UNIVERSITI SAINS MALAYSIA

LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA *PENDEK*

FINAL REPORT OF SHORT TERM RESEARCH PROJECT

Sila kemukakan laporan akhir ini melalui Jawatankuasa Penyelidikan di Pusat Pengajian dan Dekan/Pengarah/Ketua Jabatan kepada Pejabat Pelantar Penyelidikan

1. Nama Ketua Penyelidik:
Name of Research Leader

☐ Profesor Madya/ *Assoc. Prof.* ☑ Dr./ *Dr.* ☐ Encik/Puan/Cik *Mr/Mrs/Ms*

2. Pusat Tanggungjawab (PTJ):
School/Department
Jabatan Patologi Kimia

3. Nama Penyelidik Bersama:
Name of Co-Researcher
Dr Win Mar Kyi & PM Dr Mohamed Rusli Abdullah

4. Tajuk Projek:
Title of Project
Urinary Screening to determine the positivity of DMB and High Voltage (resolution) Electrophoresis of Mucopolysaccharidosis in suspected metabolic disorders children in HUSM

5. Ringkasan Penilaian/*Summary of Assessment*:	Tidak Mencukupi *Inadequate*		Boleh Diterima *Acceptable*	Sangat Baik *Very Good*	
	1	2	3	4	5
i) Pencapaian objektif projek: *Achievement of project objectives*	☐	☐	☐	√	☐
ii) Kualiti output: *Quality of outputs*	☐	☐	☐	✓	☐
iii) Kualiti impak: *Quality of impacts*	☐	☐	☐	✓	☐
iv) Pemindahan teknologi/potensi pengkomersialan: *Technology transfer/commercialization potential*	✓	☐	☐	☐	☐
v) Kualiti dan usahasama : *Quality and intensity of collaboration*	☐	☐	☐	✓	☐
vi) Penilaian kepentingan secara keseluruhan: *Overall assessment of benefits*	☐	☐	☐	✓	☐

6. **Abstrak Penyelidikan**
(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Inggeris**. Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

Abstract of Research
(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English).
This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

Please see attachment

7. **Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.**
[Sila gunakan kertas berasingan]
Applicant are required to prepare a Comprehensive Technical Report explaning the project.
(This report must be appended separately)

Senaraikan kata kunci yang mencerminkan penyelidikan anda:
List the key words that reflects your research:

Bahasa Malaysia	*Bahasa Inggeris*
	DMB (Dimethylene blue)
	HRE (high resolution electrophoresis)
	MPS (mucopolysaccharidosis)

8. **Output dan Faedah Projek**
Output and Benefits of Project

(a) * **Penerbitan Jurnal**
Publication of Journals
(Sila nyatakan jenis, tajuk, pengarang/editor, tahun terbitan dan di mana telah diterbit/diserahkan)
(State type, title, author/editor, publication year and where it has been published/submitted)

1. 16th National Conference on Medical & Health Sciences 2011
"Urinary Screening of MPS in Cases of Suspected of IEM children in HUSM"
Page 87-93 ISBN 978-967-5547-3-5 (Proceeding & Poster presentation)
2. 14th National Conference on Medical & Health Sciences 2009
A case of Hunter syndrome (Poster presentation)

(b) **Faedah-faedah lain seperti perkembangan produk, pengkomersialan produk/pendaftaran paten atau impak kepada dasar dan masyarakat.**
State other benefits such as product development, product commercialisation/patent registration or impact on source and society.

Optimizing the method of DMB and electrophoresis for the diagnosis of MPS are currently being used for diagnostic service for in house patients and other ministry of health hospital. Reference method to other universities who are embarking in MPS screening. Please see Standard Operating Procedure (Attachment)

* Sila berikan salinan/*Kindly provide copies*

(c) **Latihan Sumber Manusia**
Training in Human Resources

i) Pelajar Sarjana:
Graduates Students
(Perincikan nama, ijazah dan status)
(Provide names, degrees and status)

En Saruddin Abbas – Master of Science (Biomedicine - UKM) - Graduated

ii) Lain-lain:
Others

The methods - DMB & HRE for MPS are currently being used for M. Pathology Students and Medical Laboratory Technologist training in the metabolic laboratory.
(Please see attachment – Prosedur Penyaringan MPS)

9. **Peralatan yang Telah Dibeli:**
Equipment that has been purchased

None

Tandatangan Penyelidik
Signature of Researcher

18/9/12
Tarikh
Date

Komen Jawatankuasa Penyelidikan Pusat Pengajian/Pusat
Comments by the Research Committees of Schools/Centres

Boleh dibentangkan
Penyelidik digalakkan untuk menerbitkan
hasil penyelidikan di dalam final manuscript.

Tutup goan.

9/9/13

PROFESOR (DR) NIK SORIANI YAACOB
Chairman Of Research commitee
School Of Medical Sciences
Health Campus
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan.

