UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI
PENYELIDIKAN
LAPORAN AKHIR

URINARY SCREENING TO DETERMINE THE POSITIVITY OF DMB AND HIGH VOLTAGE ELECTROPHORESIS OF MUCOPOLYSACCHARIDOSIS IN SUSPECTED METABOLIC DISORDERS CHILDREN IN HUSM

PENYELIDIK

DR. JULIA OMAR

PENYELIDIK BERSAMA

-

2013

UNIVERSITI SAINS MALAYSIA

Jabatan Patologi Kimia
Department of Chemical Pathology

Jabatan Patologi Kimia

Pusat Pengajian Sains Perubatan
Kampus Kesihatan
Universiti Sains Malaysia
16150 Kubang Kerian,
Kelantan, Malaysia.
Tel : 609 767 6504
Fax: 609 765 3370 / 609 767 3333
www.medic.usm.my

3 Oktober 2013

Angie Chong Phey Yuen
Pegawai Penyelidik
Unit Pengurusan Geran & Pembangunan Penyelidikan
Universiti Sains Malaysia
Aras 6 Bangunan Canselori
11800 Pulau Pinang

LAPORAN AKHIR GERAN PENYELIDIKAN JANGKA PENDEK

Tajuk : **Urinary Screening to Determine the Positivity of DMB and High Voltage Electrophoresis of Mucopolysaccharidosis in Suspected Metabolic Disorders Children in HUSM.**

No. Akaun : **304/PPSP/6131512**

.....

Adalah dengan segala hormatnya perkara di atas dirujuk dan Rujukan surat tuan 2007/337 (P2805) yang bertarikh 25 September 2013 adalah berkaitan.

Bersama-sama ini disertakan satu set penerbitan untuk makluman serta tindakan pihak tuan selanjutnya.

Sekian, terima kasih.

"BERKHIDMAT UNTUK NEGARA"
`Memastikan Kelestarian Hari Esok'

Sciknow Publications Ltd.
Health Care

HC 2013, 1(1):1-6
DOI: 10.12966/hc.XX.XX.2013

Urinary Screening of Mucopolysaccharidoses (MPS) in Cases Suspected of Inborn Errors of Metabolism Children in HUSM

J.Omar[1,*], S.Abbas[1], Azizah Shaari[1], Zalina Yaacob[1], Norizam Yusof[1], Win Mar Kyi[1], M.R.Abdullah[2]

[1]Department of Chemical Pathology &[2]Department of Community Medicine, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan

[*]Corresponding Author (Email: julia@kb.usm.my)

Abstract

Introduction: Mucopolysaccharidoses (MPS) is a disease of inborn errors of metabolism (IEM). It constitutes a large and heterogenous subgroup among the lysosomal storage diseases. For the detection of this disease, urinary glycosaminoglycan (GAG) is measured by dimethlmethylene blue (DMB) assay and high resolution electrophoresis (HRE) is done to characterize the different types of MPS.

Objective: To screen for MPS in all urine samples suspected of inborn errors of metabolism sent to the metabolic laboratory from 1998 to 2008 and to look into the association between the positivity of DMB assay and HRE in the same sample

Group Method: All urine samples sent to the metabolic laboratory that fulfilled the inclusion criteria were analyzed for GAG and were further subjected to HRE. Measurement of observed agreement using Cohen's kappa coefficient (k) was used for the association between both DMB and HRE method.

Results: A total of 134 urine samples were obtained however only 90 samples were analyzed. 28 (31.1%) of the samples had normal GAG levels, 59 (65.6%) had GAG levels between 1-2 folds above normal limits and 3 (3.3%) samples had more than 2 folds increment above the normal limits for age. Three samples showed abnormal bands when subjected to HRE. Poor association between both methods (k = 0.027) was observed.

Conclusion: This study shows that all samples with high index of suspicion of MPS and elevated values of GAG of two folds or more should be subjected to HRE. Characterization of MPS through HRE gives the clinician some presumptive diagnosis and prognosis in managing these patients.

Keywords - Glycosaminoglycans (GAGs), Mucopolysaccaridoses (MPS), Dimethylmethelene Blue (DMB), High Resolution Electrophoresis (HRE)

1. Introduction

Glycosaminoglycans (GAGs) are complexes of polysaccharides. They are found in small amounts in the mammalian urine as chondroitin sulfate (CS), dermatan sulfate (DS), heparin sulfate (HS), keratan sulfate (KS) and heparin (Hep). They serve as structural and protective function and are found in various tissues such as cartilage, bone, cornea, synovial fluid and many others.

