Editorial

Neonatal Screening – A Global Perspective

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This supplement issue of the *Annals Academy of Medicine Singapore* represents a collection of nearly two-thirds of the lectures presented at the 6th Asia-Pacific Regional Meeting of the International Society for Neonatal Screening, held in Singapore from 29 August to 1 September 2007. The theme for the meeting was "Improving Child Health Through Universal Neonatal Screening".

In her keynote lecture Professor Bridget Wilcken noted the success of neonatal screening programmes over the past 40 years in reducing mental retardation and other paediatric health problems, and emphasised the importance of congenital hypothyroidism screening for any country that is contemplating starting screening programmes, to reduce the devastating burden of this readily treatable condition. It is heartening to know that healthcare authorities in Bangladesh, China and Pakistan (Hasan, Gu, Afroze) are doing precisely that.

Hearing impairment is the commonest congenital disorder that can be detected and treated early through universal newborn hearing screening, resulting not only in improved speech, but also cognitive development for the affected children. Similar to many other human health problems, a multidisciplinary approach to its management is necessary for the holistic care of the hearing impaired (Lim, Hayes, Nie, Lim and Daniel, Reyes).

Since the pioneering work in the 1990s by Millington and Chace[1,2] in developing tandem mass spectrometry (MS/MS), this technique has gained acceptance in many developed countries for detecting inborn errors of metabolism (IEMs), represented classically by phenylketonuria. A simple blood spot could potentially screen for more than 25 IEMs. However, debate still surrounds issues like what conditions to include in the screening panel and its cost-effectiveness (Therrell and Wilcken, Padilla and Lam). Most centres would screen for phenylketonuria and medium-chain acyl-coenzyme A dehydrogenase deficiency. Success of screening programmes to a great extent depends on getting the processes right, and one prerequisite would be quality assurance through regular audits (Fletcher, Hsiao). For many countries, epidemiological studies of IEMs are few. National centres should therefore be created to reap economies of scale in view of the relative rarity of IEMs (Yamaguchi, Gu, Thong).

Neonatal screening has come a long way since Robert Guthrie first started phenylketonuria screening using the bacterial inhibition assay in the 1960s.[3] Screening for congenital hypothyroidism and glucose-6-phosphate dehydrogenase (G6PD) deficiency is efficacious because these two conditions are relatively prevalent (congenital hypo-thyroidism universally, G6PD deficiency in East Asia), the tests cheap and treatment fairly straightforward with good outcomes.

In contrast even as a group the incidence of IEMs is quite uncommon (about 1:4,000 to 1:5,000 from a full panel of acylcarnitines and amino acids using MS/MS).[4] The screening technique is expensive and management of affected infants is much more involved for the physician and the family. Hence, for developing countries IEM screening will probably have to come after immunisation and infectious diseases in terms of national healthcare priorities. For existing screening programmes the challenge must be to continually ensure adequate specialist follow-up for patients with these individually rare, lifelong disorders.

The Asia-Pacific region has a long history in neonatal screening. In 1965 the late Emeritus Professor Wong Hock Boon initiated cord blood G6PD screening in Singapore, and that virtually eliminated kernicterus which was then the commonest cause of first week deaths in newborn babies.[5] Professor Naruse (see Oration) began neonatal screening

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in Tokyo in 1966. His foresight and subsequent efforts have enabled neonatal screening meetings in the Asia-Pacific region to continue to flourish, with the aim of fostering regional cooperation and assistance.

We hope the articles in this issue, we hope, will give readers a global perspective surrounding neonatal screening, and also reinforce the importance of secondary prevention (identifying a disease in its earliest stages before symptoms appear) in improving the health of children.

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Keynote Lecture

Improving Child Health – Newborn Screening for All?

Bridget Wilcken,[1] *AM, MB ChB, FRACP*

Abstract

Over the last 40 years newborn screening has been an undoubted success and many thousands of children have been saved from mental retardation and other problems because of early diagnosis of their disorders. Now many diseases can be diagnosed early by newborn screening and many more are on the horizon. It must be a long-term goal to extend newborn screening tests to all children but, in areas of the world where healthcare delivery is insufficient, solving other health problems has to take precedence over introducing newborn screening. If it is decided to introduce newborn screening in a region where currently there is none screening for congenital hypothyroidism alone should be started before anything else at all is attempted so that proper systems can be put in place. There is an exciting future for newborn screening ahead. If new programmes are approached with proper caution maximal benefit will be achieved from newborn screening, which is one of the few clearly effective preventive strategies in healthcare.

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Key words: Hypothyroidism, Opportunity costs, Tandem mass spectrometry

Congenital hypothyroidism occurs worldwide, and is relatively frequent, so it is no surprise that newborn screening for this disorder is the most widespread testing in use. "*No type of human transformation is more distressing to look at than an aggravated case of cretinism*". Thus wrote Sir William Osler in 1897.[1] It is interesting to note that the knowledge of goitre and its successful treatment by seaweed was known in China by 1600 BC.[2] In Europe, very much later, the Swiss Physician Paracelsus (1483-1541) related cretinism to endemic goitre.[2] The effective treatment of hypothyroidism was (re) discovered in 1891 by George Murray,[3] who used sheep thyroid extract – and patients were also given thyroid sandwiches to eat. And Sir William Osler was able to declare, "*That we can restore to life the hopeless victims of myxoedema is a triumph of experimental medicine... The results as a rule are most astounding, unparalleled by anything in the whole range of curative medicine*".[1] But treatment didn't completely address the problem of congenital hypothyroidism, as some intellectual deficit is permanent if treatment is not offered early. This of course is only one of the many disorders that need to be identified in the newborn period for treatment to be effective.

