Data Article

Demographic, laboratory findings and diagnostic evaluation among high risk patients with mucopolysaccharidosis in Malaysia

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A B S T R A C T

This article contains information related to a recent study “Selective screening for detection of mucopolysaccharidoses (MPS) in Malaysia; A Two-year Study” Affandi et al., 2019. Any patient registered under government healthcare facilities in Malaysia and fit at least two inclusion criteria were included in this selective screening. Urine and blood from these high risk patients were obtained and analysed for glycosaminoglycans (GAGs) level before characterization using high resolution electrophoresis (HRE). Thereafter, enzyme assay for different types of MPS based on result of HRE were determined using specific substrate. Demographic data as well as laboratory findings were tabulated and analysed. The data of this study demonstrate between clinical presentation and laboratory findings among high risk patients of MPS and can be employed to improve diagnosis of MPS.

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1. Data

This article presents data from high-risk patients profiling of mucopolysaccharidoses in Malaysia from 2014 to 2016 related to the recent study described by Omar A. [1]. Mucopolysaccharidoses (MPS) is a group of disorders characterised by the accumulation of glycosaminoglycans (GAGs) in tissues and organs due to defects in specific enzymes contained within lysosomes. Chondroitin sulphate (CS), dermatan sulphate (DS), heparan sulphate (HS), keratin sulphate (KS) and hyaluronic acid (HA) are subtype sulphated polysaccharides of GAGs [12]. MPS, like other type of inherited metabolic disorders, is still remains under diagnosed by physician due to its rareness and complexity of symptom presentation [8].

Table 1 summarizes the demographic data of 58 patients included in this selective screening. This data is useful to understand the pattern of these patients as some of the patients showed prominent symptom of MPS however was found to be negative during screening and confirmation. Hepatomegaly and dysmorphic features are the most common symptoms among high risk patients during the study period. Clinicians and physicians would find this data useful for them to investigate any suspected MPS patients in future.