GAGs are degraded in the lysosomes within the cells by various enzymes. Deficiencies of these enzymes results in accumulation of GAGs in the cells. Accumulation of GAGs within the cells in organs causes multiple organ dysfunctions and leads to excretion of high amounts of GAGs in the urine.

The disease of this disorder, known as Mucopolysaccharidoses (MPS), constitutes a large and heterogenous subgroup among the lysosomal storage diseases. MPS predominantly occurs between 9 months to 4 years of age. The commonest symptoms are coarse facies, organomegaly, growth retardation, bone abnormalities and in some cases neurological degeneration. MPS children have a wide range of clinical symptoms depending on the individual disorder and degree of severity.

Laboratory screening for detection and differentiation of the different types of MPS is important in the management of MPS. The commonest screening method employed is by measuring urinary GAG, using 1, 9 dimethlmethylene blue (DMB) assay developed by Whitley et al [1]. This assay is simple, sensitive and suitable for screening. The values obtained through this method are aged-dependent.

In our metabolic laboratory, samples with high levels of GAGs in the urine are further subjected to high resolution electrophoresis (HRE). Characterization of MPS by HRE method helps clinicians in making a presumptive diagnosis on the type of MPS since enzyme assays are not available in the country.

The objective of this study was to screen all urine samples suspected of IEM sent to the metabolic laboratory from 1998 to 2008 for MPS. We also examined for possible association

between the positivity of DMB and HRE in the same sample group to look for an agreement between the two methods.

2. Materials and Methods

2.1 Urine Samples

All urine samples of patients suspected to have IEM received at the metabolic laboratory HUSM from the year 1998 to 2008 were analyzed. The urine samples were frozen and kept at -70°C without preservatives. They were thawed on the day of analysis.

MPS-positive sample was obtained from a MPS II patient confirmed through enzymatic assay done at an IEM reference laboratory in Taiwan. The sample was kept frozen -70°C until analyzed. The MPS-negative samples were obtained from normal children collected from the nursery of HUSM which consist of children from the age of 1 to 5 years and students of primary and secondary schools.

2.2 1, 9-Dimethylene Blue (DMB) Assay

The DMB assay used was based on non-automated method by Whitley *et al.*[1] with modifications described by de Jong *et al.* [2]

2.3 Assay Procedure

The chemicals were obtained from Sigma Chemical Co. and BDH Chemicals limited. All the assays were in duplicates and were analyzed on PharmaSpec UV- 1700 spectrophotometer from Shidmadzu, Japan.

The stock color solution was prepared by dissolving 12.2mg of DMB in 1ml of 95% ethanol and 0.2M of sodium formate buffer (pH3.5). Immediately prior to analysis, 10ml of stock dye solution was added to 90ml of sodium formate buffer (pH3.5). Aqueous standard solution of heparan sulphate was prepared and used as internal quality control. Heparan sulphate was chosen as internal standard based on stability described in de Jong et al. [2]. Standard curve was plotted with various concentration starting from 25,50,75,100,125,150 to 200 ug/ml.

The thawed urine samples (40ul) were mixed in 1 ml of colour reagent and the absorbance was read immediately at 525nm. All results were expressed in mg/L and the ratio of quantitative GAG to urine creatinine was expressed in mg/mmoL creatinine.

2.4 Quantification of Creatinine

Biochemical analysis for creatinine estimation was performed on automated chemistry analyzer, Hitachi 912, Boehringer Mannheim from Jerman using Jaffe method. Urine samples with a creatinine value of less than 0.88mmol/L were excluded from the study and low volume urine samples which were insufficient to perform the assay were also excluded from this study.

2.5 High Resolution Electrophoresis (HRE)

For the electrophoresis, cellulose acetate Titan III was obtained from Helena Laboratories, United States and the electrophoresis set used was Multiphor III with EPS 350 XL from Pharmacia Biotech from Sweden.

High-resolution electrophoresis method used to separate fractions of GAGs was based on Hopwood and Harrison method.

MPS-positive sample was applied and other standards were spiked into one of the urine sample. MPS-negative sample were also applied as negative control. All the urine samples were subjected to HRE

2.6 Statistical Analysis

SPSS version 12.0 was used to analyze the data obtained and measurement of observed agreement for categorical data using Kappa method was used to look into the association between the positivity of DMB and HRE.

3. Results

Out of a total 134 urine samples obtained, only 90 samples fulfilled the study inclusion criteria and were screened for MPS by the DMB method. The age of the patients ranges from 1 day to 16 years old.

Out of 90 samples, 28 (31.1%) samples had normal GAG concentration and 62 (68.9%) samples had high GAG concentration (i.e. above the action limits for age).