A Potted History of Newborn Screening

Dr Robert Guthrie's seminal paper on the feasibility of mass screening for phenylketonuria (PKU), using a bacterial inhibition assay and dried blood spot samples, was published in 1963[4] and this can reasonably be regarded as the birth of newborn screening. While some other bacterial inhibition assays were used for the diagnosis of other rarer disorders such as Maple Syrup Urine Disease and homocystinuria the next significant step forward was the description in 1973 and an assay for thyroxine, using dried blood spots.[5] This work on congenital hypothyroidism earned Dr Jean Dussault the Order of Canada in 1989. Other disorders which were able to be screened for in the late 1970s and 1980s included cystic fibrosis, congenital adrenal hyperplasia, biotinidase deficiency, and sickle cell disease. Not all of these were widely adopted. Many screening programmes were confined to screening for PKU and hypothyroidism.

Two advances then had a considerable impact. The use of dried blood spots in DNA analysis was first reported in 1987,[6] and initially mutation analysis was applied as a second tier test for haemoglobinopathies and later for cystic fibrosis screening. The full impact of DNA

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possibilities in newborn screening is yet to be felt. Unquestionably, the most significant advance has been the application of tandem mass spectrometry to newborn screening, with the ability to test for 30 or 40 disorders in a single test, using a single 3 mm blood spot.[7] This single advance has completely changed the face of newborn screening.

The Undoubted Successes of Newborn Screening

Because of PKU screening, thousands have been saved from severe mental retardation. Where screening is undertaken many doctors have never seen an untreated patient, and are shocked at their first encounter with a patient born before screening started – often mute, wheelchair bound, aggressive, and miserable. Now, children and young adults with PKU generally have normal development. Dietary treatments have improved, but are still somewhat onerous, and young women with PKU must adhere to diet very strictly before conception to avoid foetal damage, but there is no doubt about the overwhelming success of screening. Hypothyroidism screening too has been a clear success. Over the years, some 200 million newborns have been screened worldwide, almost all getting treatment by 2 to 3 weeks of age, and largely achieving normal growth and development. Early detection of sickle-cell anaemia has resulted in a saving of lives, as shown by a Cochrane review.[8] The rationale of screening for cystic fibrosis was under attack for some time. Two randomised controlled trials and many observational studies have now shown great benefits in nutrition and growth, reduced mortality, pulmonary benefits, and even a benefit in cognitive function.[9] The new expanded newborn screening by tandem mass spectrometry is also now being evaluated, with clear benefits so far demonstrated for only two or three conditions.[10-12] This may be largely due to the rarity of detectable disorders, and the consequent problem with showing benefit at an early stage. More outcome studies are certainly needed. But this type of screening for a large number of rare disorders has caught the imagination of the newborn screening community. Almost everyone seems keen to introduce the technology.

Should We Have Newborn Screening for All?

This conference has been running in the Asia-Pacific region for many years, which shows how the region, with over half the world's population, and half the world's births, values newborn screening. There is some form of newborn screening in 20 different Asian-Pacific countries: 13 offer some or complete screening for hypothyroidism, and at least 6 have screening programmes with full population coverage. In the recent review of screening in the area, only 4 countries appeared to have no newborn screening at all.[13] So it is reasonable to ask what should be aimed at for a region like the Asia-Pacific region? What for other parts of the world? Certainly it is important to aim for improved child health, but where does newborn screening fit into that overarching aim?

My hospital and University in association with the Hoc Mai Foundation have been collaborating with Dien Bien province to help with the development of healthcare, and my colleague, Professor Elizabeth Elliott, shared with me some of the experiences of the team. In this province of North-West Vietnam, as in many similar developing areas, there is a very high perinatal mortality rate, the main causes being prematurity, asphyxia, unidentified foetal death and birth defects. There is also a high maternal death rate, due mainly to haemorrhage, sepsis, and uterine rupture. In this region, the problems in health care delivery are overwhelming – lack of medications and equipment, infrastructure problems – water, sanitation, electricity - lack of training opportunities, language barriers, and huge problems of access. These are physical: poor transport infrastructure, and also cultural: women often being reluctant to consult male doctors. The common medical problems encountered are no surprise – infection, trauma, and malnutrition. But one of the commoner problems here is hypothyroidism. So is there a place in this very poor region for newborn screening? At present it seems as though other problems must be much more pressing.

