

Study of Inherited Metabolic Disorders in Singapore – 13 Years Experience

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Abstract

Introduction: Recommended by the National Advisory Council of the Disabled, the Ministry of Health of Singapore supported a nationwide study of inherited metabolic disorders (IMDs). When the 5-year project ended, investigations were provided as a diagnostic service. This paper documents our 13-year experience. **Materials and Methods:** Patients with symptoms suggestive of an IMD were referred. Investigations on heparinised blood and/or urine included amino acid analysis using a Beckman 6300 Amino Acid Analyser, organic acids analysis using a Hewlett-Packard gas chromatography and mass spectrometry, mucopolysaccharides quantitative assay and high-resolution electrophoresis, sugars by thin-layer chromatography. **Results:** Of the 3656 patients studied from 1992 to 2005, IMDs were found in 127 (77 males; 50 females; age range, 1 day to 56 years). Their ethnic distribution was: 55.1% Chinese, 19.7% Malays, 11.0% Indians, 11.0% other races and 3.2% unknown. IMD diagnosed comprised 41 (32.3%) organic acidurias, 34 (26.8%) amino acidaemias/acidurias, 14 (11.0%) urea cycle defects, 15 (11.8%) mucopolysaccharidoses, 6 (4.7%) carbohydrate disorders and 17 (13.4%) others. Twenty-three (18.1%) cases were diagnosed during the neonatal period and 36 (28.3%) after the age of 13. **Conclusion:** Positive detection rate was 3.5% and 48 IMDs were found. Significant proportion of cases had late-onset IMDs. Early identification of IMDs permits timely management, genetic counselling and prenatal diagnosis.

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Key words: Aminoacidurias, Inborn errors of metabolism, Mucopolysaccharidoses, Organic acidurias, Urea cycle defects

Introduction

Inborn errors of metabolism (IEMs) described in the early 1900s by Garrod were due to a block in a metabolic pathway, arising from an enzyme deficiency which led directly to the disruption of cellular metabolism.¹ However 40 years later, it was discovered that many inherited diseases were not due to a block in a metabolic pathway but caused by abnormality of a transport process affecting movement of specific metabolites within the vascular compartment, within the cell and across the cell membrane: uptake into the cells may be altered by a defect in a membrane receptor. In addition, there are other diseases in which the basic disorder is in other important biological functions: for example, in cell structure, in blood coagulation and in the oxygen carrying capacity of haemoglobin. Together with the IEMs described by Garrod, they are now collectively known as inherited metabolic diseases or disorders (IMDs).

The types of disorders and gene mutations vary widely in different ethnic groups and locations. Genetic factor,

hormonal status, diets, infection and stress influence the nature and severity of clinical manifestation which may appear as early as the newborn period and as late as the fourth and fifth decades of life. The same IMD may present in different forms at different stages of life. Some patients have persistent symptoms while others may have intermittent symptoms. Severity and timing of clinical presentations vary both between and within individuals. Clinical presentation of patients with IMDs is often non-specific and suggestive of a variety of non-genetic conditions, including viral and bacterial infection, poisoning, malignancy, metabolic and organ dysfunction due to other diseases, and physical abnormalities due to other causes. It is important for clinicians to be aware of the presence of IMDs in our population and include them as possible diagnoses, in order for them to be “discovered”.

IMDs are present in every population but are perceived to be very rare because they were not recognised and not documented, especially in many Asian countries. Prevalence

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of IMDs in various populations has been underestimated, except where large population screening was conducted. Incidence between different disorders and populations is extremely variable. For example, the frequency for phenylketonuria (PKU) has been reported as 1 in 5000 in Dublin compared with 1 in 200,000 in Japan.²⁻⁸ Clinical severity is extremely varied between different disorders and populations. IMDs most likely to present in the neonates and young children are those due to defects in the metabolism of amino acids, organic acids, carbohydrates, purines and pyrimidines and urea cycle defects.⁹⁻¹¹

Currently, only 2 screening tests are performed on all babies in all 50 states of USA: PKU and hypothyroidism. There has been a suggestion that cystic fibrosis, congenital adrenal hyperplasia, G6PD deficiency and others be added to the screening profile.¹² Recent advances in Tandem Spectrometry (MS/MS) technology has facilitated the detection of multiple metabolic disorders simultaneously on a single specimen and has been attracting increasing interest. In 2000, the National Newborn Screening and Genetics Resource Center, in collaboration with the Centers for Disease Control and Prevention (CDC) and the Health Resource and Service Administration, convened a workshop to examine effective integration of MS/MS technology into newborn screening programmes. However, decision would take time because of the complexity of introducing such a programme.¹³ Due to the lack of resources many countries in Asia, with the exception of Japan, do not have screening programme nor laboratory diagnostic service for IMDs. Therefore, there is a paucity of data on large-scale studies in Asia.