TANDATANGAN PENGERUSI
JAWATANKUASA PENYELIDIKAN
PUSAT PENGAJIAN/PUSAT
Signature of Chairman
[Research Committee of School/Centre]

9/9/13

Tarikh
Date

BORANG LAPORAN HASIL PENYELIDIKAN
PPSP

Tajuk geran: **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

Jenis geran: Geran jangka pendek

Tempoh geran: 24 bulan

Jenis laporan: Laporan Kemajuan ☐ Alatan di beli ☐ Ya:nyatakan.....

Laporan Akhir*: ☑ ☐ Tidak

OBJEKTIF SPESIFIK KAJIAN (sama pt dalam proposal asal)	**SECARA RINGKAS TERANGKAN PENCAPAIAN/HASIL**	**OBJEKTIF TERCAPAI ATAU TIDAK**
1. To assess the agreement of DMB and high voltage electrophoresis as a screening tool for MPS.	Telah dapat menilai *the agreement of DMB and high voltage electrophoresis as a screening tool for MPS*	Tercapai
2. To determine the prevalence of MPS through urinary screening of urine samples sent to the metabolic laboratory by using DMB and high voltage electrophoresis.	Telah mendapat prevalence MPS melalui ujian saringan urin yang dihantar ke makmal tetapi ujian ulangan menggunakan sampel baru dari pesakit tidak diperolehi disebabkan pesakit telah meninggal dunia.	Tercapai
3.		
4.		

- *Laporan Akhir perlu disertakan salinan manuskrip dan surat yang dihantar kepada mana-mana jurnal untuk penerbitan.*

Nama Penyelidik Utama (PI): Dr Julia Omar t.t.:

Tarikh: 07/03/12

DR. JULIA OMAR
Ketua Jabatan Patologi Kimia
Pusat Pengajian Sains Perubatan
Kampus Kesihatan,
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan.

UNIVERSITI SAINS MALAYSIA

SHORT TERM GRANT

TECHNICAL REPORT

Project Title:	Urinary Screening to determine the positivity of DMB and High Voltage (resolution) Electrophoresis of Mucopolysaccharidosis in suspected metabolic disorders children in HUSM
Investigator:	Dr Julia Omar
Grant A/C No:	304/PPSP/6131512

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ACKNOWLEDGEMENT

This project was carried out under the financial support of USM Short Term Grant (304/PPSP/6131512). I would like to thank the Department of Chemical Pathology for providing the facilities to carry out this study especially in the metabolic laboratory. Contribution by En Saruddin and his team were very much appreciated.

ABSTRACT

Mucopolysaccharidoses (MPS) is a disease of inborn errors of metabolism (IEM) and constitute 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. The aim of this study was to screen for MPS in all urine samples suspected of inborn errors of metabolism sent to the metabolic laboratory from 1998 to 2008 and look into the agreement between the positivity of DMB assay and HRE in the same sample group. Measurement of observed agreement using Cohen's kappa coefficient (k) was used for the association between both DMB and HRE method. The second aim of this study was to determine the prevalence of MPS through urinary screening by using these two methods.

A total of 134 urine samples were obtained however only 90 samples fulfilled the inclusion criteria and were analysed for GAG. Out of those, 28 (31.1%) 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 out of the 90 samples showed abnormal bands when subjected to HRE.

In this study, the prevalence percentage obtained was 68.9% based on DMB method and 3.3% for HRE method. Weak association was seen between both methods (k = 0.027) and samples with high GAG levels did not exhibit positive HRE as expected.

This study concludes that all samples with high index of suspicion of MPS must be screen through both methods, DMB and HRE. However elevated value of GAG of two folds or more should be scrutinized for the possibility of MPS.

ABSTRAK

Mukopolisakaridosis(MPS) adalah penyakit ralat metabolik semulajadi yang merangkumi sekumpulan penyakit heterogenus penyakit simpanan lisosomal. Untuk pendiagnosan penyakit ini, ujian penaksiran glikoaminoglikan (GAG) melalui kaedah dimetilmetilina biru (DMB) dan elektroforesis tinggi (ERT) dijalankan untuk pengkelasan MPS. Kajian ini dijalankan untuk menyaring semua sampel urin yang diterima di makmal metabolik untuk penyakit MPS dari tahun 1998 hingga 2008 di samping melihat korelasi positif yang terdapat di antara dua kaedah ini iaitu DMB dan ERT.

Terdapat sebanyak 134 sampel urin tetapi hanya 90 sampel yang memenuhi kriteria kajian. 28 (31.1%) sampel mempunyai aras GAG yang normal, 59 (65.6%) aras GAG 1-2 kali ganda lebih tinggi dan 3 (3.3%) mempunyai aras GAG melebihi 2 kali ganda dari normal. Tiga daripada 90 sampel mempamerkan jaluran tidak normal bila menggunakan kaedah ERT.

