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Inherited disorders - Metabolic disorders

Cod: W007

PREVALENCE OF SICKLE CELL TRAIT IN THE SOUTHERN SUBURB OF BEIRUT , LEBANON

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OBJECTIVE:
The objective of this study was to assess the prevalence, gender differences, and time trends of Sickle Cell Trait in the Southern Suburb of Beirut, Lebanon; as well as to highlight the importance of screening for Sickle Cell Trait carriers in this population.
Another objective was to describe a New screening technique for Sickle Cell Trait carriers.

METHODS:
This was a retrospective cohort study carried out at a private laboratory in the Southern Suburb of Beirut, Lebanon between 2002 and 2014. The sickling test was carried out for each patient using two methods: the Classical "sodium metabisulfite sickling test", and the New "sickling test method" used in the private lab. As a confirmatory test, Hemoglobin electrophoresis was run on a random sample of 223 cases which were found to be positive using both sickling tests.
RESULTS:
A total of 899 cases were found to be positive for the sickle cell trait out of 184,105 subjects screened during the 12-year period, prevalence = 0.49% (95% CI: 0.46 - 0.52). Among the total sample, females were found to have higher prevalence, where no time trend over the studied period was noted. The haemoglobin electrophoresis method confirmed the results of this new sickling test technique among the random sample of the 223 cases.

CONCLUSION:
We found that the prevalence of sickle cell trait is lower as compared to other Arab countries, higher in females,with no significant time trend. The New sickling test was found to be an accurate, simple and cheap test that could be easily added as a requirement for the Prevalence of Sickle Cell Trait in the Southern Suburb of Beirut, Lebanon.

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RESEARCH INTO LYSOSOMAL STORAGE METABOLISM USING PLASMA LIPID CHARACTERIZATION BY LC-DIFFERENTIAL MOBILITY SPECTROMETRY-MS/MS

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Introduction

Research into plasma sphingolipids and determining the concentrations of such is of growing importance in the clinical research laboratory, particularly within groups researching Lysosomal Storage Metabolism. Current methods of analysis primarily involve either enzyme activity procedures or derivatization of compounds prior to analysis. Direct analysis of these groups can be complex due to extensive structural homogeneity between individual compounds. We investigate here the possibility to use Differential Ion Mobility to separate and quantify these compounds under simple HPLC conditions.

The plasma lipids lysosphingomyelin (SPC), Glucosylsphingosine (Glu-SPH), Psychosine (Gal-SPH) and Globotriasosylsphingosine (Lyso Gb3) were analysed. Research indicates that levels of these lipids are elevated in the lysosomal storage conditions Niemann-Pick C, Gaucher, Krabbe and Fabry respectively. C17-SPC was added as an internal standard.

Materials and Methods

Sample Preparation:
Extraction was achieved by protein precipitation/direct injection approach utilizing 100µL of plasma

HPLC Conditions:
Short chromatography was provided by a C8 column and a gradient of acidified acetonitrile/water

MS/MS Conditions:
Sciex Triple Quad™ 6500+ fitted with SelexION® DMS Technology, operating in Positive Low Mass MRM

Results

• Ion Mobility can separate the stereoisomers Glu-and Gal-SPH in extracts without the need for chromatographic separation
• Ion Mobility has been shown to remove interferences that can cause to misinterpretation of the results
• Results and statistics in plasma show good Accuracy (88 – 105%) Precision (1.5 – 19%)and Linearity (>0.997) with sensitivity of all compounds in plasma significantly below 0.1ng/ml

Conclusions

• We have presented here proof-of-concept results for the direct analysis of a series of sphingolipids in plasma using a simple protein precipitation, standard chromatography and Differential Ion Mobility Spectrometry.
• The proposed method offers advantages over enzyme activity methods as incubation is unnecessary.
• The proposed method shows significant advantages over advanced chromatographic separation techniques and uses standard, conventional and short HPLC.
• LC-DMS-MS/MS shows good analytical performance across relevant concentration ranges.
• Research into these compounds as biomarkers of lysosomal storage metabolism is ongoing.