I visited Northern Thailand in 1995 to look at their screening programmes. Here, the general health problems were far fewer. A pilot programme for PKU and hypothyroidism screening had been in place since 1992. There was village-based follow-up in this rural area (as in other similar areas) which worked very well, and iodination of salt was also done at a local level to combat iodine deficiency. Thailand now has a national programme with near-complete coverage. The many programmes in the region have recently been excellently reviewed.[13]

The Hard Questions

- Should there be newborn screening in every country now? Clearly there should be sometime in the future.
- How do we balance the opportunity costs? What other healthcare will suffer if money is spent on screening?
- Is it right to promote newborn screening in areas where healthcare is very poor?
- Can we ensure equal access? Or should screening be offered, as a start, to those who can pay?
- If newborn screening is undertaken, can follow-up be achieved?
- Can identified patients be treated? Are there a trained medical and auxiliary staff? Are the appropriate medications available and affordable?

If it is decided to introduce newborn screening in a region where currently there is none, it seems that a programme of screening for congenital hypothyroidism alone should be started before anything else at all is attempted. Certainly tandem mass spectrometry screening should not be adopted too early. Screening for hypothyroidism is known to be effective, and the condition is endemic throughout the world. Treatment is usually easily available and cheap, and is easy to administer. If screening for hypothyroidism is implemented first, systems can be developed for staff training, sample taking and dispatch, keeping records, follow-up of cases, and all the other aspects that make up a newborn screening programme. Proceeding slowly in this way enables the development of a firm basis for expansion later.

There is indeed an exciting future ahead. New treatments are already pointing to new possibilities for screening, some of which may be quite complex. So there is a clear need to get current endeavours right now. There needs to be great care taken with decisions on when to start a new programme, with the knowledge of what must be in place before a consideration is given to starting. Evaluation of benefits and drawbacks to individual programmes is extremely important, and going hand in hand with that is the need to have the courage to stop programmes that, after a good trial, are seen to be ineffective.

Screening can certainly improve child health – we have all seen that, and everyone deserves a good start in life. If new programmes are approached with proper caution maximal benefit will be achieved from newborn screening, which is one of the few clearly effective preventive strategies in healthcare.

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Towards Universal Newborn Screening in Developing Countries: Obstacles and the Way Forward

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Abstract

Newborn Screening is a well recognised public health programme aimed at the early identification of infants who are affected by certain genetic/metabolic/infectious conditions. Early identification of these conditions is particularly crucial, since timely intervention can lead to a significant reduced morbidity, mortality, and associated disabilities in affected infants. Establishing sustainable newborn screening programmes in developing countries poses major challenges as it competes with other health priorities – infectious disease control, immunisation, malnutrition, etc. Despite this, it is imperative that developing countries recognise the importance of newborn screening based on experiences on both developed and developing countries in saving thousands of babies from mental retardation, death and other complications. Some of the critical factors necessary for a successful national newborn screening programme are inclusion of newborn screening among government priorities, funding (including the possibility of newborn screening fees), public acceptance, health practitioners cooperation, and government participation in institutionalising the newborn screening system. This paper presents a historical review of 4 eras of newborn screening in the Asia Pacific, discusses enabling factors leading to successful newborn screening programme implementation, and identifies obstacles that threaten the programme implementation in developing countries.

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Key words: Asia Pacific, Newborn screening, Screening

Introduction

In most economically developed countries, newborn bloodspot screening (NBS) using biochemical markers to detect certain congenital conditions is a public health measure aimed at the early identification and management of affected newborns.[1] Since the Asia Pacific region is vast and diverse, the development of newborn screening in the region has been likewise varied. The Asia Pacific Region includes countries varying widely in size from very small countries (Singapore, New Zealand) to extremely large countries (China, Mongolia). It includes both economically developed (Japan, Taiwan, Korea, Singapore, Australia, New Zealand) and economically developing countries (the rest of the Asia Pacific Region). Asia Pacific countries have faced and continue to face many challenges in implementing newborn screening including differences in language and culture, extremes in geography (large numbers of islands and many mountainous regions), poor economies, and unstable governments. In countries with depressed or developing economies, particularly in Asia, newborn screening and other forms of infant screening compete with other health priorities, i.e. control of infectious diseases, immunisation and malnutrition, etc. In some of the more progressive developing countries, NBS is now emerging as a priority.

Eras of Newborn Screening

Pre Guthrie Era. Prior to bloodspot newborn screening, newborn screening was performed using urine testing in New Zealand, Australia and Japan.[2-4] In 1964, urine testing for PKU attained 80% coverage in New South Wales, Australia.[3] However, due to the reported increase in false-negative results and the difficulties in collecting a satisfactory specimen, urine screening was subsequently replaced with dried bloodspot screening method introduced by Dr Robert Guthrie in the 1960s.[5]

First Asia Pacific Screening Era. One of the first national NBS programmes in the world was developed in New

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Zealand. It began as a pilot study for phenylketonuria (PKU) in 1966 and became a national screening programme in 1970. Nearby country, Australia started its pilot study of PKU screening in 1967 and it also progressed to national in coverage in 1970. Japan also began a pilot study in 1967, but it did not develop into a national programme until 1977. A pilot screening programme for glucose-6-phosphate dehydrogenase (G6PD) deficiency using cord blood began in Singapore in 1965 and developed into a national programme in 1970. Subsequently, this cord blood screening programme expanded to include screening for congenital hypothyroidism (CH).[1] It has remained cord blood based until recent expansions to metabolic conditions required a transition to dried blood on filter paper.

