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Selective screening for detection of mucopolysaccharidoses in Malaysia; A two-year study (2014–2016)

Affandi Omar[a], Julaina A. Jalil[a,*], Norashareena M. Shakrin[a], Lock H. Ngu[b], Zabedah M. Yunus[a]

[a] Biochemistry Unit, Specialised Diagnostic Centre, Institute for Medical Research, Ministry of Health Malaysia, Jalan Pahang, 50588 Kuala Lumpur, Malaysia
[b] Genetic Department, Kuala Lumpur Hospital, Ministry of Health Malaysia, Jalan Pahang, 50586 Kuala Lumpur, Malaysia

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ABSTRACT

Introduction: Mucopolysaccharidoses (MPS) are a group of inherited disorders caused by the deficiency of a specific lysosomal enzyme involved in glycosaminoglycans (GAGs) degradation. This enzyme deficiency leads to accumulation of GAGs in the lysosomes, resulting in organ dysfunction and enlargement. We aimed to detect cases of MPS in patients with suggestive signs and symptoms.

Methods: This was a 2-year cross-sectional study conducted during June 2014 to May 2016. Urine and whole blood samples were taken from high-risk MPS patients. All urine samples were analysed for GAGs and characterised by high resolution electrophoresis (HRE). Whole blood was collected in ethylenediaminetetraacetic acid (EDTA) tube and analysed for specific enzymes based on the clinical history and HRE findings.

Results: From the 60 samples tested, 15 were positive for MPS; (Type I = 1), (Type II = 4), (Type IIIA = 3), (Type IVA = 1), (Type VI = 6). The overall prevalence of MPS among high-risk Malaysian patients was 26% (95% CI 14.72% to 37.86%). One patient had mucolipidosis. The mean age of patients when diagnosed was 5 years old. Patients with MPS were more likely to present with hepatosplenomegaly compared to other symptoms (OR = 0.974, $p < .05$).

Conclusion: One in 4 high-risk patients was diagnosed with MPS being MPS type VI the most common among Malaysian patients. Hepatosplenomegaly was the most common symptom. Patients with suspected MPS should be screened by urinary GAGs analysis and diagnosis confirmed by enzyme activity analysis.

1. Introduction

Mucopolysaccharidoses (MPS) are a group of inherited disorders caused by deficiency of specific lysosomal enzyme involved in glycosaminoglycans (GAGs) degradation, including dermatan sulphate (DS), heparan sulphate (HS), keratan sulphate (KS), chondroitin sulphate (CS) and/or hyaluronic acid (HA). Deficiencies of one of these enzymes lead to accumulation of GAGs in many tissues and organs and, in most cases, with increased excretion in urine [19].

There are seven types of MPS related to the deficiency of different enzymes, α-L-iduronidase (MPS I Hurler, Scheie, Hurler/Scheie), iduronate-2-sulphatase (MPS II), heparan *N*-sulphatase (MPS IIIA), α-*N*-acetylglucosaminidase (MPS IIIB), acetyl-CoA-glucominidase acetyltransferase (MPS IIIC), *N*-acetylglucosamine 6-sulfatase (MPS IIID), galactose 6-sulphate sulphatase (MPS IVA), β-galactosidase (MPS IVB), arylsulphatase B (MPS VI), β-glucuronidase (MPS VII) and hyaluronidase 1 (MPS IX). Common features include organomegaly, dysostosis multiplex and dysmorphic facies [19].

MPS can be described as chronic and progressive disorders and typical symptoms depending on the severity of each one which could include organomegaly, dysostosis multiplex, joint mobility abnormalities and characteristic facial features. Hearing, vision, cardiovascular and respiratory function may also be affected. Different types of MPS can present with overlapping clinical signs and symptoms. However, some have specific presentations that can be use for early diagnosis.