Table 2 focuses on demographic data in study population. Male to female ratio is almost proportionate and eliminates gender bias. For ethnicity, we have divided the major races in Malaysia into 6 subcategories: Malay, Chinese, Indian, natives from Sabah and Sarawak region and indigenous people (Orang Asli). We also classified origin hospital of these patients into different regions. For instance, central region comprises any hospital in Federal Territory of Kuala Lumpur and Putrajaya, State of Selangor and State of Negeri Sembilan; northern region (State of Perak, Pulau Pinang, Kedah and Perlis); East Coast region (State of Kelantan, Terengganu and Pahang); Southern (State of Melaka and Johor); Sarawak region and Sabah region. We believe this demographic data would be useful among policy makers to decide whether selective screening should be conducted in selected regions or the whole country.
| No | Region       | Age (Year) | Symptom                      | GAGs value (mmol/g creat) | GAGs level (based on age) | HRE band                           | Diagnosis           |
|----|--------------|------------|------------------------------|---------------------------|----------------------------|------------------------------------|---------------------|
| 1  | Northern     | 1.42       | Hepatomegaly, Dysmorphic     | 53.3                      | Abnormal                   | Trace amount of DS band.           | Normal              |
| 2  | East Coast   | 2.00       | Hepatomegaly, dysmorphic     | 35.34                     | Abnormal                   | Normal pattern                     | Normal              |
| 3  | Northern     | 5.08       | Dysmorphic, achondroplasia   | 15.27                     | Abnormal                   | Trace amount of HS band            | Normal              |
| 4  | Sarawak      | 6.00       | Pectus carinatum, short stature | 13.76                     | Normal                     | Normal pattern.                   | Normal              |
| 5  | Sarawak      | 2.00       | Pectus carinatum, short stature | 18.55                     | Normal                     | Normal pattern.                   | Normal              |
| 6  | Northern     | 5.00       | Hepatomegaly, Dysmorphic     | 19.72                     | Abnormal                   | Normal pattern.                   | Normal              |
| 7  | Northern     | 1.42       | Hepatomegaly, Splenomegaly   | 34.97                     | Abnormal                   | Normal pattern.                   | Normal              |
| 8  | Northern     | 56.00      | Dysmorphic, short stature    | 6.86                      | Normal                     | Normal pattern.                   | Normal              |
| 9  | Northern     | 16.00      | Dysmorphic, short stature, kyphosis | 5.36                      | Normal                     | Trace amount of HS band            | Normal              |
| 10 | East Coast   | 1.83       | Dysmorphic, hepatosplenomegaly | 28.02                     | Abnormal                   | Trace amount of HS band            | Normal              |
| 11 | Southern     | 9.00       | Dysmorphic, Clawed hand, kyphoscoliosis | 10.35                     | Normal                     | Normal pattern                     | Normal              |
| 12 | Northern     | 0.25       | Hepatomegaly, umbilical hernia | 44.08                     | Abnormal                   | Normal pattern.                   | Normal              |
| 13 | East Coast   | 0.05       | Dysmorphic                   | 76.33                     | Abnormal                   | Increase of HS band                | Normal              |
| 14 | East Coast   | 4.92       | Dysmorphic                   | 1.84                      | Normal                     | Increase HS band                   | Normal              |
| 15 | Sabah        | 2.25       | Hepatomegaly, Dysmorphic     | 18.51                     | Normal                     | Normal pattern.                   | Normal              |
| 16 | East Coast   | 14.00      | Eye lesions, dysmorphic      | 7.49                      | Normal                     | Trace amount of HS                 | Normal              |
| 17 | Central      | 8.00       | Joint contracture            | 11.89                     | Normal                     | Normal pattern.                   | Normal              |
| 18 | Sarawak      | 3.00       | Hepatomegaly, Dysmorphic     | 9.61                      | Normal                     | Trace amount of HS band            | Normal              |
| 19 | Northern     | 0.17       | Hepatomegaly, Dysmorphic     | 44.82                     | Abnormal                   | Normal pattern.                   | Normal              |
| 20 | Central      | 4.00       | Dysmorphic                   | 18.54                     | Normal                     | Normal pattern.                   | Normal              |
| 21 | Central      | 0.33       | Hepatomegaly, Dysmorphic     | 34.82                     | Abnormal                   | Normal pattern.                   | Normal              |
| 22 | Central      | 0.50       | Hepatomegaly, Dysmorphic     | 47.98                     | Abnormal                   | Normal pattern.                   | Normal              |
| 23 | Southern     | 12.06      | Hepatomegaly, Dysmorphic     | 10.35                     | Normal                     | Trace amount of HS band            | Normal              |
| 24 | Central      | 4.00       | Hepatomegaly, Dysmorphic     | 8.28                      | Normal                     | Normal pattern.                   | Normal              |
| 25 | Sabah        | 1.25       | Dysmorphic, hepatosplenomegaly | 16.72                     | Normal                     | Normal pattern.                   | Normal              |
| 26 | Central      | 2.00       | Dysmorphic, hepatosplenomegaly | 15.95                     | Normal                     | Trace amount of HS band            | Normal              |
| 27 | Northern     | 0.67       | Dysmorphic, hepatosplenomegaly | 42.36                     | Abnormal                   | Normal pattern.                   | Normal              |
| 28 | Southern     | 7.42       | Dysmorphic, genu valgum/bowing legs | 11.76                     | Normal                     | Marked increase in CS band         | Normal              |
| 29 | Northern     | 7.00       | Kyphoscoliosis               | 92.82                     | Abnormal                   | Presence of HS band                | Normal              |