Second Asia Pacific Screening Era. Twenty years later after the development of the Guthrie process, newborn screening began in Malaysia in 1980, with cord blood screening for G6PD deficiency. In India, a pilot study occurred in Bangalore, screening for various amino-acidopathies, homocystinuria (HCY), hyperglycinemia, maple syrup urine disease (MSUD), PKU. Taiwan began limited screening for CH, PKU, and G6PD deficiency in 1981, which became a national programme in 1985. China also began screening for CH, PKU, and galactosemia (GAL) in 1981, but legislation of national expansion did not exist until 1995. Hong Kong started a national programme of cord blood screening for G6PD deficiency and CH in 1984.[1]

Third Asia Pacific Screening Era. A third era of NBS in the Asia Pacific appears to have occurred during the 1990s following the initiation of CH screening in much of the economically developed world. Screening for CH began in South Korea and the iodine-deficient areas of Thailand as pilot studies were carried out in 1991 and 1992 respectively. The Thailand pilot project became a national screening programme in 1996. In South Korea, nationalisation of screening occurred in 1997. NBS began in the Philippines as a pilot project for 5 conditions [PKU, CH, congenital adrenal hyperplasia (CAH), galactosemia (GAL), and homocystinuria] in 1996 and became a national programme when a screening law was passed in 2004.[1]

Fourth Asia Pacific Screening Era. The fourth NBS era began in the late 1990s with pilot NBS programmes for CH in Mongolia, Vietnam, Bangladesh and Indonesia in 1999, Myanmar in 2000, Sri Lanka in 2005, Pakistan in 2006 and Palau in 2008.[7] Laos has expressed interest on a pilot CH screening project during a recent workshop entitled 'Consolidating Newborn Screening Efforts in the Asia Pacific' held in the Philippines[6] There is still no screening programmes in Nepal and Cambodia. Aside from Guam, Saipan (part of the Federated States of Micronesia) and Palau (personal communication–Ms Berry Watson), there is little newborn screening activity in the rest of the Islands in the Pacific Basin.[6]

Burden of CH in Asia

NBS for CH began in developed programmes in the 1970s. As programmes began to develop in Asia during the 1990s, screening for CH became an important consideration, particularly since many areas in the developing world are iodine deficient. Globally, the incidence of CH approaches 1:3000, with substantially higher prevalence in iodine deficient areas, sometimes in excess of 1:900.[7] With annual births of 66.9 million of babies in Asia, there are at least 22,200 potential new cases of CH every year and probably even more, given the limited documented areas of iodine deficiency in Asia. Of the 66.9 million annual births in Asia, only 10% are screened for CH; thus, only a small proportion of new CH cases even have the chances for identification.

Enabling Factors and Obstacles

In a survey conducted among newborn screening managers in the Asian countries, the following factors were identified as integral to the success of full population newborn screening: (i) government prioritisation; (ii) full or partial government financing; (iii) public education and acceptance; (iv) health practitioner cooperation/ involvement; and (v) government participation in institutionalising a newborn screening system. Among the countries that lack total coverage, the following obstacles are most often cited: (i) poor economies; (ii) insufficient health education; (iii) lack of government support; (iv) early hospital discharge; and (v) a large numbers of out-of-hospital births.[1]

Geography. Geographical location is a hindrance for some countries, i.e. the Philippines (7107 islands) and Indonesia (13,667 islands) are huge archipelagoes, and Mongolia has 90% of its land as either pasture or desert.

Population and number of births. Of the 133 million births worldwide, 66.9 million occur in Asia. Eighty percent (80%) of these are born in 5 countries: India (25 million), China (17 million), Pakistan (4.7million), Indonesia (4.5 million), and Bangladesh (3.7 million).[6] These 5 Asian birthing giants account for over two-thirds of all people living in rural areas without access to proper sanitation, children who suffered from malnutrition, people living on less than US$1 a day and rampant tuberculosis cases.[8] These 5 giants collectively have only reached <1% of screening coverage. Table 1 shows that the countries with lower annual births yet higher coverage for NBS. Singapore, Hong Kong, Taiwan, Australia, New Zealand have almost 100% coverage. Mongolia is the only country with low NBS coverage and with a low birth rate. This shows that

other than population and birth rates, there are other factors that contribute to a successful NBS programme.

General health priorities. All countries with an Infant Mortality Rate (IMR) of less than 10 per 1000 live births have achieved better than 90% screening coverage of their newborn population. Of the remaining countries with higher IMRs, Thailand (IMR 13 per 1000) is the only one that has achieved a high rate of newborn coverage (97%). There are other factors responsible for this success and most notably is the high level of government financial support.

Government support and integration in the health delivery system. Integration into the national health delivery system was cited as the single most critical element among survey responders. All countries with coverage of at least 90% have fully integrated NBS into the health delivery system, including the payment scheme for a NBS fee. Payment is either covered by government, insurance or out-of-pocket expense of the family (Table 1).