MPS are difficult to be recognised in children and young adults with less pronounced clinical presentation. Despite having some unique presentations like claw hands, hearing loss, vision loss, reduced lung function, obstructive sleep apnoea, recurrent ear and respiratory tract infections, umbilical and inguinal hernias, spinal cord compression, and hip surgery, it is difficult to suspect MPS in a young patient [11].

Accurate and timely diagnosis of MPS is crucial due to the availability of treatment for some types of MPS. Choices of treatment include bone marrow transplantation or enzyme replacement therapy in MPS I (laronidase), MPS II (idursulfase or idursulfase beta®), MPS IVA (elosulfase) and MPS VI (galsulfase). In 2017, vestronidase-alfa has been

* Corresponding author.
E-mail address: julaina@imr.gov.my (J.A. Jalil).

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approved by Food and Drug Administration (USA) to treat patients with MPS VII.

Analysis of deficient enzyme is the usual method to diagnose specific types of MPS. However, methods for enzyme assay are expensive and laborious, and it is not practical to examine all of the 11 enzymes for every clinically suspected patient [8]. Therefore, preliminary screening with analysis of urinary GAGs could specify which enzyme should be assayed. When available, molecular genetics analysis enables the identification of pathogenic variants, which is useful for confirmation of diagnosis, carrier identification, prenatal diagnosis and genetic counseling.

There is limited epidemiological data from Malaysia on the number of cases of MPS; individually or cumulatively. One report from the National Referral Centre at Hospital Kuala Lumpur showed 38% of the patients referred were diagnosed as MPS Type II [12]. This project was designed to screen and diagnose MPS patients in high-risk population to ensure accurate and timely diagnosis for treatment and genetic counseling.

In the current article, we identified the common presentations for patients suspected to have MPS, reported the cases of different types of MPS in high-risk patients and compared the findings with data available from other countries.

2. Methods

2.1. Study design

This is a prospective cross-sectional study involving samples from high-risk children and young adults for MPS conducted during June 2014 to June 2016.

2.2. Study population

We requested samples from patients at all hospitals in Malaysia who met the inclusion criteria.

2.3. Operational definitions

We defined the inclusion criteria as patients having at least two features of MPS: (1) abnormal facial features such as macrocephaly or coarse face; (2) corneal clouding or loss of visual acuity; (3) hearing impairment and recurrent middle ear infections; (4) recurrent respiratory tract infection; (5) valvular heart disease or heart murmur; (6) recurrent inguinal or umbilical hernia; (7) hepatosplenomegaly; (8) at least two symptom of musculoskeletal problems: (a) evolving joint contracture without obvious signs of inflammation, (b) joint laxity, (c) gibbus, (d) cervical spine stenosis and/or cord compression, (e) kyphosis or scoliosis, (f) pectus carinatum, (g) bilateral hip dysplasia, (h) progressive genu valgum after age of 3 years old, (i) short stature of unknown reason, or (j) carpal tunnel syndrome. We excluded patients presented with intellectual and developmental disabilities from this study.

2.4. Sample size

We calculated a sample size of 60 patients to achieve 90% confidence interval.

2.5. Sample collection

The pediatrician or medical officer in each participating hospital collected 20 mL samples of first morning urine and 6 mL of whole blood in ethylenediaminetetraacetic acid (EDTA) tube from each patient.

2.6. Materials

We used Modified Lowry Protein Assay kit (Thermo Scientific, USA) for protein quantitation. Enzymes substrate, 4-methylumbelliferone-α-L-iduronide (MPS I), 4-methylumbelliferone-β-D-galactopyranide-6-sulphate (MPS IVA), 4-methylumbelliferone-β-D-galactopyranoside (MPS IVB) and 4-methylumbelliferone-β-D-glucuronide (MPS VII) were obtained from GlycosynthTM (Cheshire, UK); 4-methylumbelliferone-β-D-galactopyranoside 4-MU-α-Iduronate-2-sulphate (MPS II) and 4-Methylumbelliferyl 2-Sulfamino-2-deoxy-α-D-glucopyranoside sodium salt (MPS IIIA) from Toronto Research Chemicals (Ontario, Canada); 4-methylumbelliferone-α-D-N-Acetylglucosamide sodium salt (MPS IIIB) from Moscerdam (Oegstgeest, Netherlands) and ρ-nitrocatechol sulphate dipotassium salt (MPS VI) from Sigma (USA).