(continued on next page)
| No | Region      | Age  | Symptom                                | GAGs value (mmol/g creat) | GAGs level (based on age) | HRE band                  | Diagnosis                      |
|----|-------------|------|----------------------------------------|---------------------------|---------------------------|---------------------------|--------------------------------|
| 30 | Northern    | 10.00| Dysmorphic, hepatosplenicomegaly       | 13.33                     | Abnormal                  | Presence of mild HS band  | Normal                         |
| 31 | Northern    | 0.33 | Dysmorphic, hepatosplenicomegaly,      | 29.91                     | Normal                    | Increase of CS band with  | Normal                         |
|    |             |      | respiratory distress                   |                           |                           | presence of mild HS band  |                                |
| 32 | Northern    | 0.33 | Corneal clouding                       | 43.02                     | Abnormal                  | Mild increase of DS band  | Normal                         |
|    |             |      |                                        |                           |                           | and trace amount of HS    |                                |
|    |             |      |                                        |                           |                           | band                       |                                |
| 33 | Southern    | 6.00 | Dysmorphic                             | 7.93                      | Normal                    | Presence of trace amount  | Normal                         |
|    |             |      |                                        |                           |                           | of HS and DS band         |                                |
| 34 | Northern    | 14.00| Dysmorphic, short stature, kyphosis    | 3.81                      | Normal                    | Mild increase of HS band  | Normal                         |
| 35 | Southern    | 0.02 | Dysmorphic                             | 40.92                     | Abnormal                  | Trace amount of HS band   | Normal                         |
| 36 | Sabah       | 15.00| Dysmorphic, hepatosplenicomegaly      | 53.41                     | Abnormal                  | Marked increase of HS    | Normal                         |
|    |             |      |                                        |                           |                           | band                       |                                |
| 37 | Southern    | 11.42| Dysmorphic, Clawed hand                | 6.65                      | Normal                    | Increase of DS band and  | Normal                         |
|    |             |      |                                        |                           |                           | trace amount of HS band   |                                |
| 38 | Sabah       | 3.00 | Dysmorphic, hepatosplenicomegaly,      | 19.7                      | Abnormal                  | Presence of trace HS      | Normal                         |
|    |             |      | Corneal clouding                       |                           |                           | band                      |                                |
| 39 | Sarawak     | 0.16 | Dysmorphic, hepatosplenicomegaly,      | 67.76                     | Abnormal                  | Presence of HS band       | Normal                         |
|    |             |      | Corneal clouding                       |                           |                           |                            |                                |
| 40 | Northern    | 1.58 | Dysmorphic, gibbus, pectus carinatum   | 56.13                     | Abnormal                  | Presence of KS band       | Normal                         |
| 41 | Central     | 3.00 | Dysmorphic, respiratory problem        | 108.74                    | Abnormal                  | Increase of DS band and   | Normal                         |
|    |             |      |                                        |                           |                           | trace HS band             |                                |
| 42 | Northern    | 0.83 | Dysmorphic, eye lesions                | 42.86                     | Abnormal                  | Presence of DS band and   | Normal                         |
|    |             |      |                                        |                           |                           | trace amount of HS band   |                                |
| 43 | Sarawak     | 4.00 | Dysmorphic, asymptomatic (sibling      | 76.28                     | Abnormal                  | Increase of DS and HS     | MPS I                          |
|    |             |      | screening)                             |                           |                           | band                      |                                |
| 44 | Central     | 0.17 | Dysmorphic, asymptomatic (sibling      | 185.35                    | Abnormal                  | Increase DS and HS band   | MPS II                         |
|    |             |      | screening)                             |                           |                           |                            |                                |
| 45 | Central     | 1.0  | Dysmorphic, hepatosplenicomegaly       | —                         | —                         | —                          | (Result of GAGs screening and | MPS II                         |
|    |             |      |                                        |                           |                           | —                          | HRE characterization were    |                                |
|    |             |      |                                        |                           |                           | —                          | performed in University     |                                |
|    |             |      |                                        |                           |                           | —                          | Hospital)                      |                                |
| 46 | Central     | 2.50 | Dysmorphic, hepatosplenicomegaly       | 92.37                     | Abnormal                  | Presence of DS and HS     | MPS II                         |
| 47 | Central     | 2.00 | Dysmorphic, Hepatomegaly               | 44.31                     | Abnormal                  | Presence of HS band       | MPS IIIA                       |
| 48 | Central     | 1.58 | Dysmorphic, Hepatomegaly               | —                         | —                         | —                          | (Result of GAGs screening and | MPS IIIA                       |
|    |             |      |                                        |                           |                           | —                          | HRE characterization were    |                                |
|    |             |      |                                        |                           |                           | —                          | performed in University     |                                |
|    |             |      |                                        |                           |                           | —                          | Hospital)                      |                                |
| Patient Type | Region | Score | Diagnosis Details | Score | Abnormality | Pattern Details | Disorder |
|-------------|--------|-------|-------------------|-------|-------------|----------------|----------|
| Central     | 3.12   | Scoliosis, Claw hand | 12.44 | Normal | Normal pattern | MPS IVA |
| Central     | 7.00   | Respiratory distress, dysmorphic | —    | —    | HS prominent (Result of GAGs screening were performed in University Hospital) | MPS IVA |
| Central     | 1.08   | Dysmorphic, Hepatomegaly | 27.64 | Abnormal | Normal pattern | MPS VI |
| Central     | 7.33   | Dysmorphic, Hepatomegaly | 69.54 | Abnormal | Presence of DS band with trace amount of HS band | MPS VI |
| Northern    | 3.25   | Dysmorphic, corneal clouding | 51.7  | Abnormal | Presence of DS band | MPS VI |
| Central     | 0.18   | Dysmorphic, respiratory problem | 100.97 | Abnormal | Normal pattern | MPS VI |
| Northern    | 2.83   | Dysmorphic, macrocephaly | 19.27 | Abnormal | Normal pattern | MPS VI |
| Northern    | 2.83   | Dysmorphic, macrocephaly | 60.25 | Abnormal | Normal pattern | MPS VI |
| Central     | 0.5    | Dysmorphic, hepatosplenomegaly | 28.41 | Normal | Presence of HS band | MPS VI |
| Central     | 0.21   | Dysmorphic, hepatosplenomegaly | 39.75 | Abnormal | Normal pattern | MPS VI |
Table 2
Demographic data of high risk patients of selective screening for mucopolysaccharidoses (MPS) in Malaysia (2014–2016).