Legislation. While most of the developed programmes in the region have successfully accomplished health care integration without requiring legislation, at least 2 of the developing programmes (China and the Philippines) have found national legislation to be necessary. In China,

Table 1. Programme Demographics in Asia Pacific

Country	Thousand births[a]	Infant mortality rate (under 1)[a]	Reported programme coverage in 2006	Source of payment for newborn screening fee[1]
Australia	250	5	100%	Government
Bangladesh	3,747	54	<1%	Government
Cambodia	429	98	0	?
China	17,310	21	25%	Family
Hong Kong (China)[b]	65[b]	1.8[b]	99%	Government
India	25,926	43	<1%	Family
Indonesia	4,495	18	<1%	Family
Japan	1,162	2	>99%	Government
Korea (South)	457	3	94%	Government
Korea (North)	342	22	?	?
Laos	205	35	0	?
Malaysia	547	5	95%	Government/Private
Mongolia	58	26	<1%	Grant
Myanmar	976	40	<1%	Government
Nepal	787	40	?	?
New Zealand	54	4	100%	Government
Palau[c]	0.385[c]	13.7[c]	0	Government
Pakistan	4773	57	<1%	?
Philippines	2018	15	10%	Family /Ins
Singapore	39	1	>99%	Family 40%
Sri Lanka	329	11	<1%	Government
Taiwan[d]	287[d]	6.3[d]	>99%	Family
Thailand	1009	13	97%	Government
Vietnam	1648	15	<1%	Government
Totals	66,913			
World Statistics	133,801			

[a] Source: UNICEF 2006 The State of the World's Children 2007. New York: UNICEF, 102-105. (Available at: http://www.unicef.org/sowc/archive/ENGLISH/The%20State%20of%20the%20World%27s%20Children%202007.pdf)

[b] Source: Hongkong Statistics. http://www.censtatd.gov.hk/FileManager/EN/Content_811/health.pdf(2006)

[c] Source: Palau Statistics. https://www.cia.gov/library/publications/the-world-factbook/print/ps.html

[d] Source: Taiwan Statistics. http://indexmundi.com/taiwan/birth_rate; http://indexmundi.com/taiwan/population.html (2006)

Presidential Order No 33 (1994) Article 24 states that "medical and health institutions shall gradually develop medical and health care services such as the screening of newborn babies".[9] In the Philippines, Republic Act 9288 or the Newborn Screening Act of 2004 Article 1 Section 3 states that "every newborn must be given access to newborn screening" and article 3 states that "any health practitioner who delivers, or assists in the delivery, of a newborn in the Philippines shall, prior to delivery, must inform the parents or legal guardian of the newborn of the availability, nature, and benefits of newborn screening".[10]

Health providers. Some healthcare providers have insufficient knowledge, interest and commitment to newborn screening. In most developing programmes, there are very few specialists to whom referrals can be made once newborns have been confirmed to be positive for a particular disease.

Parents and family. In beginning programmes, parents and family members remain unaware of the benefits of NBS as well as consequences of late diagnosis for the disorders in the panel. Hence, they remain uncooperative to the screening process. The early discharge policy in the developing countries, contribute to missed opportunities for the newborns as parents decide not to screen their babies for any disorders after being discharged.

Partnership. All sectors must be empowered to participate in the implementation of the NBS programme, i.e. paediatricians, obstetricians, midwives, neonatologists, geneticists, endocrinologists, nurses, community health workers, hospital administrators and policy makers.

Operations and infrastructure. For countries with beginning pilot projects, operations at either hospital or community setting, may need guidance from neighbouring countries with more developed programmes. Off-site newborn screening laboratories may be a consideration for small populations and countries without ready infrastructure. A guidance book entitled *Screening of Newborns for Congenital Hypothyroidism Guidance for Developing Programmes*[7] provides step-by-step instructions for the organisation of a screening programme.

The Way Forward

Experience from successful programmes showed that integration into the national health delivery system is the single most critical element. All beginning programmes must work towards this end. Integration must include a payment scheme to ensure ready acceptance by the families. The pilot projects must work within the framework of a national programme with a national committee taking charge of the different phases of implementation. As of 2007, only 10% of the newborn population in the Asia Pacific Region is being screened. As newborn screening competes with other priorities of the developing countries, there is a need for an international body to convince national governments that NBS is important for their population. Though small in numbers, the countries in the Pacific Basin must give sufficient attention to NBS as their problems are similar to all of the developing programmes.

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Improved Health and Development of Children who are Deaf and Hard of Hearing Following Early Intervention