In Singapore in the early 1960s, studies performed to identify the cause of hyperbilirubinaemia, kernicterus and death in infants,¹⁴⁻¹⁸ revealed a high incidence of G6PD deficiency (over 3%) and led to the establishment of a screening programme for detecting G6PD deficiency in newborns in 1965. In the early 1980s, studies performed to assess the thyroid status in newborns resulted in the establishment of a newborn screening programme in mid-1990s.¹⁶⁻¹⁹

At the recommendation of the National Advisory Council on the Disabled, which has been concerned with the problems and welfare of the physically and mentally handicapped, the Ministry of Health (MOH) established a national laboratory in early 1992 for the study of inherited metabolic disorders other than G6PD deficiency, thalassaemias and hypothyroidism which already had their respective screening programmes. The objective was to determine the incidence and diversity of other metabolic disorders especially those leading to mental retardation, physical disability and other severe clinical consequences. The strategy for prevention of IMDs is the definitive

identification of these diseases, followed by genetic counselling for the affected families and prenatal diagnosis for future pregnancies where possible. A national committee comprising paediatricians and neonatologists from major public hospitals and experienced laboratory staff designated for the study was appointed.

As the biochemical basis of IMDs is wide ranging and there is a bewildering array of specialised tests, our approach was to maximise the cost-effectiveness of the allocated funds and the diagnostic potential of the range of tests to be introduced. After much deliberation and advice from experts in the field, we concluded that the use of an amino acids analyser, an organic acids analyser and instruments for the quantitative assay and separation of mucopolysaccharides and carbohydrates would enable us to diagnose a wide variety of disorders of intermediary metabolism i.e., amino acids and organic acids disorders, urea cycle defects and mucopolysaccharidoses (MPSs). The tests were, however, not targeted for the full range of lysosomal storage disorders (except some MPSs), peroxisomal disorders and mitochondrial disorders as these require the use of a far wider range of instruments and methods such as enzyme analyses and DNA mutation studies and would incur higher cost. Where possible, such cases would be referred to well-established laboratories overseas.

Methods

Dry and wet chemistry qualitative tests were used for screening various metabolites in fresh random urine specimens: Ames Reagent Strips, Multistix, Sulphite Test reagent strips (Merckoquant Sulfite Test strip, E Merck, Darmstadt, Germany), Clinitest for reducing substances (Clinitest reagent tablets, Bayer Diagnostics, Australia), ferric chloride test for phenylketones,²⁰ cyanide nitroprusside tests for cystine and homocystine,²¹ silver nitroprusside test for homocystine,²² nitrosonaphthol tests for tyrosine,²³ cupronic test for histidine,²⁴ 2,4-dinitrophenylhydrazine (DNPH) test for α -keto acids,²⁵ Selivanoff's test for fructose,²⁶ spot test for methylmalonic acid,²⁷ ammonium-silver nitrate test for homogentisic acid,²⁸ thin-layer chromatography for mono-, di- and oligosaccharides.²⁹ These screening tests were performed on all urine specimens.

A Vitros 950 Analyser (Ortho-Clinical Diagnostics Inc, a Johnson-Johnson Co, Rochester, USA) was used for plasma ammonia and lactate assay while a Sigma reagent kit (Sigma Diagnostic Inc, St Louis, MO, USA) was used for blood pyruvate measurement. A Beckman 6300 series high-performance amino acid analyser was used for the quantitation of amino acids in plasma, urine and cerebrospinal fluid (Spino Division, Beckman Instruments, Palo Alto, California, USA). Analyses were performed according to the instrument manufacturers' specified

protocol. Urine creatinine assay was performed by a Beckman LX20-PRO analyser (Beckman Coulter, Palo Alto, California, USA). Amino acids concentrations in urine were expressed as $\mu\text{mol}/\text{mmol}$ creatinine. A gas chromatography-mass spectrometry (GC-MS) system comprising a 5890A Series II chromatograph and a 5971A mass spectrometer (Hewlett-Packard, Analytical Product Group, Delaware, USA) was used for the analysis of urinary organic acids. Trimethylsilyl derivatives of organic acids were prepared as described by Sternowsky et al,³⁰ and identification of the derivatives were made according to the methods described by Chalmers and Lawson,³¹ Lefevre et al³² and Toshimitsu Niwa.³³ Quantitation and identification of glycosaminoglycans (GAG) in urine were made by a direct dye-binding assay procedure,^{34,35} and high-resolution electrophoresis respectively.³⁶ Detection and identification of monosaccharides, disaccharides and oligosaccharides in urine was achieved by thin layer chromatography. A Sigma reagent kit (Procedure No. 195, Sigma Diagnostic Inc, St Louis, USA) was used for erythrocyte galactose-1-phosphate-uridyl transferase measurement. Serum biotinidase activity was assayed according to the method of Pettit and Wolf.³⁷