Kajian ini menunjukkan peratus prevalens menggunakan kaedah DMB adalah 68.9% dan melalui kaedah ERT adalh 3.3%. Korelasi antara dua kaedah ini adalah sangat lemah (k = 0.027).

Rumusan dari kajian ini menunjukkan bahawa untuk kes yang mempunyai indeks kesangsian yang tinggi untuk penyakit MPS harus menjalai ujian saringan DMB dan ERT. Penaksiran GAG yang melebihi 2 kali ganda aras normal harus diberi perhatian yang tinggi untuk kemungkinan penyakit MPS.

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.

OBJECTIVES

The objectives of this study were

i. To screen all urine samples suspected of IEM sent to the metabolic laboratory from 1998 to 2008 for MPS and assess the agreement of DMB and HRE as a screening tool for MPS

ii. To determine the prevalence of MPS through urinary screening of urine samples sent using DMB and HRE

MATERIALS AND METHODS

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.

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]

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.

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.

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

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.

RESULTS AND DISCUSSION

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).

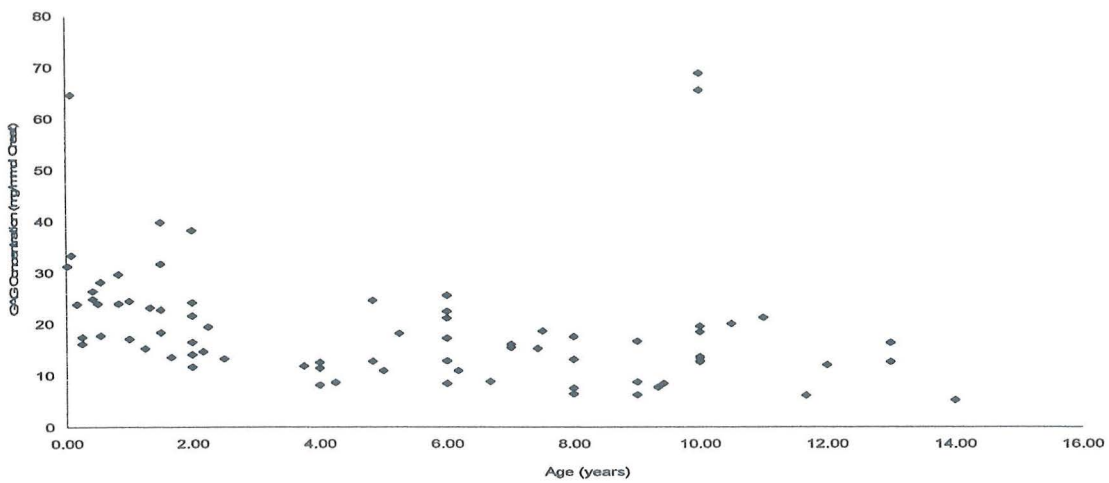

Fig. 1: Distribution of GAG levels in the 90 urine samples analyzed

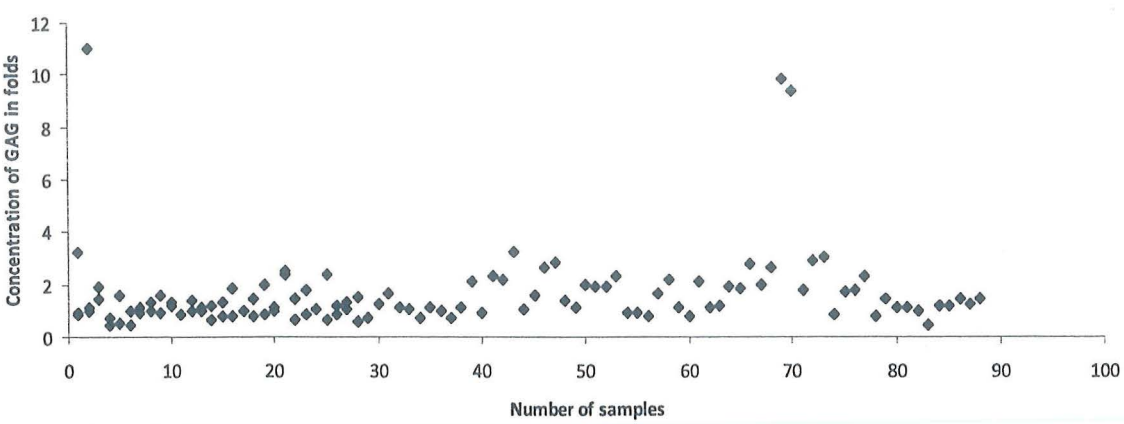

Fig.2: Increment of GAG concentration (in folds) in the urine sample analyzed

All the 90 samples were further subjected to high resolution electrophoresis. 87 (96.7%) samples showed chondroitin sulfate (CS) predominance which is normally found in normal subjects. 3 (3.3%) samples showed abnormal bands. One of the samples had heparan sulfate (HS) predominance as in figure 3. Two other samples had dermatan sulfate (DS) predominance as in figure 4 and 5.