2.7. Quantitation and characterization of urinary GAGs

We performed the quantitative analysis of total GAGs using dimethylmethylene blue method and qualitative analysis using high-resolution electrophoresis (HRE) as described by Nor [13]. We interpreted the results based on the amount of excretion of total GAGs and pattern of the specific GAGs detected by HRE: MPS I and II (DS and HS), MPS III subtypes (HS), MPS IV subtypes (KS), MPS VI (DS) and MPS VII (CS, DS and HS) [18]. MPS IX cannot be identified by urinary GAG analysis.

2.8. Analysis of enzyme activity in leukocytes

Enzymes activity analysis was performed in plasma or leukocytes. We extracted the leukocytes from EDTA blood by differential centrifugation as described by van Diggelen [21]. Plasma and leukocytes pellet were kept frozen at −80 °C until enzyme assays. The resulting leukocyte pellet was added 500 μL of deionised water before sonicated in ice for two 5-second bursts at 5 micro/amplitude and then centrifuged. The supernatants were kept in ice before analysis. Modified Lowry method was used to determine protein concentration. We adopted enzyme assays methods from Lysosomal Laboratory of Willink Biochemical Genetics, St. Mary's Hospital, Manchester, UK with one modification which is performing the assays in microtiter plates instead of test tubes. We also used the method described by Hopwood [12] for the enzyme assays needed to identify MPS I, MPS II [22], MPS IIIA [24], MPS IIIB [25], MPS IVA [21], MPS IVB [6], MPS VI [2], and MPS VII [3]. We also measured total β-hexosaminidase [14], β-mannosidase [15] and α-mannosidase [18] in plasma to identify mucolipidoses if all enzyme levels were found normal.

We performed enzyme analysis based on the qualitative results of HRE. Specified volumes of sample (plasma or leukocytes) were mixed with specific buffer and specific artificial substrates were tagged to a fluorescence compound, depending on the enzyme being assayed. The mixtures were incubated at specified times and the reactions were terminated by adding stop buffer. The enzymes in the sample reacted with the artificial substrate and released the fluorescence compound. We measured this compound using Tecan (Switzerland) fluorometer. We prepared different concentrations of the fluorescence compound as standards, plotted the curves, and used it for calculation of product amount. Enzyme activities in plasma were calculated based on the amount of fluorescence compound (product) being released per mL per hour (nmol/mL/h). Enzyme activities in leukocytes were calculated based on the amount of product being released per mL per mg protein per hour and the unit was nmol/mL/mg protein/h.

2.9. Statistical analysis

We analysed the data using IBM SPSS Statistics version 14 (SPSS Inc., Chicago, IL, USA). Descriptive analysis was performed and the results were presented as mean ± standard deviation (SD). Testing for

statistical significance ($p < .05$) of the relationship between diagnosis status and prominent symptoms was performed using Fisher Exact Test. We estimated the prevalence based on the number of high-risk patients over the total number samples obtained in this study and expressed in percentage. We calculated the relative frequency as the number of positive patients in MPS subtype over the total of positive cases and also expressed in percentage.

Diagnostic test performance analysis including sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) with the respective two-sided 95% confidence intervals (CI) were calculated using MedCalc diagnostic test evaluation online calculator (www.medcalc.org/calc/diagnostic_test.php).