The Department of Chemical Pathology of the Adelaide Women's and Children's Hospital; Peroxisomal Diseases Laboratory, Kennedy Institute for Handicapped Children, North Broadway, Baltimore, USA; Oliver Latham Laboratory, Sydney, Australia; Royal Children's Hospital, Melbourne, Australia; the Metabolic Laboratory at Baylor College of Medicine, Houston, Texas, USA; and the Institute of Genetics, National Yang Ming University, Taiwan assisted us with specific enzyme and metabolite analyses and mutation studies for confirmation of MPS, Niemann-Pick Disease and galactosialidosis, peroxisomal disorder, Pearson Marrow-Pancreas syndrome, mitochondrial disorder, sulphite oxidase deficiency, propionic aciduria, adrenoleucodystrophy and atypical hyperphenylalaninaemia.

Patients

Prior to the commencement of the nationwide study, a brochure providing the objectives and details of the study and criteria of patient selection was distributed to all paediatricians and neonatologists in Singapore. They were invited to refer patients with symptoms suggestive of an IMD to the coordinating centre for clinical examination and laboratory investigations. Physicians for adult patients were also welcome to refer patients if there was strong indication to do so. During the national study period between 1992 and 1997, a complete range of tests was performed on every patient who developed symptoms suggestive of an IMD. The referring physician was required to provide full clinical history, presenting symptoms and

clinical observations with detailed information on potential metabolic triggers on a standard form containing a list of questions.

Specimen Collection

Blood (with/without appropriate anticoagulant and preservative), cerebrospinal fluid and random urine specimens, collected as far as possible during the acute phase of the disease and prior to treatment, were transported at 4°C to the laboratory promptly. Although it was desirable to obtain blood and urine specimens simultaneously for laboratory testing, sometimes only blood or urine specimen was received.

Results

(A) Results for the Ministry of Health (MOH) study

From early 1992 to mid-March 1997, 1075 patients were studied and 56 (37 males; 19 females; male-to-female ratio, 1.95) were diagnosed with IMDs giving a positive detection rate of 5.2%. This is most likely an underestimate because urine specimens were only available from 448 patients and blood specimens could not be obtained from some patients. The types of IMDs diagnosed included: 6 amino acidaemia/acidurias (10.7%), 5 urea cycle disorders (8.9%), 14 organic acidurias (25%), 8 MPSs (14.3%), 6 carbohydrate disorders (10.7%) and 17 miscellaneous disorders (30.4%). The age of presentation of these disorders is summarised in Table 1. The highest percentage of patients with late manifestation (45.5%) and diagnosed after 10 years of age was found in the amino acids/urea cycle disorders. Detailed and specific diagnoses of these cases are included in the collective results for the 13-year duration.

Of the 56 affected patients, 35 (62.5%) were Chinese, 12 (21.4%) were Malays, 5 (8.9%) were Indians and 4 (7.1%) belonged to other ethnic minorities. A high proportion of patients with an IMD (14 of 56 patients or 25.0%) were

Table 1. Age of Presentation of Various IMDs in the MOH Study

Types of IMDs	Age of Presentation
Organic aciduria	2d, 2d, 4d, 6d, 5w, 5m, 9m, 1y, 1y1m, 1y1m, 2y5m, 4y4m, 7y, 38y
Amino acidaemia/urea cycle disorders	2d, 21d, 1y6m, 1y11m, 3y5m, 5y, 11y, 15y, 23y, 30y, 52y
Mucopolysaccharidosis	11m, 1y8m, 1y11m, 2y8m, 4y11m, 5y2m, 5y3m, 13y
Carbohydrate disorders	23d, 1m5d, 5m28d, 8m, 5y, 16y
Miscellaneous	2d, 4d, 29d, 6m, 7m, 8m, 1y10m, 2y1m, 2y2m, 6y, 7y, 7y, 9y, 11y, 2 (teenage brothers, exact age not given), 17y

d: days; IMD: inherited metabolic disorder; m: months; MOH: Ministry of Health; w: weeks; y: years

found to be products of consanguineous marriages. The incidence of consanguinity was found to be highest in Malays (9 of 14 patients, or 64.3%), followed by Indians (3 of 14 patients, or 21.4%) and Chinese (2 of 14 patients, or 14.3%). Consanguinity as a predisposing factor for the development of IMDs was observed in 57.1% (8 of 14 cases) of the organic acidurias, 25.0% (2 of 8 cases) of the MPSs, 17.6% (3 of 17 cases) of the miscellaneous group, 9.1% (1 of 11 cases) of the amino acidurias and urea cycle defects and none of the carbohydrate disorders. Results of part of this study have been previously reported.³⁸⁻⁴² At the end of the 5-year study, few patients referred for investigations provided detailed family history. Therefore, prevalence of consanguinity in patients with IMDs could no longer be studied.