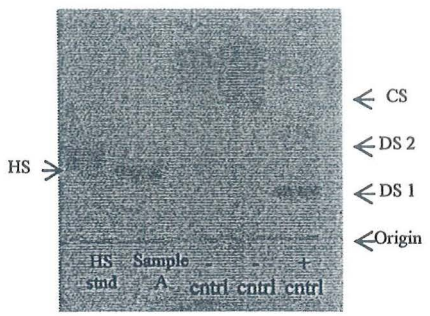

Fig. 3. HRE of a sample A with abnormal band of heparan sulphate (HS) predominance.

CS: Chondroitin sulfate DS: Dermatan sulfate
HS: Heparan sulfate Cntrl: Control

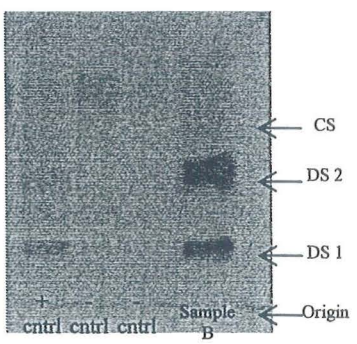

Fig. 4. HRE of a sample B with abnormal band of dermatan sulphate (DS1 & DS2) predominance.

CS: Chondroitin sulfate DS: Dermatan sulfate
HS: Heparan sulfate Cntrl: Control

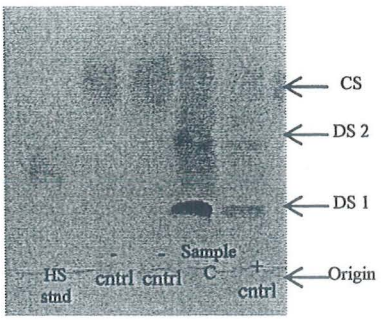

Fig. 5. HRE of a sample C with abnormal band of dermatan sulphate (DS1 & DS2) predominance.

CS: Chondroitin sulfate DS: Dermatan sulfate
HS: Heparan sulfate Cntrl: Control

All three patients with the abnormal bands detected had more than 2 folds increment in their GAG values and among the three, two (sample B and C) had more than nine folds increment. The prevalence percentage obtained was 68.9% based on DMB method and 3.3% for HRE method.

Measurement of observed agreement using Cohen's kappa coefficient (k) showed that the value of k was 0.027. This demonstrates a weak correlation between both DMB and HRE method as the samples with high GAG levels did not exhibit positive HRE as expected.

DISCUSSION

The measurement of GAG by DMB assay is a long established technique [2 - 4] It is known for it simple and rapid technique however further identification of the GAG compounds would enable in assisting physicians to characterize the disease in accordance to the clinical manifestations.

In view of the different methods that are available in performing GAG electrophoresis, we have decided in our laboratory to use cellulose acetate electrophoresis optimized by Gray G *et al.* [5] since it is not expensive and easily available.

Our findings demonstrated that GAG values above two fold increment need to be looked into and correlated with the clinical findings of the patient. This is in view of the positive bands found in these 3 patients. However there were various studies conducted by Hopwood and Harisson [6], de Jong *et al.* [3,7,8] Gray G *et al.* [5] and Tan *et al.* [9] which found patients excreting normal limits of GAG had the disorder of MPS. This study also noted that those patients whom had 9 fold increment of GAG as in our study were likely to have MPS.

We were not able to re-assay and reconfirm the three positive samples with the abnormal bands as these three patients had expired without a conclusive diagnosis.

In view of our findings and findings of other researchers, we have taken measures to run GAG and HRE in all samples received with the suspicion of MPS in our metabolic laboratory. The laboratory has opted to pay particular attention to those samples with high GAG values above two folds. Perhaps the next line of testing would be for those patients with a GAG level above 2 folds, enzymatic testing should be considered as an urgent testing even though treatment through enzyme replacement therapy is not readily available for all type of MPS.

CONCLUSION

Our results showed that GAG quantification and HRE is useful, inexpensive and practical first-line screening test when MPS is suspected clinically. It provides some presumptive diagnosis on the type of MPS for the clinicians in managing MPS patients. Our metabolic laboratory has started using both methods as the first line of screening for MPS. Special attention is taken when the clinical suspicion is strong and when the GAG values are 2 folds or more for the age limit. However normal quantitative GAG excretion alone cannot rule out a diagnosis of MPS.

Further enzymatic studies should be considered in samples with abnormal bands found on HRE. Till enzymatic assays are available in the country, both GAG and HRE method would be useful to characterize our MPS patients and in helping clinicians to manage them.