3. Results

60 patients, comprising 35 males and 25 females with mean age of 4.8 ± 1.02 years and age range of 1 day to 56 years were included in this study. There were 40 (67%) Malay patients, 16 (27%) Chinese patients and 4 (3%) Indian patients. We identified twelve prominent symptoms including hepatomegaly (10/60), hepatosplenomegaly (19/60), genu valgum (1/60), kyphoscoliosis (1/60), dysmorphic/coarse facies (16/60), short stature (2/60), corneal clouding (2/60), pectus carinatum (3/60), kyphosis (2/60), claw hand (2/60), scoliosis (1/60) and joint contracture (1/60) (Table 1). We observed a significant association between hepatosplenomegaly and diagnosis status (Fisher's Exact test, $p = .002$). The risk estimate showed that patients with MPS were more likely to present with hepatosplenomegaly compared to other symptoms (OR = 0.974). The remaining prominent symptoms did not present any association with diagnosis status.

3.1. Screening of MPS by GAGs quantitation and HRE analysis

We performed urinary GAGs quantitation and HRE analysis in urine from 56 patients: the remaining 4 did not have sufficient urine. Based on the HRE pattern, 31/60 (51.7%) samples showed normal results while 25/60 (41.7%) samples were found to be abnormal. The most common types of MPS suggested from HRE results was MPS III (A, B, C or D) (9/60) followed by results suggestive of MPS I, II or VI (5/60). HRE results suggesting MPS I or II, MPS VI or unable to rule out MPS III were found in 2 patients respectively. One patient was suspected for MPS VII, MPS III or MPS VII and MPS I or VI respectively (Table 2).

3.2. Confirmation by enzyme assay

We tested all 25 patients with abnormal HRE results for enzyme assay analysis. We also performed enzyme assays for MPS I, MPS II, MPS IIIA, MPS IIIB, MPS IVA, MPS IVB, MPS VI and MPS VII in patients with normal results and insufficient urine. Fifteen were found positive for MPS and 1 patient was positive for mucolipidosis. The most common type of MPS identified was MPS type VI (6/60) followed by MPS II (4/60) and MPS IIIA (3/60). There was 1 case each reported for MPS I, MPS IVA and mucolipidosis. We observed equal number of male and female patients diagnosed with MPS excluding MPS II which is X-linked in inheritance. The eldest patient diagnosed was an 8 year old whilst the youngest was a 2.4 months baby (Table 3).

In our study, the screening for MPS using urine sample had a sensitivity of 87.5%, specificity of 83.3%, positive predictive value (PPV) of 49.8% and negative predictive value (NPV) of 94.6%.

Table 1
Clinical presentations of patients suspected with MPS from hospitals in Malaysia, June 2014 to June 2016.

Clinical presentations	Patients (percentage)
Hepatosplenomegaly	19 (31.7)
Dysmorphism	16 (26.7)
Hepatomegaly	10 (16.7)
Pectus carinatum	3 (5.0)
Kyphosis	2 (3.3)
Short stature	2 (3.3)
Claw hands	2 (3.3)
Corneal clouding	2 (3.3)
Genu valgum	1 (1.7)
Joint contracture	1 (1.7)
Kyphoscoliosis	1 (1.7)
Scoliosis	1 (1.7)

Table 2
HRE results for high-risk patients suspected for MPS from hospitals in Malaysia, June 2014 to June 2016.

Bands in HRE analysis	Results	Frequency (percentage)
DS, HS	MPS I, II or VI	5 (8.3)
HS	MPS III (A, B, C, or D)	9 (15.0)
CS	MPS VII	1 (1.7)
HS trace	Unable to rule out MPS III	2 (3.3)
HS, CS	MPS III (A, B, C, or D) or MPS VII	1 (1.7)
DS	MPS I or II	2 (3.3)
DS, HS trace	MPS I or VI	1 (1.7)
DS trace, HS	MPS VI	2 (3.3)
DS, CS	MPS I, II or VII	1 (1.7)
DS trace	MPS II	1 (1.7)
Insufficient urine	–	4 (6.7)

3.3. Prevalence and relative frequencies of MPS

The overall prevalence of MPS among high-risk Malaysian patients was 26% (95% CI 14.72% to 37.86%). We found that, within the group of MPS, MPS VI was the most frequent disorder, and no case of MPS VII was identified. In Taiwan and Eastern China, we identified that MPS II was the most common and MPS VII was rare (Table 4).