After the MOH-sponsored study, we were unable to document diagnoses of carbohydrate and miscellaneous metabolic disorders. Erythrocyte enzyme assay for confirmation of galactosaemia was discontinued as commercial production of test kits had ceased. Research fund was no longer available for specialised tests performed overseas for diagnosis of the other disorders. Patients requiring specialised tests for confirmation were referred directly to laboratories overseas. For the purpose of providing a complete list of the type and number of individual IMDs found, all cases of MOH study have been included in the summarised results of our 13-year experience.

Prevalence of β -aminoisobutyric Aciduria (BAIB)

Of the 448 urine specimens available for amino acids analysis, 67 or 14.9% (Chinese, 11.8%; Malays, 2.7%; Indians, 0.2%; Others, 0.2%) showed high excretion of β -aminoisobutyric acid (exceeding twice the upper limit of the reference range). β -aminoisobutyric aciduria is a benign metabolic polymorphism and reported to be present in 5% to 10% of Caucasians and 40% to 95% of Oriental populations but not in Asian-Indians.^{4,43} As β -aminoisobutyric aciduria is a benign condition, it is excluded from the list on amino acidurias and from the calculation of total number of IMDs.

(B) Inherited Metabolic Disorders in Patients from Kota Bahru, Malaysia

Urine specimens of 32 Malay patients from Kota Bahru, Kelantan, were referred to us for investigations. Tests for MPS diagnosis were specifically requested for 7 patients but no abnormality was detected. Analyses for amino acids and organic acids for 25 patients revealed the following: methylmalonic aciduria (2 cases), cystine-lysinuria (1 case), argininaemia (5 cases) and S-sulphocysteinuria (1 case). Three patients were found to have severe ketoacidosis with marked increase in β -hydroxybutyric acid but no other specific organic acid abnormality. The remaining 14 patients

did not show any abnormality. A high detection rate of at least 28.1% (9 out of 32) was noted for this pre-selected group with suspected IMDs. It is not known if consanguinity was an important predisposing factor because family histories were not available. Argininaemia affected 5 members of a family. One child died before laboratory tests could be performed. The 4 surviving children showed symptoms of varying severity, presenting with progressive spastic diplegia or tetraplegia, epilepsy, mental retardation and developmental delay. All had increased plasma arginine and urinary arginine, orotic acid and uracil. All cases from Malaysia have been excluded from the summarised results of our 13-year study presented below. They were also excluded from calculation of percentage distribution of positive cases among various ethnic groups in Singapore.

(C) Results of 13-year Study (inclusive of the Ministry of Health Study)

During the 13-year period from early 1992 to July 2005, a total of 3656 patients (excluding 32 Malaysian patients) were studied. Of these, 127 (77 males; 50 females; male-to-female ratio, 1.54) were diagnosed as having an IMD, giving a positive detection rate of 3.5%. The distribution of positive cases among the Chinese, Malays, Indians, other races and unknown was 55.1%, 19.7%, 11.0%, 11.0% and 3.2%, respectively. Compared with the ethnic distribution of the population in Singapore which was 76.0% Chinese, 13.8% Malays, 8.4% Indians and 1.8% other races, IMDs are present in a disproportionately high percentage of non-Chinese. The types and number of IMDs are shown in Table 2 and age of presentation and diagnosis is summarised in Table 3.

Specific diagnoses of IMDs diagnosed are summarised below.

Organic Acidurias diagnosed comprised: 10 (24.4%) glutaric aciduria type II, 8 (19.6%) propionic aciduria, 7 (17.1%) methylmalonic aciduria, 5 (12.2%) glutaric aciduria type I, 3 (7.4%) 3-methyl-glutaconic aciduria, 2 (4.9%)

Table 2. Types and Number of IMDs Diagnosed from 1992 to 2005

Type of IMDs	No.	%
Organic acidurias	41	32.3
Amino acidurias/acidurias	34	26.8
Urea cycle defects	14	11.0
Mucopolysaccharidoses	15	11.8
Carbohydrate disorders	6	4.7
Others	17	13.4
Total number diagnosed	127	100.0