REFERENCES

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2. de Jong, J.G., Wevers, R.A. & Liebrand-van Sambeek, R. Measuring urinary glycosaminoglycans in the presence of protein: an improved screening procedure for mucopolysaccharidoses based on dimethylmethylene blue. *Clin. Chem.* 1992; **38**: 803-807.
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10. Scriver, C.R., Beaudet, A.L., Sly, W.S. & Valle, D. The metabolic and molecular bases of inherited disease, Ed. ke-8. New York: Mc Graw-Hill. 2001.
11. de Lima, C.R., Baccarin, R.Y. & Michelacci, Y.M.. Reliability of 1,9-dimethylmethylene blue tests in comparison to agarose gel electrophoresis for quantification of urinary glycosaminoglycans. *Clin. Chim. Acta.* 2007; **378**(1-2): 206-215.

PENYATA KEWANGAN

UNIVERSITI SAINS MALAYSIA
JABATAN BENDAHARI
KUMPULAN WANG PENYELIDIKAN GERAN USM(304)
PENYATA PERBELANJAAN SEHINGGA 29 FEB. 2012

Jumlah Geran:	RM	20,000.00	Ketua Projek:	DR JULIA OMAR
Peruntukan 2007 (Tahun 1)	RM	10,000.00	Tajuk Projek:	Urinary Screening to Determine the Positivity of DMB and High Voltage Electrophoresis of Mucopolysaccharidosis in Suspected Metabolic Disorders Children in HUSM
Peruntukan 2008 (Tahun 2)	RM	10,000.00		
Peruntukan 2009 (Tahun 3)	RM	0.00	Tempoh:	15 Mac 2007 - 14 Nov 09
			No.Akaun:	304/PPSP/6131512

Kwg	Akaun	PTJ	Projek	Donor	Peruntukan Projek	Perbelanjaan T'kumpul Hingga Tahun Lalu	Peruntukan Semasa	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
304	11000	PPSP	6131512		-	-	-	-	-	-	-
304	14000	PPSP	6131512		-	-	-	-	-	-	-
304	15000	PPSP	6131512		-	-	-	-	-	-	-
304	21000	PPSP	6131512		500.00	-	500.00	-	-	-	500.00
304	22000	PPSP	6131512		-	-	-	-	-	-	-
304	23000	PPSP	6131512		-	-	-	-	-	-	-
304	24000	PPSP	6131512		-	-	-	-	-	-	-
304	25000	PPSP	6131512		-	-	-	-	-	-	-
304	26000	PPSP	6131512		-	-	-	-	-	-	-
304	27000	PPSP	6131512		9,500.00	18,606.05	(9,106.05)	-	400.00	400.00	(9,506.05)
304	28000	PPSP	6131512		-	-	-	-		-	-
304	29000	PPSP	6131512		10,000.00	900.00	9,100.00	-		-	9,100.00
304	32000	PPSP	6131512		-	-	-	-	-	-	-
304	35000	PPSP	6131512		-	-	-	-	-	-	-
304	A11102	PPSP	6131512		-	-	-	-	-	-	-
					20,000.00	19,506.05	493.95	-	400.00	400.00	93.95

PUBLICATIONS

POSTER PRESENTATION:

1. 14TH NATIONAL CONFERENCE ON MEDICAL AND HEALTH SCIENCES 2009
2. 16TH NATIONAL CONFERENCE ON MEDICAL AND HEALTH SCIENCES 2011

PROCEEDING:

1. 16TH NATIONAL CONFERENCE ON MEDICAL AND HEALTH SCIENCES 22-23 JUNE 2011

UNIVERSITI SAINS MALAYSIA

COMPENDIUM OF ABSTRACTS

14TH NATIONAL CONFERENCE ON MEDICAL AND HEALTH SCIENCES

Transforming Research For Sustainable Health

21 - 22 MAY 2009

HEALTH CAMPUS
UNIVERSITI SAINS MALAYSIA
KUBANG KERIAN, KELANTAN

PM 25

A case of Hunter Syndrome

Julia Omar, Saruddin Abbas, Mohd Adi Firdaus Tan Abdullah, Azizah Shaari, Norizam Yusof, Zalina Yaacob*

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Introduction: Mucopolysaccharidosis (MPS) is rare genetic disorder. It is a lysosomal storage disease caused by a defect in the gene coding for the lysosomal enzyme resulting in the cells of affected individuals, inability to produce the enzyme or produce in low amounts. As a result, the lysosome is unable to induce the stepwise degradation of glycosaminoglycans(GAGs) namely dermatan sulfate and heparan sulfate. These GAGs which are constituents of extracellular matrix, joint fluid and connective tissue accumulate progressively in the lysosome causing cell, tissue and organ dysfunction. The pathologic manifestations of MPS involve most organ systems. Some individuals suffer from developmental delay and physical symptoms in the first two years of life or suffer progressive mental retardation.

