspicion, the previously performed clinical exome was re-examined, leading to the identification of a second variant in the PAH gene. This intronic variant, c.1066-11G>A, associated with abnormal splicing of this gene, was subsequently confirmed through Sanger sequencing.

CONCLUSIONS

The patient is a carrier of two heterozygous variants. Confirmation of a trans configuration would establish a diagnosis of PKU. As his father is unavailable for testing, identifying one of the two variants in the mother would validate the biallelic origin of the disease. To date, this genetic study remains pending. This case highlights the importance of considering intronic variants that affect splicing in autosomal recessive diseases, particularly when clinical suspicion is high.

Metabolic diseases

P1803

PROSPECTIVE SCREENING OF WILSON DISEASE IN PRIMARY SCHOOL CHILDREN USING SPOT URINE: AN UNEXPECTED SUCCESS IN CASE DIAGNOSIS IN A PILOT STUDY

N. Tang [3], J. Hui [1], I. Chan [1], R. Chiang [1], A. Wang [1], K. Belaramani [2], A. Li [1], C. Fung [2], A. Kwok [2]
[1]*Chinese University of Hong Kong*
[2]*Hong Kong Children Hospital*
[3]*Li Ka Shing Institute of Health Sciences, Chinese University of Hong Kong*

BACKGROUND-AIM

Wilson disease (WD) is prevalent in the Chinese population. However, universal screening for WD, particularly for newborns, poses challenges because prior exposure and accumulation of tissue copper are minimal before birth. Recently, an ultra-sensitive assay for ATP7B protein was used in NBS.
WD symptoms typically occur after puberty in most patients, as tissue copper takes time to accumulate to toxic levels. This 10-year time gap provides a unique opportunity for screening and pre-symptomatic treatment with zinc.
In our prior retrospective study, we developed reference ranges for spot urine indexes for children. These cut-off values were evaluated in a new prospective cohort, leading to diagnosis of 2 asymptomatic WD.

METHODS

Over 200 healthy children aged between 4-11 years were recruited, among them 194 returned at least one spot urine sample. And 176 also returned a 24-hour urine collection. Urine copper was measured by ICPMS by Agilent 7700. Urine creatinine and osmolality were measured in the hospital lab. Samples with high urine total protein were excluded as ceruloplasminuria leads to false positive urine screening.

RESULTS

Prior developed cut-off values for 3 spot urine indexes were evaluated: spot urine copper>0.5 umol/L, copper to osmolality ratio>0.00085 and copper to creatinine>0.1 umol/mmol. Several other children with proteinuria were picked up. 11 children exceeded at least one cut-off and were called back for blood ceruloplasmin and copper as second-tier test (call back rate ~6%). Two children (siblings) had very low ceruloplasmin and were confirmed WD by mutation.

CONCLUSIONS

Spot urine copper in children is useful in universal screening of WD. This small pilot study unexpectedly diagnosed 2 patients despite a small sample. Urine copper 0.5>umol/L is the most reliable screening cut-off.

Metabolic diseases

P1804

ANALYSIS OF AMINO ACIDS IN CEREBROSPINAL FLUID BY LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY: ASSESSMENT OF LIMITS OF QUANTIFICATION.

L. Peris Serra [1], G. Gonzalez Silva [1], M. Macaya Font [1], J. Arnedo Alcántara [1], J.A. Arranz Amo [1], R. Ferrer Costa [1], C. Carnicer Caceres [1]

[1]*Inborn Errors of Metabolism Laboratory .Clinical Biochemistry Department. Vall d'Hebron University Hospital. Clinical Biochemistry, drug delivery and therapy Research Group. Vall d'Hebron Research Institute (VHIR). Vall d'Hebron Barcelona Hospital Campus.*

BACKGROUND-AIM

Measurement of amino acids in cerebrospinal fluid (CSF) is useful for the diagnosis of several inherited metabolic disorders. Concentrations of most amino acids in CSF are significantly decreased compared to those in plasma (ranging from 1 to 25%), and quantification of concentrations below the lower limit of the reference interval (LoQ) is crucial for detecting some of these disorders. At present, there are no commercial kits specifically designed for CSF matrix. Instead, commercial plasma kits are used for this purpose, which are adapted to its higher concentrations. Therefore, it is important to ensure reliable quantification in the CSF concentration ranges. Hence, the aim of this study is to establish the LoQs from the material supplied in the plasma kit, to adjust the measurement ranges for CSF.

METHODS

Quadruplicate determinations from 1/2 to 1/14 dilution from the lowest calibator and control levels were performed in the amino acids with most clinical interest in CSF. LoQ calculations were estimated according to CLSI EP17-A2:2012 guideline and was set based on the precision and relative deviation from nominal concentration criteria (CV < 20% and dev < 15%, respectively). The Nexera X2 Chromatograph coupled to a Shimadzu LCMS-8050 MS/MS (Shimadzu, Japan) and the commercial kit MassTox® Amino Acid Analysis in plasma/serum - LC-MS/MS (Chromsystems® Gräfelfing,Germany) were used.

RESULTS

The LoQ for aminoacids (mean in µmol/l) that met specifications were set at: alanine (23.6), asparagine (8.7), phenylalanine (5.6), GABA (0.1), glycine (13.8), lysine (4.5), serine (14.8), leucine (3.3), valine (12.0), ornithine (5.0), arginine (16.2), glutamine (176.9), isoleucine (10.9), proline (21.4), glutamic acid (40.9). For these amino acids the estimated LoQs were significantly lower than the concentrations of the lowest calibrator or control levels established in the plasma kit.

CONCLUSIONS

The findings of this study demonstrate that the adjustment of the measurement range for the amino acids evaluated has been accomplished, leading to a decrease in the minimum concentration at which they can be quantified in accordance with the established acceptance criteria. This has resulted in an enhancement in the estimation of reference intervals for the majority of amino acids in CSF.