IMD: inherited metabolic disorder

(Note: total number studied = 3656; positive detection rate = 3.5%)

Table 3. Age of Symptom Manifestation and Diagnoses

Age	No.	%
1 month or less (neonatal)	23	18.1
Between 1 month and 13 years	68	53.5
Below 13 years (before puberty)	91	71.7
More than 13 years (after puberty)	36	28.3

D-2-hydroxyglutaric aciduria and 1 case (2.4%) each of alcaptonuria, glycerol kinase deficiency, holocarboxylase synthetase deficiency, hyperoxaluria, 2-ketoadipic aciduria and orotic aciduria. The 41 cases included 14 diagnosed during the 5-year MOH study, but excluded 2 cases of methylmalonic aciduria from Malaysia.

Amino Acidaemias/Acidurias diagnosed comprised: 8 (23.5%) cystine-lysinuria, 6 (17.7%) hyperornithinaemia with gyrate atrophy (HOGA), 6 (17.7%) phenylketonuria, 4 (11.8%) maple syrup urine disease (MSUD), 3 (8.8%) non-ketotic hyperglycinaemia, 2 (6.9%) homocystinuria, 2 (5.9%) tyrosinaemia type I and 1 case (2.9%) each of atypical phenylketonuria, iminoglycinuria and s-sulphocysteinaemia. The 34 cases included 6 diagnosed during the 5-year MOH study, but excluded 1 case each of cystine-lysinuria and s-sulphocysteinuria from Malaysia.

Urea Cycle Defects diagnosed comprised 7 (50.0%) ornithine transcarbamoylase deficiency, 6 (42.9%) citrullinaemia (argininosuccinate synthetase deficiency) and 1 (7.1%) argininosuccinate lyase deficiency. The 14 cases included 5 diagnosed during the 5-year MOH study but excluded the family of 5 with argininaemia from Malaysia.

Mucopolysaccharidoses diagnosed comprised 4 (26.7%) MPS type III (Sanfilippo disease), 3 (20%) MPS type I, II, VI, 3 (20%) MPS type IV (Morquio syndrome), 2 (13.3%) MPS type I (Hurler syndrome), 2 (13.3%) MPS type II (Hunter syndrome) and 1 (6.7%) MPS type VI (Maroteau-Lamy syndrome). The 15 cases included 8 from the 5-year MOH study. Diagnoses were confirmed by enzyme assays performed by a laboratory for genetic study in Australia, except 3 cases whose GAG studies indicated MPS type I, II or VI and whose specimens were unavailable for enzyme studies.

Carbohydrate Disorders diagnosed comprised 1 case each of galactosaemia (galactose-1-P-uridyl transferase deficiency), galactosaemia (patient died before enzyme assay could be performed), galactosaemia (not due to galactose-1-P-uridyl transferase deficiency; specimen unavailable for confirming epimerase or kinase deficiency by overseas reference laboratory), glycogen storage disease type I (Von Gierke), glycogen storage disease type II (Pompe's Disease), glycogen storage disease (biopsy

specimen unavailable for enzyme study). All cases were diagnosed during the 5-year MOH study. After this period, we could no longer perform test for enzyme assay for galactosaemia as commercial production of test kits had ceased, and we had no funds to pay for specialised tests performed overseas for diagnosis of glycogen storage disease.

Miscellaneous Disorders diagnosed comprised 3 (17.6%) cases of x-linked adrenoleukodystrophy, 2 (11.8%) cases each of galactosialidoses, Lesch-Nyhan syndrome, lipodystrophy, Lowe syndrome and 1 (5.9%) case each of familial lipoprotein lipase deficiency, mitochondrial disorder with severe lactic acidemia, Niemann-Pick disease type C, Pearson marrow-pancreas syndrome, porphyria and sulphite oxidase deficiency. All cases were diagnosed during the 5-year MOH study. After the study, research funds were unavailable for specialised tests performed overseas for diagnosis of miscellaneous IMDs.

Persistent or repeated episodes of marked lactic acidemia were observed in a number of patients. We were successful in diagnosing secondary lactic acidosis associated with a variety of organic acidurias. However, we were unable to perform tests to confirm primary lactic acidosis due to disorders of pyruvate metabolism, gluconeogenesis, tricarboxylic acid cycle turnover and the electron transport chain. These congenital lactic disorders include deficient activity of the pyruvate dehydrogenase complex, pyruvate carboxylase and multicarboxylase deficiency, phosphoenol-pyruvate carboxykinase and fructose-1,6-diphosphatase deficiency, disorders of the cytochromes and respiratory chain.