Case report: A 2-year old Malay boy, youngest of 6 siblings was noted by the parents to have coarse facies resembling the brother who died at the age of 11 years old. The child was born full term with good birth weight. He had normal developmental milestone except for delay in speech. The child presented with recurrent episodic wheeze and progressive abdominal distension. Clinically he has coarse facies with frontal bossing and hepatosplenomegaly. Urinary glycosaminoglycan(GAG) estimation was done and noted to be high. The high resolution electrophoresis showed abnormal bands. Further evaluation for enzymatic studies done in Taiwan showed low level of iduronate sulfatase enzyme which confirms the diagnosis of MPS II or also known as Hunter Syndrome

Conclusions: The clinical presentation and investigation results in this case are characteristic of mucopolysaccharidosis. Any child who presents with chronic and progressive coarse facies with multiple clinical features should be investigated for MPS.

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Compendium of Abstracts

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TRAINING OF HUMAN RESOURCE

URINARY SCREENING OF MUCOPOLYSACCHARIDOSES (MPS) IN CASES SUSPECTED OF INBORN ERRORS OF METABOLISM CHILDREN IN HUSM

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ABSTRACT

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. The objective of the present study was 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. 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. A total of 134 urine samples were obtained however only 90 samples were analysed. 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. 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)*

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 heparin sulphate was prepared and used as internal quality control. Heparin 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.

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.

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.

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.

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 ranged 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).

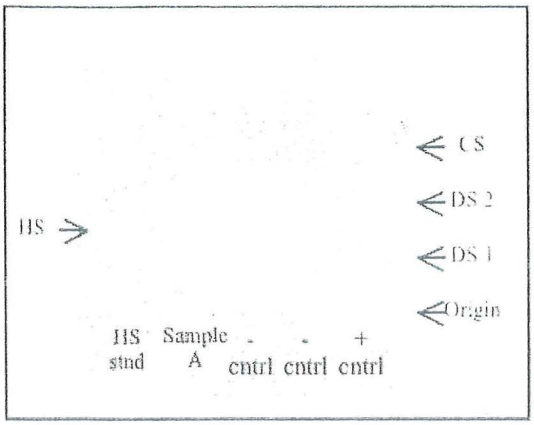

Fig. 3. HRE of a sample A with abnormal band of heparan sulphate (HS) predominance.

CS: Chondroitin sulfate DS: Dermatan sulfate
HS: Heparan sulfate Cntrl: Control

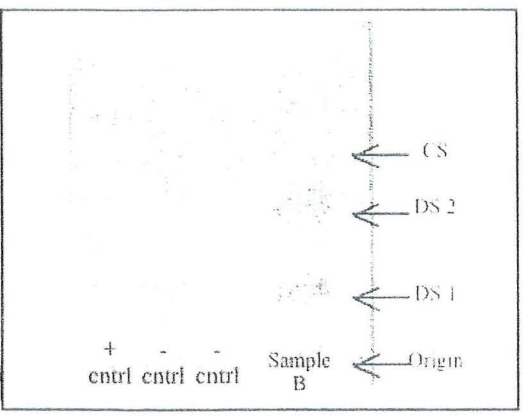

Fig. 4. HRE of a sample B with abnormal band of dermatan sulphate (DS1 & DS2) predominance.

CS: Chondroitin sulfate DS: Dermatan sulfate
HS: Heparan sulfate Cntrl: Control

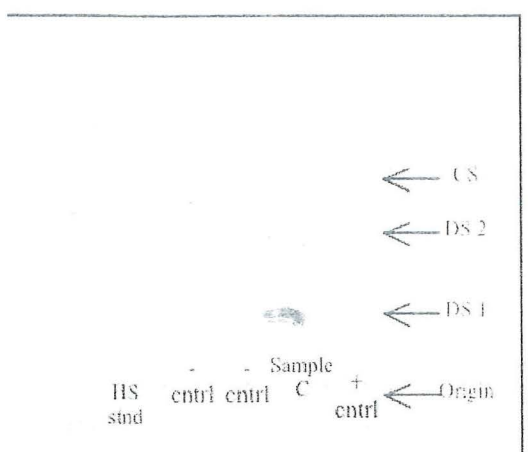

Fig. 5. HRE of a sample C with abnormal band of dermatan sulphate (DS1 & DS2) predominance.

'S: Chondroitin sulfate DS: Dermatan sulfate
S: Heparan sulfate Cntrl: Control

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

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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 analysed. 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.