4. Discussion

Diagnosis of MPS is a challenge for both physicians and laboratory personnel. In usual practice, quantitative analysis of GAGs using a dye binding assay is followed by electrophoresis, enabling separation of different GAGs species. In our study, only 56/60 patients' samples were performed for GAGs quantitation and separation with HRE. We did not manage to get sufficient urine volume in the other four patients due to condition of patient (age less than one year) and decided to proceed to enzyme assay for MPS I, MPS II, MPS IVA, MPS IVB, MPS VI and MPS VII. We assayed for MPS IIIA and MPS IIIB if all mentioned enzymes were within normal range.

GAGs quantitation and separation of GAGs species using HRE only cover distinct groups of MPS and often carry the risk of false negative or positive. Faint or mild DS and HS pattern maybe misdiagnosed as MPS I or MPS II or MPS VI. Therefore, this screening must be followed by enzyme assay analysis [10]. Our study using urine as screening method

Table 3
Different types of MPS identified in high-risk patients suspected for MPS from hospitals in Malaysia, June 2014 to June 2016.

	Subject (number)	Gender		Mean age ± Standard error (years) (Min–Max)
		Female	Male	
No abnormality	44	20	24	5.60 ± 1.34 (0.02–56.00)
MPS I	1	1	0	4
MPS II	4	0	4	2.92 ± 1.76 (0.17–8.00)
MPS IIIA	3	1	2	1.40 ± 0.41 (0.61–2.00)
MPS IVA	1	0	1	0.19
MPS VI	6	2	4	2.85 ± 0.99 (0.19–7.33)
Mucolipidoses	1	1	0	2.5

Table 4
Relative frequencies of MPS in Malaysia from June 2014 to June 2016 and other countries.

Disorders	Malaysia		Taiwan		Eastern China		UAE*		Northern Portugal		The Netherlands	
	(2014–2016)		(1984–2004)		(2000 – 2012)		(1995–2010)		(1962–2001)		(1970–1996)	
	N	%	N	%	N	%	N	%	N	%	N	%
MPS I	1	6.7	7	6.4	31	16.4	4	17.4	16	30.2	82	31.4
MPS II	4	26.7	68	61.8	90	47.6	2	8.7	21	39.6	52	20.0
MPS IIIA	3	20.0	5	4.5	7	3.7	0	0	0	0.0	93	35.6
MPS IVA	1	6.7	21	19.1	51	27.0	4	17.4	6	11.3	22	8.4
MPS VI	6	40.0	9	8.2	8	4.2	13	56.5	10	18.9	6	2.3
MPS VII	0	0	0	0	2	1.1	0	0	0	0.0	6	2.3
All types	15	100	110	100	189	100	23	100	53	100	261	100

* UAE: United Arab Emirates.

has shown high performance with high sensitivity of 87.5%, specificity of 83.3% and PPV and NPV of 66.7% and 94.6% respectively. A similar study of 2 years MPS screening program in Spain reported a PPV of 24% and NPV of 100% [5]. We managed to diagnose 15 cases of MPS in only 60 patients.

We found a case of MPS IVA patient with normal urinary GAGs level. It is known that MPS IV patients often result as false negative at screening test [23]. The amount of GAGs was within range but the electrophoresis showed presence of KS band. This again demonstrates the importance of combining screening and enzyme assay method to diagnose any types of MPS.

We also found 6 cases of MPS VI with normal level of GAGs and HRE pattern. Diluted or non-first morning urine can give rise to the results. Request for repeat urine sample or further testing with enzyme assay and/or molecular analysis can assist in the diagnosis if there is clinical suspicion of MPS VI.

Patients with intellectual and developmental disabilities are expected to be irreversible with treatment, as present treatment formulation does not cross blood brain barrier. Therefore, they were not included in this study.