High level of ethanolamine was found in blood/urine of several patients, the significance of which was not clear and these cases remained unconfirmed as some of the patients died and others did not return for follow-up investigations. Increase urine excretion of ethanolamine is found in ethanolaminosis due to hepatic ethanolamine kinase deficiency. However secondary increase is also observed in some newborns and is associated with liver cirrhosis, hyperlysinaemia and hypersarcosinaemia.⁴⁴

Proficiency Testing Programme for Laboratory Diagnosis of IMDs

Our laboratory participated in external proficiency testing programmes conducted by the Human Genetics Society of Australasia and the College of American Pathologists. Urine and/or plasma specimens from actual patients were provided for testing and participating laboratories are required to provide diagnoses based on tests results obtained. Our laboratory provided correct diagnoses for about 82.0% of patient specimens in both programmes which is above the average rate of 72.4% obtained by all participating

laboratories. Experience with these programmes is given in a separate paper.⁴⁵

Discussion and Conclusion

The results of our study showed that a wide variety of IMDs are present in Singapore. Organic aciduria accounted for the largest proportion of cases referred to our laboratory because of symptoms suggestive of an IMD (32.3%). This was followed by amino acidurias or acidurias (26.8%), MPSs (11.8%) and urea cycle defects (11.0%). A heterogeneous group of disorders accounted for the remaining 28.1% of cases. A preponderance of male over female patients (males-to-female ratio, 3) was observed. Incidental observation of a high prevalence of β -aminoisobutyric aciduria confirmed previous reports that this benign disorder is common in Oriental populations.^{4,43} In a 2-year study conducted by the Metabolic Laboratory of the Institute of Clinical Genetics in Rotterdam serving a population of 3 million people in the south-western part of The Netherlands, the observation was: organic aciduria (19.8%), amino acidurias/acidurias including some urea cycle defects (64.0%), carbohydrate, lysosomal storage and peroxisomal disorders (16.2%).⁴⁴

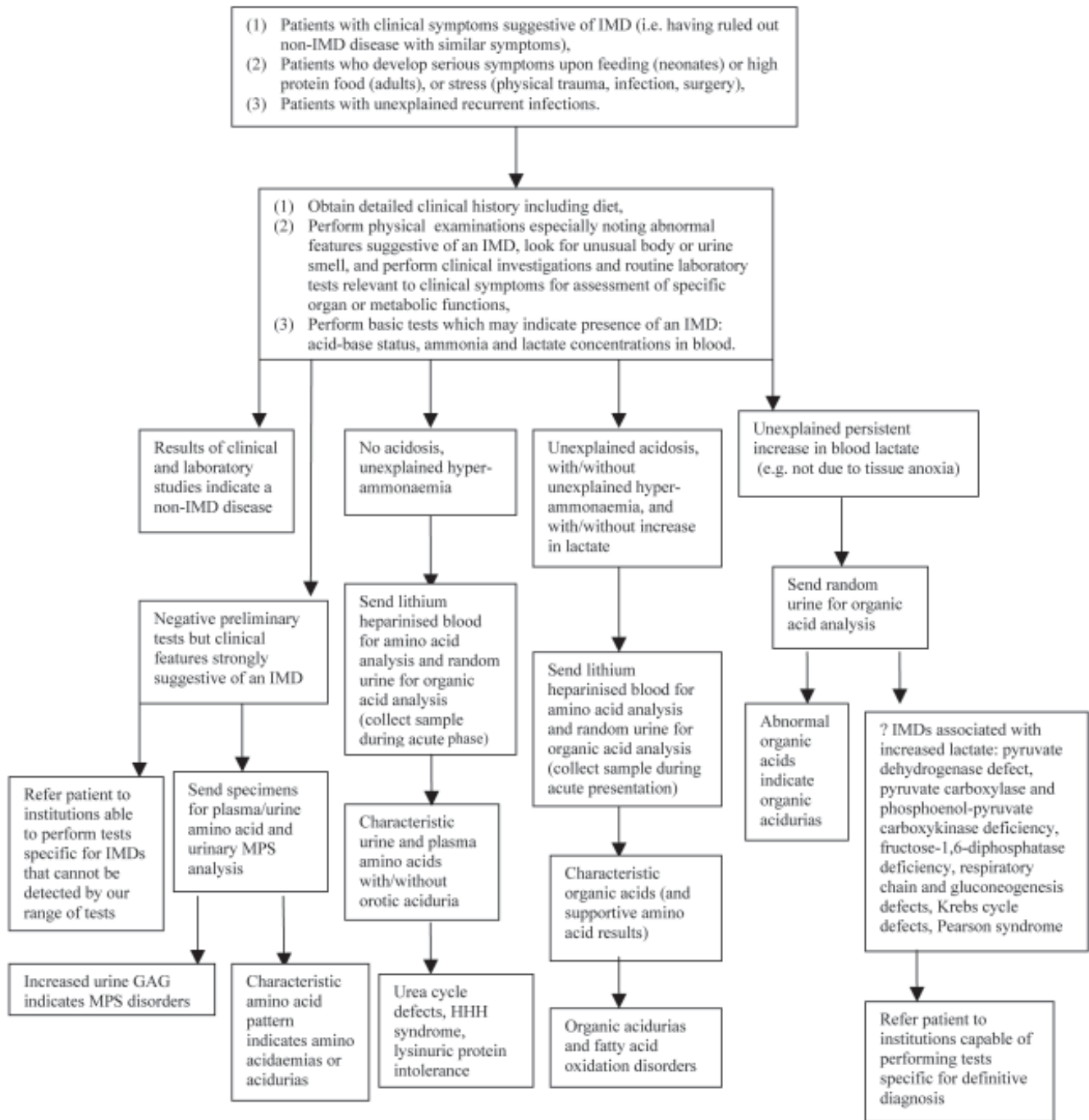
Based on the results of our 13-year study, the positive detection rate in high-risk patients was 3.5%. The apparent difference between this detection rate and the 5.2% observed for the 5-year MOH study period was due to the inclusion of a heterogeneous group of carbohydrate and miscellaneous disorders in the calculation of detection rate for the earlier study. When research funds were exhausted, we were unable to bear the cost of patient investigations performed by laboratories overseas. This clearly shows that the overall detection rate observed is lower than the true rate due to our failure in providing appropriate tests for some patients. We believe that even the rate of 5.2% is still an underestimate due to a number of reasons which include: inadequate awareness and knowledge of IMD manifestations, lack of awareness of the availability of laboratory testing service, inappropriate specimen collection and inability to provide appropriate tests.