PROCEEDING

KAEDAH DIMETILMETILENA BIRU (DMB) DAN ELEKTROFORESIS RESOLUSI TINGGI (ERT) DALAM PENDIAGNOSAAN MUKOPOLISAKARIDOSIS (MPS)

SARUDDIN BIN ABBAS

TESIS YANG DIKEMUKAKAN UNTUK MEMENUHI SEBAHAGIAN DARIPADA SYARAT MEMPEROLEHI IJAZAH SARJANA SAINS KESIHATAN (SAINS BIOPERUBATAN)

FAKULTI SAINS KESIHATAN BERSEKUTU
UNIVERSITI KEBANGSAAN MALAYSIA
KUALA LUMPUR

2008

PENGHARGAAN

Dengan nama Allah Yang Maha Pemurah Lagi Maha Penyayang. Alhamdulillah. Syukur ke hadrat Allah s.w.t. kerana dengan rahmat dan izinNya, maka dapatlah saya menyempurnakan tesis ini. Semoga ilmu yang diperolehi akan diberkati.

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Semoga kalian semua akan mendapat keberkatan dan keredhaan dariNya.

Saruddin Bin Abbas

2008

MAKMAL METABOLIK

JABATAN PATOLOGI KIMIA

UNIVERSITI SAINS MALAYSIA

PROSEDUR PENYARINGAN MUKOPOLISAKARIDOSIS (MPS)

- **DIMETHYLMETHYLENE BLUE DYE (DMB) METHOD**
- **HIGH RESOLUTION ELECTROPHORESIS (HRE) METHOD**

MAKMAL METABOLIK JABATAN PATOLOGI KIMIA	
Mucopolysachharidoses (MPS)	**Titles: SCREENING FOR MPS** • **DIMETHYLMETHYLENE BLUE DYE (DMB) METHOD** • **HIGH RESOLUTION ELECTROPHORESIS (HRE) METHOD**

1.0 REAGENT AND STANDARD PREPARATION

No	Activity
1.1	**DIMETHYLMETHYLENE BLUE DYE (DMB) METHOD** ***Principle*** This is a direct method for quantifying excessive urinary GAG excretion expolid the specific binding of 1,9 dimethymethylene blue to sulfated glycosaminoglycan **Materials** **i. Sodium Format Buffer (0.2 mmol/L, pH 3.5)** • Dissolve 0.0136g Sodium Format and top up to 1000ml water **ii. DMB Dye Reagent-Stock Solution** • Dissolve 0.0122g DMB in 1ml of 95% ethanol dilute to 100ml with sodium format buffer **iii. DMB Dye Reagent-Working Solution** • Dilute 10ml of DMB dye stock solution to a final volume of 100ml sodium format buffer **iv. Glycosaminoglycans Standard (1mg/ml)** • Dilute 40µl Chondroitin Sulphate Type A (CSA) – (Sigma 4134) 25mg/ml with 960µl water **v. Working Standard Solution (100µg/ml)** • Dilute 100µl of CSA (1mg/ml) with 900µl of water

Prepared by	**Tan Say Koon / Dr Julia Omar**
Approved by	**Dr. Julia Omar**

MAKMAL METABOLIK JABATAN PATOLOGI KIMIA	
Mucopolysachharidoses (MPS)	**Titles: SCREENING FOR MPS** • **DIMETHYLMETHYLENE BLUE DYE (DMB) METHOD** • **HIGH RESOLUTION ELECTROPHORESIS (HRE) METHOD**

2.0 PROCEDURE

No	Activity
2.1	**Methodology** i. Warm up the spectrophotometer and set to a wavelength of 595nm. ii. Blank the spectrophotometer with distill water. iii. Use 1x DMB as next blank. iv. If the absorbance reading is more than 0.9 then the spectrophotometer is ready to use. v. Change the spectrophotometer to wavelength 525nm. Use the DMB working solution as the blank. vi. Mix 1 ml of 1x DMB and 40µl of test urine or standard in a disposable cuvette. Mix thoroughly with pipetor until colour is homogenous. Take the absorbance reading within 30 minutes. vii. The calculation for DMB assay: $\frac{\text{OD sample}}{\text{OD standard}} \times 100 =$ GAGmg/L / Urine Creatinine (mmol/L) = _____**GAG mg/mmol Creatinine**

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MAKMAL METABOLIK JABATAN PATOLOGI KIMIA	
Mucopolysachharidoses (MPS)	**Titles: SCREENING FOR MPS** • **DIMETHYLMETHYLENE BLUE DYE (DMB) METHOD** • **HIGH RESOLUTION ELECTROPHORESIS (HRE) METHOD**