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Abstract

Infants begin to learn language in the earliest months of life. In the absence of early identification and intervention, infants who are deaf or hard-of-hearing experience significant and lasting deficits in language learning, academic achievement, social-emotional development, and quality of life. Evidence is mounting that early identification of infants who are deaf or hard of hearing through newborn hearing screening and intervention by the age of 6 months improves developmental outcomes for these children, especially in the area of language proficiency. Newborn screening programmes, including newborn hearing screening, are typically public health activities aimed at the early identification of infants who are affected by certain congenital disorders: including genetic, metabolic, haematologic, and infectious diseases. Early identification of these conditions is critical, as timely intervention can lead to a significant reduction of morbidity, mortality and associated disabilities in affected infants. For infants with hearing loss, the goal of early identification is to provide early intervention leading to language development and academic achievement commensurate with cognitive ability, and ultimately an improved quality of life. For newborn hearing screening, the definition of early intervention is initiation of intervention by the age of 6 months. Initiatives for newborn hearing screening have spread to every continent and many countries now have well-developed, comprehensive programmes of screening, diagnosis, and early intervention for childhood hearing loss. Although no exact statistics currently exist, tens of millions of infants probably receive newborn hearing screening each year. Because the developmental effects of early intervention for hearing loss are improved and sustained language development, large-scale studies documenting the language outcomes in early-identified children take decades to collect. Furthermore, because full-scale implementation of universal newborn hearing screening has occurred only within the last 5 years in most countries, many early-identified children are still at preschool-age. Thus, documenting these youngsters' language development is a work in progress. Despite these limitations, evidence is mounting that early intervention for childhood hearing loss improves the developmental outcome of these children.

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Key words: Hearing loss, Infant, Language, Newborn hearing screening

Language Development in the Earliest Months of Life

Although normally-developing infants appear to learn their mother tongue effortlessly, they are actively engaged in language-learning from the birth through early childhood. Linguists, social scientists, and developmental psychologists have extensively studied language development in 2 domains: the infant's perception of the acoustic stream of speech which comprises language, and the infant's production of non-speech and speech-like sounds and words. During the first year of life, rapid changes in both perception and production are observed.

For perception, research has shown that, at birth, babies can distinguish virtually all the phonetic units used in languages; they are language universalists. Of the 600 consonants and 200 vowel sounds in the world's languages, babies can appreciate all these sounds. However, because each language uses a unique set of only about 40 specific language units, or phonemes, by 6 months of age, the infant begins to develop phonetic perception specific to his or her mother tongue.[1] By 8 months of age, babies demonstrate knowledge of the supersegmental features of speech, such as stress patterns, and by 9 months of age, normally-hearing infants recognise language-specific sound combinations.

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By 1 year of age, perception of acoustic distinctions of non-native language consonants declines, and babies have become language specialists.

Production of language also progresses rapidly during the first year of life. Up until about 3 months of age, babies produce only non-speech sounds; cries and grunts. At 3 months of age, vowel-like sounds are produced which rapidly evolve into consonant-vowel repetitive babbling with syllabic timing patterns, termed canonical babbling. This represents an important milestone linking perception and production. By the age of 10 months, infants combine sounds into mother-tongue specific speech sounds, usually resulting in the infant's first native language word at the age of 12 months.

Recent neuropsychological and brain imaging research has shown that language acquisition involves *neural commitment*. Exposure to language produces dedicated neural networks that code speech patterns: the "native language neural commitment" theory of language development. As the distribution of speech patterns that a baby hears stabilises in neural representation, the period of learning novel speech patterns begins to close, resulting in a sensitive language-learning period. As summarised by Kuhl,[2] "...an absence of early exposure to patterns that are inherent in natural language ~ whether spoken or signed ~ produces life-long changes in the ability to learn language..."

Effect of Congenital Hearing Loss on Language Development

Congenital hearing loss has a devastating effect on this normal process of speech perception and speech production. Children who do not hear from birth do not experience the normal stimulation needed to develop neural networks specific to language learning. Research has shown that deaf infants are significantly delayed in onset canonical babbling.[3] Furthermore, there is a clear correlation between age of onset of canonical babbling and age at which auditory amplification is provided to hearing-impaired infants[4] proving the importance of hearing to speech development.

If mastery of the rules of language does not occur within the "sensitive period," the probability that the child can adequately develop this skill is significantly diminished. Research from national surveys of deaf children in the United States has shown that, on the average, children with hearing loss have a 20 to 40 point discrepancy between verbal and performance intelligence scores. In addition, only 40% of deaf children at high school graduation demonstrate reading levels at the fourth grade equivalent or above.[5] Thus, the lifetime effects of congenital hearing loss cascade from delayed language development into (i) poor literacy skills, (ii) decreased academic success, (iii) limited job opportunities, especially in a world economy highly dependent on oral and written communication,(iv) social-emotional problems,(v) lowered lifetime economic success, and ultimately, and (vi) reduced quality of life.

Interventions for Congenital Hearing Loss

There are multiple effective interventions for congenital hearing loss. The 3 principle interventions are (i) parent education and support, (ii) amplification and cochlear implants, and (iii) early intervention.

Parent education and support includes training the family on the effects of congenital hearing loss, facilitating development of a language-rich environment in the home setting, helping families advocate for and obtain the resources needed for their child's optimum development, and training parents to recognise developmental milestones. Research has shown that high levels of family involvement in children's intervention programmes correlate with positive outcomes for these children.[6] In addition, Calderon[7] documented that maternal communication skills are predictive of language development and literacy competency for children who are deaf.

Conventional hearing aids provide infants who are hard of hearing, that is, infants with residual hearing, access to sound. Contemporary hearing aids are digitally-programmable, flexible, electroacoustic devices with adaptive listening environment features such signal-to-noise reduction capabilities. Hearing aids may be fitted to infants as young as 2 to 3 months of age.