In comparison, the Dutch laboratory which studied 1726 patients in 1985 and 1986 reported 111 positive cases and a positive detection rate of 6.4%.⁴⁴ A higher positive rate is expected of the laboratory in Rotterdam as it was able to perform a wider range of specialised investigations such as those for lysosomal storage diseases and peroxisomal disorders. The laboratory was also equipped to measure acyl-carnitines, imidazoles, purines and pyrimidines. The laboratory has a much longer history and accumulated experience, starting as part of the routine clinical laboratory in 1971 and developed into a full Metabolic Laboratory in an institute dedicated to the diagnosis and management of

genetic disorders in 1979. From the larger number of patients studied within 2 years, there is obviously a far greater awareness of the presence of IMDs in the population. Experienced physicians for laboratory investigations helped to reduce false negative test results. Differences between Dutch and Singapore populations may also contribute to difference in positive detection rate. We can expect to see an increase in the rate of positive detection with further accumulation of experience and expertise, introduction of more sophisticated technology and wider range of investigations, increase in awareness of the presence of IMDs among clinical colleagues, greater experience in patient selection and improvement in specimen collection.

It is clear that an appropriate laboratory testing service and expertise in interpretation of test results are essential for IMD diagnosis. On the question of the need and efficacy of a neonatal screening programme, the MOH was silent for some time after the last report on the study was submitted. In view of the wide range of disorders, high cost compared with majority of routine tests, and higher cost compared with existing newborn screening programmes for G6PD deficiency and hypothyroidism, the MOH was hesitant in supporting a newborn screening programme. However, during the past few years, there has been rapid advancement in methods and instrumentation, in particular Tandem Mass Spectrometry, which is now capable of detecting about 40 IMDs in a single blood sample spotted on filter paper. In response to a new proposal to reassess the need for routine neonatal screening in Singapore, the MOH approved the purchase of a Tandem Mass Spectrometry system for a new research project on neonatal screening, which would be jointly conducted by the KK Women's and Children's Hospital and the National University Hospital.

Unfortunately, unlike screening for G6PD deficiency and congenital hypothyroidism, a screening programme for a wide variety of IMDs as described in this paper during the neonatal period is unlikely to yield positive findings for all cases. Our results showed that only 18.1% of patients manifested severe symptoms and were diagnosed below 1 month of age. The majority were diagnosed after the neonatal period, 53.5% between 1 month and puberty (13 years), and 28.3% from puberty to the fifth decade of life (Table 3). Apparently, patients who have late clinical manifestation did not experience major health problems earlier in life. However, once clinical symptoms appear, these may be followed by repeated episodes with increasing frequency and/or severity, with fatal consequences. In the absence of clinical symptoms, abnormal metabolites may not be detectable or only present at very low concentrations. Therefore, neonatal screening is likely to miss a good number of patients who do not have neonatal IMDs. Education of our clinical colleagues and a good laboratory



HHH: Hyperomithinaemia-hyperammonaemia-homocitrullinuria

Fig. 1. Suggested algorithm for patient investigations.

testing service are important to cater to the diagnosis of such patients.