3.0 REAGENT PREPARATION

No	Activity
3.1	**HIGH RESOLUTION ELECTROPHORESIS (HRE) METHOD** **Principle** The migration of charged colloidal particles or molecules through a solution under the influence of an applied electric field usually provided by immersed electrodes **Materials** **i. Citrate Buffer 0.054 M, pH 4.8** • Dissolve 15.88g Trisodium citrate dehydrate in approximately 800ml water • Adjust pH 4.8 with 1.43 M Citric acid (300g/l) • Dilute to 1 litre **ii. CPC/citrate** • Dissolve 0.1g Cetylpyridium chloride in 100 ml citrate buffer **iii. Lithium chloride 2 mol/L** • Dissolve 8.5g of Lithium Chloride in 100ml water **iv. Barium acetate 1 mol/L, pH 5.0 aqueous** • Dissolve 255.43g in 800ml water • Adjust to pH 5.0 with glacial acetic acid • Dilute to 1 litre **v. Barium acetate 0.1 mol/L, pH 5.0 aqueous** • 25.54g in 800ml water. • Adjust to pH 5.0 with glacial acetic acid • Dilute to 1 litre **vi. Barium acetate 0.1 mol/L, pH 5.0, 15% ethanol** • Take 50ml from no.4 + 400ml of 15% ethanol and make up to 500ml

	vii. **Barium acetate 0.1 mol/L, pH 5.0, 50% ethanol** • Take 50ml from no.4 + 400ml 50% ethanol and make up to 500ml viii. **Alcian blue 8 GX : 0.25% w/ V water** • 0.5g alcian blue dissolve in 200ml water ix. **Phenol red** • Dissolve 5mg of phenol red in 10ml water x. **Acetic acid : 1% v/v**

Prepared by	**Tan Say Koon / Dr Julia Omar**
Approved by	**Dr. Julia Omar**

MAKMAL METABOLIK JABATAN PATOLOGI KIMIA	
Mucopolysachharidoses (MPS)	**Titles: SCREENING FOR MPS** • **DIMETHYLMETHYLENE BLUE DYE (DMB) METHOD** • **HIGH RESOLUTION ELECTROPHORESIS (HRE) METHOD**

4.0 PROCEDURE

No	Activity
4.1	**Preparation of Sample** **Methodology** a. Centrifuge urine at 3100 rpm at 20°C for 10 minutes. b. Add equal volume of supernatant and CPC-Citrate c. Vortex the mixture for 10 seconds d. Incubate the mixture at 37°C for 30 minutes e. Centrifuge mixture at 3100 rpm at 20°C for 10 minutes. f. Decant and drain inverted on 3 MM Whatman paper for 30 minutes. g. Dissolve in 150µl Lithium chloride and vortex for 10 seconds. h. Add 800µl Ethanol and vortex for 10 seconds. Let it stands for 5 minutes. i. Centrifuge at 3100 rpm at 20°C for 10 minutes. j. Decant and drain inverted on 3 MM Whatman paper for 10 minutes. k. Dry precipitate at 37°C in dry block heater under the nitrogen gas. l. Dissolve precipitate in 20µl phenol red solution. m. Mix and stand for 10 minutes.

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MAKMAL METABOLIK JABATAN PATOLOGI KIMIA	
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5.0 PREPARATION AND PROCEDURE

No	Activity
5.1	**Preparation for HRE analysis** **Methodology** a. Fill the HRE chamber with 250ml 1 M Barium acetate, pH 5.0 aqueous (buffer) b. Set the cooling temperature for the HRE platform at 12-15°C. **Pre-run preparation** a. Soak cellulose acetate plate in 0.1 M Barium acetate, pH 5.0 aqueous for 10 minutes. b. No sample should be applied on the cellulose acetate plate. c. Run electrophoresis on the cellulose acetate plate at 200V for 30 minutes. **Plate preparation** a. Soak cellulose acetate plate in 0.1 M Barium acetate, pH 5.0 aqueous for 10 minutes. b. Blot and apply 10µl prepared sample from HRE precipitate. **HRE analysis** a. Stage 1 i. Run the electrophoresis plate at 200V for 7 minutes. ii. After the 7 minutes, soak the plate in 0.1 M Barium acetate, pH 5.0, 15% Ethanol for 2 minutes and blot the plate. iii. Proceed to stage 2 b. Stage 2 i. Run the electrophoresis plate at 200 V for 30 minutes.

ii. After 30 minutes, soak the plate in 0.1 M Barium acetate, pH 5.0, 50% Ethanol for 2 minutes and blot.

iii. Proceed to stage 3

c. Stage 3

i. Run the electrophoresis plate at 300 V for 30 minutes.

ii. After 30 minutes, stain the plate with Alcian blue for 15 minutes and blot lightly.

iii. Destain in 3% acetic acid.

iv. Wash in deionsed water and blot.

v. Destain in 3% acetic acid until clear.

vi. Read and analyze image.

Clearing procedure

a. Dehydrate cellulose acetate plate twice in absolute Methanol for 2 minutes.

b. Soak plate in clearing solution in 10 minutes.

c. Drain off excess solution by draining.

d. Put plate in the oven of 60°C for 15 minutes.

STANDARD OPERATING PROCEDURE

MUCOPOLYSACCHARIDOSIS SCREENING