The interpretation of HRE results may vary as it also relies on clinical presentation of the patients. For example, in HRE results with presence of DS and HS, MPS VI is still possible if there was history of fever or patients taking antibiotics. MPS II is an X-linked inheritance disorder therefore we did not suggest this diagnosis in female patients. We also received samples sent by clinical geneticist or pediatrician whom already stated the most probable diagnosis for their patients. Specific type of MPS was suggested in those patients.

We managed to diagnose three patients with MPS III, all with MPS IIIA, after testing their enzyme level. All these three patients without intellectual and developmental disabilities, showed presence of HS band in HRE with normal enzyme level of MPS I, MPS II, MPS IV, MPS VI and MPS VII. Enzyme assay for MPS IIIA revealed undetectable activity. Currently, there is no available treatment for MPS IIIA but with the diagnosis of MPS IIIA in those patients, genetic counseling can be given to the parents before conceiving the next child.

One (1) patient with normal HRE findings showed increased activity of total β-hexosaminidase in plasma and diagnosed as having mucolipidosis. Mucolipidosis is not categorized as MPS. However, patients with mucolipidosis have clinical and biochemical features of both the mucopolysaccharidoses and the sphingolipidoses [20]. Mucolipidoses should be excluded in high risk patients with normal HRE pattern by assaying their total β-hexosaminidase, β-mannosidase and α-mannosidase levels.

We found our patients with MPS commonly presented with hepatosplenomegaly. Patients with MPS types that demonstrate increase dermatan sulfate (DS) and heparan sulfate (HS) GAGs usually presents with hepatosplenomegaly as those types of GAGs are observed to accumulate in liver and spleen cells.

The estimated prevalence of MPS among high-risk Malaysian patients was almost 1 in 4 (~25%). Since this prospective study targeted high-risk patients and was not a comprehensive MPS screening, the methodology of calculating prevalence described by Pinto [16] cannot be applied. Therefore, we used relative frequencies as an indicator to compare with other countries or studies.

We observed a high relative frequency of MPS VI in Malaysia. A similar finding was also reported in UAE and Northern Portugal [1,17]. Studies from other Asian countries, Taiwan [9] and eastern China [4] had contrasting finding and demonstrated MPS II as the most common type. In Malaysia, MPS II was the second most frequent type of MPS but our study was done for two years only. MPS III is the most common MPS type in Australia and the Netherlands. Those 2 countries are different in terms of demographic, geographic and genetic makeup from our population.

The distribution of MPS I and MPS IIIA compared to other types of MPS in Malaysia is comparable with Taiwan, Eastern China and UAE. This may be due to Malaysian population who are ethnically diverse, with some ancestries from mainland China and India with the local Malay dominant.

In conclusion, MPS disorder can be considered not that uncommon in Malaysia with 1 in 4 cases among high-risk patients. Through this selective screening over a two-year period, we identified 15 cases of MPS and 1 of mucolipidosis in Malaysia. We recommend that data from this study be used as pilot for a future larger study aiming to establish the prevalence of MPS in Malaysia.

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Conflict of interest

Affandi Omar, Julaina A. Jalil, Norashareena M. Shakrin, Lock H. Ngu and Zabedah M. Yunus declare that they have no conflict of interest.

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Compliance with ethical standards

We carried out this study in accordance with the Declaration of

Helsinki of the World Medical Association. The protocol was approved by the Medical Research & Ethic Committee (MREC) and Ministry of Health Malaysia (NMRR-14-340-19843).

Patient consent statement

All participants/guardians signed the provided informed consent form.

Authors' contribution

Affandi Omar carried out the quantitative analysis studies, performed the statistical analysis and drafted the manuscript. Zabedah M. Yunus participated in the design of the study and coordination and helped to draft and proof the manuscript. Julaina A. Jalil, Norashareena M. Shakrin and Lock H. Ngu helped to draft the manuscript.

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