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Cod: W010

NEWBORN SCREENING FOR CONGENITAL HYPOTHROIDISM IN TURKEY: A REGIONAL EVALUATION.

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Congenital hypothroidism (CH) is the most common congenital endocrine disorder and the most important cause of preventable mental retardation. It is important to begin the treatment within 2 weeks before the development of brain damage. TSH based newborn screening programs are shown to be useful for implementing early treatment of CH. Besides permanent CH, primer neonatal TSH levels are useful for detecting transient CH and iodine status of the study area. In this study, regional results of CH screening program in Turkey between 2006 – 2015 were assessed retrospectively. We have evaluated the results of Marmara, Central Anatolia, Aegean and Mediterranean regions in which our laboratories are located.
Screening was based on TSH determination in dried blood spot specimens taken from heel capillary blood sample and TSH limits determined to be 10 μu/mL for cut off point and 25 μu/mL for clinical decision point. TSH was measured using enzyme immune assay (EIA). Blood spot TSH data for 65.857 newborns during this time period were evaluated. Permanent or transient CH was determined according to the results of thyroid function tests. Confirmed CH cases were based on local endocrinologists' report and initiation of thyroxine treatment.

The frequency of neonatal TSH levels were found to be under the cut off level of 10 μu/mL in 63513 (96,44%), between 10 – 25 μu/mL in 2287 (3,47%) and above the level of 25 μu/mL in 57 (0,09%) babies, respectively. Recall rate was 3,5%. CH cases of neonatal TSH levels greater than 25 μu/mL was 26. The incidence of CH of this group was 1:2532. There were no significant differences in the number of congenital hypothyroidism between males and females (P> 0.05). Gender ratio was 7/6 (female/male).

The preliminary results of our study indicate that the incidence of CH in our region is higher than the worldwide reports as has been proved by preceding studies. Iodine deficiency, dyshormonogenesis, highly consanguineous population may contribute to the high incidence of CH in Turkey. Newborn screening of CH must be developed for detecting true cases and TSH cut off point must be reviewed for decreasing redundant recall rate. Further studies are needed to identify other factors associated with increasing incidence of CH.

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THE SWEAT TEST FOR CYSTIC FIBROSIS DIAGNOSIS AND THE INFLUENCE OF SALBUTAMOL

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Introduction
Nowadays, the diagnosis of cystic fibrosis is based only on the sweat test which is carried out in Algiers, Oran and Constantine. Most of the children who come for the sweat test, have respiratory manifestations and are under Salbutamol. The dominance of the respiratory pattern, the significant number of patients under Salbutamol "Ventolin" and the recommendation of a therapeutic window annoying for the sick have formed the starting point for our study which main objective is to confirm or deny the interference of this medicine on the dosage of sweat chlorides.
Materials and Method
-In Béni-Messous laboratory, Algiers,the sweat test is performed in three steps:
. Stimulation of the sweating: Method of Gibson and Cooke: the stimulation of the sweating is done by iontophoresis to pilocarpine
. Collection of the Sweat: on paper Wattman
. Dosage: After the step of stimulation, the dosage of chloride ions is performed by titrimetry of Schales and Schales (mercurimetry)
Results
- It is observed that the percentage of positivity in our study population during a year is 1%.
- For the positive results, there is a slight male predominance with 6 boys and 5 girls.
- Nearly a quarter of the study population are derived from a marriage inbred.
- More than half of the patients who have come suffer from respiratory disorders.
- We note that Salbutamol therapy occupies the first place in the treatment of patients greeted with the highest percentage follow-up of the salted serum then corticosteroids
-we could include 26 cases which were under Salbutamol and for whom a second dosage outside of any treatment has been performed.
- With the help of the GraphPad Prism 6.0 and use the Mann-Whitney test, we could demonstrate that there is no significant difference between the two groups with and without Salbutamol with a P= 0.95.
Conclusion
- Based on its review key to diagnosis, represented by the sweat test, we could save 11 cases of cystic fibrosis during the period from 03/2015 to 03/2016.
- In the second part of our work, the results have shown that the Salbutamol does not overestimate the test values of the sweat and does not interfere on the rate of sweat chlorides (P=0.95), therefore the use of the therapeutic window for this bronchodilator is not necessary in the case of the completion of the sweat test.