| Variables          | Frequency | Percentages |
|--------------------|-----------|-------------|
| Gender             |           |             |
| Male               | 31        | 53.4        |
| Female             | 27        | 46.6        |
| Ethnicity          |           |             |
| Malay              | 36        | 62.1        |
| Chinese            | 16        | 27.6        |
| Indian             | 2         | 3.4         |
| Sabah Native       | 1         | 1.7         |
| Sarawak Native     | 1         | 1.7         |
| Indigenous People  | 2         | 3.4         |
| Region             |           |             |
| Central            | 19        | 32.8        |
| Northern           | 20        | 34.5        |
| East Coast         | 4         | 6.9         |
| Southern           | 6         | 10.3        |
| Sarawak            | 5         | 8.6         |
| Sabah              | 4         | 6.9         |

Table 3
Glycosaminoglycans (GAGs) distribution between age group in high risk patients of selective screening for mucopolysaccharidoses (MPS) in Malaysia (2014–2016).

| Parameters          | Age group          | Median | Minimum value | Maximum value |
|--------------------|--------------------|--------|---------------|---------------|
|                    | Less than 1 year   | 43.02  | 29.91         | 76.33         |
|                    | 1–4 years          | 19.13  | 8.28          | 108.74        |
|                    | 4–9 years          | 12.83  | 1.84          | 92.82         |
|                    | More than 9 years  | 7.49   | 3.81          | 53.41         |

Table 3 below describes distribution of GAGs among different age groups in study population. Each age group is carefully divided according to age group in GAGs determination. The data from this table supports the facts that GAGs distribution will be decreased towards increment of age.

In general, Table 4 below describes the diagnostic test evaluation for three different approaches in our selective screening of high risk patients of MPS in Malaysia. The first approach was using only GAGs determination using DMB method, the second approach utilised characterization of GAGs using High Resolutions of Electrophoresis (HRE) and the last approach was carried out using a combination of GAGs determination using DMB and GAGs characterization using HRE. The first approach showed high sensitivity but poor specificity while the second approach revealed high specificity but poor sensitivity. By using both analytical methods, we managed to achieve satisfactory performances of sensitivity and specificity (more than 80%). We believe this information will be beneficial to laboratory personnel in order to evaluate their performance and capabilities of current methods.

Table 4
Diagnostics test evaluation for distinctive approaches of selective screening for MPS in high risk patients in Malaysia (2014–2016).

| Parameters          | GAGs         | HRE          | Combination   |
|--------------------|--------------|--------------|---------------|
|                    | Value 95% CI | Value 95% CI | Value 95% CI  |
| Sensitivity (%)     | 81.25 54.35 to 95.95 | 45.00 23.06 to 68.47 | 87.50 61.65 to 98.45 |
| Specificity (%)     | 47.62 32.00 to 63.58 | 81.58 65.67 to 92.26 | 83.33 68.64 to 93.03 |
| Positive predictive value (%) | 37.14 28.94 to 46.16 | 56.25 36.01 to 74.6 | 66.67 49.80 to 80.13 |
| Negative predictive value (%) | 86.96 69.61 to 95.10 | 73.81 64.84 to 81.16 | 94.59 82.62 to 98.47 |
| Disease prevalence (%) | 27.59 16.66 to 40.90 | 34.48 22.49 to 48.12 | 27.59 16.66 to 40.90 |
In conclusion, demographic data together with clinical symptoms/presentation and laboratory findings are important to assist clinician/researchers for future studies in MPS and can be employed to improve the diagnosis of MPS.