We have described a number of patients with manifestation in adulthood. Citrullinaemia due to argininosuccinate synthetase deficiency was diagnosed in 2 patients aged 25 and 52.^{46,47} Serious symptoms in a Japanese adult patient with citrullinaemia was found to be triggered by drug administered for common cold.⁴⁸ When published reports on 28 patients were reviewed by these authors, 4 categories

of probable trigger were found: stress, liver dysfunction, alcohol and drugs. Prompt recognition and treatment are essential in determining the outcome of these patients.⁴⁹ Adult-onset and asymptomatic citrullinaemia have been reported in Caucasian and Japanese patients.⁵⁰⁻⁵⁷ Liver transplantation has been a successful mode of treatment for adult-onset citrullinaemia.^{58,59} A recent report on watermelon-induced citrullinaemia highlighted the importance of obtaining comprehensive history of food

intake from patients presented for IMD diagnosis. Watermelon, a common fruit in Singapore and other Asian countries, has been found to be a natural and rich source of citrulline.^{60,61}

A variety of IMDs are associated with ocular symptoms. When several adult patients with predominant complaints of ocular symptoms were referred, they were found to have HOGA of the choroid and retina.⁶²⁻⁶⁷ Increase in ornithine is due to a deficiency of the enzyme, ornithine aminotransferase (OAT). In human eyes the activity of OAT was found to be high, suggesting a physiologically significant role of this enzyme in the eyes.⁶⁸ As ornithine is a precursor of proline, a deficiency in OAT would lead to accumulation of ornithine and decreased formation of proline. Proline supplementation has been shown to halt disease progression.⁶⁸

Glutaric aciduria type II (GAII) or multiple acyl-CoA dehydrogenase deficiency (MADD) is an autosomal recessive inherited metabolic disease in which there is a defect in the activity of many CoA dehydrogenases.⁶⁹

The clinical picture of MADD is variable. We found a 24-year-old female Chinese patient with relatively mild symptoms of exhaustion, weakness and vomiting,⁷⁰ while another 38-year-old Malay male patient with hyperammonaemia, status epilepticus and life-threatening symptoms, who died 4 days after hospital admission.

In conclusion, early diagnosis of an IMD enables timely treatment and family counselling, helps the affected patient in avoiding inappropriate diet and incorrect and potentially harmful treatment, prevents recurrence of acute disease and serious consequences, and spares the family from stress and high cost of searching for the cause of the disease and a cure. Parents could be given advice on future family planning, early disease detection and option for avoiding further birth of affected children.⁷¹ For some IMDs, simple treatment such as dietary restriction, vitamin supplementation and drug therapy can help in reducing severity of disease and disability and lessen the social and economic burden posed on family and society. In communities where consanguineous marriages are traditional or encouraged, genetic counselling and education should be helpful in preventing or reducing such marriages.

Our experience shows that the range of tests provided was helpful in diagnosing a wide range of IMDs. Laboratory staff competent in performing the specialised tests and experienced in interpretation of test results have made valuable contributions to patient diagnosis. Experienced paediatricians and neonatologists can help in reducing wasteful and non-productive laboratory investigation by their expertise in patient selection. The algorithm in Figure 1 is suggested for patient investigations.

Proper interpretation of laboratory tests result and

successful diagnosis of an IMD often requires clinical information (i.e., detailed record of patient's history, presentation and clinical examination findings), results of relevant clinical/radiological diagnostic tests, and results of routine laboratory tests, in addition to those specific for IMDs. Therefore, good communication between clinical and laboratory staff is important in improving the chances of correct diagnosis. In the event of negative laboratory findings, it is important for clinical colleagues to realise that "normal test results" do not necessarily indicate the absence of an IMD as this may not be detectable by the investigations performed. For example, very long chain fatty acids, peroxisomal, mitochondrial and respiratory chain disorders cannot be diagnosed by the methods currently used in our laboratory. If clinical manifestation strongly indicates the presence of an IMD, it would be advisable to seek assistance of well-established centres capable of providing appropriate diagnostic tests.

The treatment and prevention of IMDs could be improved by setting up a national register of families at risk. Such a register is a powerful tool for national surveillance of inherited disorders. A well-kept register allows tracking of complex and changing distributions of IMDs within the population and can identify the services needed to keep up with these changes. It can also reveal disappointing results of treatment and underscore the need for new treatment approaches within specialist centres. It can pinpoint areas where screening and counselling facilities are inadequate.

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