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LC-MS/MS MEASUREMENTS OF URINARY GUANIDINOACETIC ACID AND CREATINE: METHOD OPTIMIZATION BY DELETING DERIVATIZATION STEP.

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Creatine deficiency syndromes include three hereditary diseases affecting the metabolism of creatine (Cr): Guanidinoacetate methyltransferase deficiency, arginine glycine amidinotransferase deficiency and the deficiency of creatine transporter. These pathologies cause a brain creatine deficiency responsible of non-specific neurological impairments with mental retardation. LC-MS/MS measurements of guanidinoacetic acid (GA) and Cr in urine and plasma are useful for the diagnosis and the identification of one of the deficits. The analysis of these polar and basic molecules is difficult. For the study of these substances, which are not retained on standard column, the reference method requires a derivatization step. To overcome this long and fastidious derivatization, an ion pairing method was chosen. The principle is to add an opposite charging ion to the molecules of interest in the two mobile phases to form an uncharged complex. The opposite charging ion will interact via its hydrophobic chain with the organic stationary phase causing a stronger retention of the complex.

Material and methods:A range of calibration was chosen to frame the biological and pathological values. Those solutions, the internal quality controls and the urines of the patients were diluted to 1/20th in an aqueous solution of internal standard. After agitation, they have been analyzed by LC-MS/MS. To validate this method, the COFRAC (comité français d'accréditation) recommendations has been used as reference and adapted to the specific criteria of chromatographic dosages.

Results: The coefficient of variation (CV) realized on one day were representative of repeatability of the results while CV realized on several days were representative of reproducibility. Every of them were lower than 15% which proves the accuracy of our method. Precision, estimated by external quality samples, was good with biases lower than 15%. The comparison between our method and the reference method showed the same results. Furthermore the time to prepare the samples before LC-MS/MS was shortened, moving from 2h with the former technique to 30 min with this new method. This is a technical timesaving of 75% combined to a significant technical simplification.

Conclusion: In the dosage of GA and Cr by LC-MS/MS, the use of ion-pairing technique we have developed leads to a technical simplification of the method and to a saving of time. It appears to be an optimization of the current method.

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QUANTITATIVE ANALYTICAL METHOD FOR THE DETERMINATION OF BIOTINIDASE ACTIVITY AND DETECTION OF BIOTINIDASE GENE MUTATIONS IN IRANIAN PATIENTS

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Background: Biotinidase deficiency (BTD) is an autosomal recessive disorder of biotin metabolism. Biotin is a coenzyme that enhances the action of four enzymes that play an important role in carbohydrates, amino acid, and fatty acid metabolism. Defects in these pathways can cause severe metabolic disorder in the body. In general, biotinidase deficiency can be classified into two levels: partial and profound. The incidence of BTD is 1:40,000 to 1:60,000 births in the world, even though no convincing statistical data on the prevalence of this disorder exist in Iran. Our aim in this study was to set up a test to determine biotinidase activity in the Iran population and report BTD mutations.

Materials and Methods: Quantitative method was performed as previously described in the Laboratory Guide to the Methods in Biochemical Genetics 2008 by spectrophotometric method. To detect mutations in BTD, we performed polymerase chain reaction (PCR), followed by DNA sequencing.

Results: Reference range values were 3.81–8.25 nmol min−1.ml−1. We identified 8 BTD patients out of 47 cases with neurologic signs. We detected two mutations, c.98-104del7ins3 and R79C, in 5 patients with profound BTD, and one D444H mutation in 3 patients with partial BTD.

Conclusion: Infants suffering from BTD seem healthy during their first months of life. At present, the screening program for metabolic disorders such as BTD is in progress. The patients that are BTD deficient benefit from the availability of the tests, and consequently, receive the Biotin tablets before being clinically affected.