2. Experimental design, materials and methods

2.1. Study population and sample collection

This is a prospective cross-sectional study involving samples from high-risk children and young adults for MPS conducted over 2 years starting June 2014 to June 2016. A total of 58 urine samples for urinary GAGs quantitation and characterization and whole blood (n = 60) for enzymatic assays were received between 2014 and 2016. Urine samples (20 ml) were kept frozen while whole blood (6 ml) was processed to obtain plasma and leukocytes before stored at −80 °C. All the samples were collected from patients which had least two features of the following of inclusion criteria: (a) abnormal face features such as macrocephaly or coarse face; (b) corneal clouding or loss of visual acuity; (c) hearing impairment and recurrent middle ear infections; (d) recurrent respiratory tract infection; (e) valvular heart disease or heart murmur; (f) recurrent inguinal or umbilical hernia; (g) hepatosplenomegaly; (h) at least two symptom of musculoskeletal: (1) evolving joint contracture without obvious signs of inflammation, (2) joint laxity, (3) gibbus, (4) cervical spine stenosis and/or cord compression, (5) kyphosis or scoliosis, (6) pectus carinatum, (7) bilateral hip dysplasia, (8) progressive genu valgum after age of 3 years old, (9) short stature of unknown reason, (10) carpal tunnel syndrome. Patients presenting with mental retardation were excluded from this study.

2.2. Sample size calculation

\[
n = \frac{t^2 \times p (1-p)}{m^2} = \frac{(1.645)^2 \times 0.0021 \times (1-0.0021)}{(0.01)^2} = \frac{2.706 \times 0.000209559}{0.0001} = 56.7 \sim 57 \text{ samples}
\]

Where,
\( n = \text{required sample size} \)
\( t = \text{confidence level at 90\% (standard value is 1.645)} \)
\( p = \text{estimated prevalence of mucopolysaccharidoses worldwide in percentage (1/48,780 \times 100\% = 0.0021)} [7] \)
\( m = \text{margin of error at 1\% (0.01)} \)

2.3. Quantitation and characterization of urinary GAGs

MPS urine test includes both quantitative analysis of total GAGs using dimethylmethylene blue method (DMB) and qualitative using High Resolution Electrophoresis (HRE). In brief, 30 μL of standards and patient samples were diluted with 120 μL of deionised water. 825 μL of freshly prepared DMB was later added and mixed thoroughly and analysed by spectrophotometer, at 520 nm. Standard graphs were plotted and used to calculate the value of GAGs in patients’ samples. Method is adapted from Nor A [5]. Equal amount of urine is added to cetylpyridinium chloride (CPC) buffer where GAGs in urine is precipitated to form a complex with CPC. The resulting CPC/GAGs complexes are dissociated by addition of lithium chloride and the GAGs re-precipitated with ethanol. The GAGs precipitate was re-dissolved in 20 μL of phenol red. Electrophoresis of the recovered GAGs was undertaken on cellulose acetate using divalent ion buffer system of high ionic strength (0.1 mol/L barium acetate). High
resolution was achieved by making use of the different solubility of each GAGs in ethanol/buffer solutions of different concentrations. Interpretation is based upon the quantitative analysis of their relative amounts of excretion and pattern recognition of the specific sulphate(s) detected on HRE.

2.4. Enzyme activity in blood

Enzyme activity was performed in plasma or leukocytes. The leukocytes were extracted from EDTA blood by differential centrifugation as described by van Diggelen et al. (1990). Plasma and leukocyte pellets were kept frozen at −80 °C until analysis. The resulting leukocyte pellet was sonicated in ice for two 5-s bursts at 5 micro/amplitude followed by centrifuged. The supernatants were kept on ice before analysis. Modified Lowry method was used to determine protein concentration. For enzyme assays, methods from Lysosomal Laboratory of Willink Biochemical Genetics, St. Mary's Hospital, Manchester, UK were adapted with modification for the assays to be performed in microtiter plates. We used methods described by Hopwood et al. (1979) for MPS Type I [6], MPS II [14], MPS IVA [13], MPS IVB [4], MPS VI [2], and MPS VII [3]. Furthermore, various methods for determination of total β-hexosaminidase [9], β-mannosidase [10] and α-mannosidase [11] for diagnosis of mucolipidoses if all MPS enzyme assay were found normal.

Selected enzyme analysis was performed 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 fluorescent 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 were reacted with the artificial substrate and released the fluorescent compound. The compound was measured using fluorometer. Various concentrations of the fluorescence compound were run as standards and the curves were plotted and used for calculation of product amount. Enzyme activities in plasma were expressed as amount of fluorescent compound (product) being released per ml per hour (nmol/ml/hour). Enzyme activities in leukocytes were expressed as the amount of product being released per ml per mg protein per hour and the unit is nmol/ml/mg protein/hour.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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