For infants with minimal residual hearing, cochlear implants provide important, albeit limited, access to sound. A cochlear implant consists of a stimulating electrode array that is surgically implanted into the cochlea to provide direct electrical stimulation of the auditory (eighth cranial) nerve, and an external receiver/transmitting device that extracts critical speech elements from the spoken word and converts this acoustic stream into an electrical stream for activating the stimulating electrode array. For infants and young children, cochlear implants are typically considered after a trial with conventional hearing aids has demonstrated very limited benefit, and usually after about 1 year of age. Multiple studies have shown that children who receive early implantation obtain better language-learning results than children who are implanted at older ages.[8,9]

Early intervention for childhood hearing loss is customised to the family's desired language mode, whether native sign language or auditory-oral, spoken language. It is typically initiated as soon as the infant is confirmed with permanent hearing loss, and provided in the home-setting with guidance for developing a language rich environment. Families of children who are deaf and hard of hearing must commit to intervention throughout the child's toddler and preschool

years. Research demonstrates that early identification and early intervention improve language outcomes for children who are deaf or hard-of-hearing.[6,10-12] For example, Colin Kennedy and his colleagues in the U.K. studied language and speech outcomes in one hundred and twenty 8years-old children with bilateral permanent hearing impairment identified from a large birth cohort in southern England. Of these 120 children, about half of them were born during periods of universal newborn hearing screening and the other half were born during periods without newborn hearing screening. Using standardised assessment tools to measure language competency, the authors found that children who were exposed to universal newborn hearing screening or who had confirmation of hearing loss by 9 months of age had significantly higher scores for language, but not for speech, in mid-childhood than those who were not exposed to newborn hearing screening or whose impairment was confirmed after 9 months of age. For the early identified group, the difference in language was equivalent to 10 to 12 points in the verbal as compared to the nonverbal intelligence quotient.

How Early is "Early Enough?"

"Early intervention" for childhood hearing loss is considered intervention by the age of 6 months. Assuming high-quality intervention and optimum family commitment, is intervention by age 6 months early enough to ensure language development commensurate with cognitive ability? The medical geneticists, Morton and Nance[13] argue that newborns who fail initial screening should move immediately into audiological confirmation of hearing loss before discharge from the birth admission. By their proposal, infants who fail newborn hearing screening receive confirmatory diagnosis before age 1 month and are enrolled in early intervention by the age of 2 months. If successful, this acceleration of the identification and intervention process might:(i) reduce loss-to-follow up,(ii) improve specification of etiology of hearing loss, especially for babies with congenital cytomegalovirus, and(iii) normalise access to sound for hard of hearing infants through prompt hearing aid fitting.

For babies for whom otological and audiological diagnosis of hearing impairment is possible immediately after birth, intervention by 2 months of age should be possible. Whether families are comfortable with an accelerated diagnostic process is unknown; however, reducing that period of uncertainty that worries families today would seem beneficial.

Summary

Children who are deaf or hard-of-hearing will enjoy improved health and developmental outcomes only if early identification and intervention are effective. Evidence is emerging from well-developed and implemented programmes of universal newborn hearing screening that early identification and intervention improve language development in these youngsters, at least through mid-childhood. Through universal newborn hearing screening, the goal of language competence should be within reach for all deaf and hard-of-hearing children.

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Newborn Screening in Japan: Restructuring for the New Era

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Abstract

Nationwide neonatal mass screening for inherited metabolic diseases has started in Japan since 1977. At least 8000 children have probably been spared from handicaps resulting from such diseases over the past 30 years. Recently remarkable changes have been made to the evolving neonatal screening system. Declining birth rate and economic problems in Japan have demanded a more effective neonatal screening system. Development of new innovative screening methods and treatment tools, e.g. tandem mass spectrometry (MS/MS) technology and enzyme replacement therapy for mucopolysaccharidosis (MPS), have facilitated expansion of target diseases in neonatal screening. We have carried out pilot screening using MS/MS in 6 laboratories in Japan. The incidence of inherited metabolic diseases was found to be 1 in 9330 (65 cases out of 606,380 babies screened) during the period between 1997 and 2007. The incidence was lower than those of Europe or USA (about 1 in 4000 to 5000). The disease frequency between unscreened symptomatic cases and asymptomatic cases detected through MS/MS screening were also found to be different. In MS/MS screening, the most common organic acidemia was propionic acidemia, whereas in symptomatic cases, methylmalonic acidemia was the most common. Further study of ethnic diversity in severity of propionic academia is required. The outcomes of patients detected in the MS/MS screening were significantly favourable. The results showed the benefits of MS/MS screening. The diagnostic support network for gas chromatography-mass spectrometry (GC/MS) analysis and enzyme determination has also been developed. We have developed an automated system of GC/MS data processing and auto-diagnosis which allowed the GC/MS data processing to be extremely fast and simple. Enzyme evaluation for diagnostic support for screening, including a method using peripheral blood and high performance liquid chromatography (HPLC), and another method of in-vitro probe assay using cultured cells and MS/MS. Furthermore, re-location of screening laboratories for a more efficient screening network will be required such that at least 30,000 samples can be analysed in each laboratory.

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Key words: Neonatal mass screening, Organic and fatty acid disorder, Reconstruction of the system, Tandem mass spectrometry (MS/MS)

Introduction

The nationwide neonatal mass screening for inherited metabolic diseases started 30 years ago in Japan, and now 6 different kinds of diseases are being screened. It is estimated that at least 8000 children could have been spared from being handicapped as a result of these diseases. For the last 30 years, however, the circumstances surrounding neonatal screening have remarkably changed. Our challenges to reform neonatal screening in Japan for the new era will be discussed in this paper.

History of Neonatal Screening in Japan

In the early 1960s, the Guthrie method was developed, and inherited metabolic diseases were given much attention. In 1964, the Japanese Society of Inborn Metabolic Disease was formed, and in 1973, the Japanese Society of Mass Screening was formed. In 1977, a nationwide neonatal mass screening project which included 5 targeted diseases, was started by the Japanese government. In 1979, congenital hypothyroidism was included in the screening process, and in 1989, congenital adrenal hyperplasia was then added. On the other hand, histidinemia was eliminated from the list of targeted diseases since no clinically significant handicaps were observed. Hence, the current neonatal screening includes 6 targeted diseases.

Although mass screening for neuroblastoma using urine collected from 6-month-old children was carried out between 1984 and 2002, it is now no longer carried out

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except for a few areas in Japan. Recently, studies to expand the targeted diseases, such as organic acidemias, fatty acid disorders, Wilson disease, and so on, have been carried out.

Since the 1990s, a new innovative screening method, tandem mass spectrometry (MS/MS) was developed.[1,2] In 1997, pilot neonatal screening using MS/MS was initiated by Professor Shigematsu from the Fukui University in Japan.[3] Since 2004, an expanded pilot screening using MS/MS has gained support by receiving funds from the Ministry of Health, Labor and Welfare of Japan.

Population Dynamics and Present Situation of Neonatal Screening in Japan

The number of births in Japan for the past 50 years has declined tremendously from 2.33 million in 1950 to 1.76 million in 1977, and to 1.08 million in 2005. The infant mortality rate in 2005 was only 2.8 per 1000 births, compared to 60.1 in 1951 and 8.9 in 1977.

Table 1 shows the results of neonatal screening in Japan for the past 30 years. It shows that (i) congenital hypothyroidism was the most common disease and the most cost-effective to screen for, (ii) the incidence of phenylketonuria (PKU) was unexpectedly low (1 in 80,000) compared to western Europe (1 in 10,000), (iii) the frequency of maple syrup urine disease (MSUD) and homocystinuria was extremely low.

Table 1. Results of Neonatal Screening in Japan (as of 2007)

Disease	Incidence
1. Phenylketonuria (Biopterin disorder = 1:1,580,00)	1:80,000
2. Maple syrup urine disease	1:500,000
3. Homocystinuria	1:800,000
4. Galactosemia* (type 1 = 1: 800,000) (type 2 = 1: 600,000)	1:30,000
5. Congenital hypothyroidism	1:2200
6. Congenital adrenal hyperplasia	1:20,000

* The incidence includes transient galactosemias

Changes in Neonatal Screening during Past 30 years in Japan

During the last 30 years, remarkable changes in socio-economics affected neonatal screening in Japan (Table 2). (i) The birth rate has rapidly declined from 1.8 million in 1977 to 1.1 million in 2005. (ii) Economic problems have surfaced. These problems demanded efforts for a more effective neonatal screening programme. (iii) Patients or families' awareness of screening and treatment options are also changing. For example, emphasis on better quality of life (QOL). (iv) New innovative screening methods, such as MS/MS or hearing screening, were developed recently. (v) New methods of treatment for inherited metabolic diseases such as enzyme replacement therapy for mucopolysaccharidosis (MPS) were also established. These technological innovations have facilitated expansion of targeted diseases in neonatal screening. (vi) To respect the privacy of patients, there may be problems in the tracking of patients detected in neonatal screening. New information on treatments should be provided to patients as early as possible through continuous exchange of information between government officers, doctors, researchers, technicians, and patients' families.

Reform Project of the Neonatal Screening in Japan

We are now embarking on projects supported by funds from the Japanese government to reform the current neonatal screening system in Japan. These are as follows, (i) Investigation of the natural history of possible new targeted diseases, which may be essential for determining the benefits of neonatal screening. (ii) Development of new screening methods. (iii) Development of diagnostic support system

Table 2. Changes in Neonatal Screening in Japan during the Past 30 years

Changes during the past 30 years	Future actions
1. Declining birth rate 1.8 million (1977)→1.1 million (2005)	Improve cost-effectiveness of the screening programme
2. Economic problems	
3. Social stigma for patients and family	Improving quality of life, ethical issues
4. Newer methods of screening – MS/MS, hearing screening	Expansion of targeted diseases? Determination of cost-benefit
5. Newly developed treatment – Enzyme replacement for MPS etc.	
6. Others – Problems in tracking of patients, screening quality	Providing new information Assurance of screening quality

Table 3. Results of Screening in the Pre-symptomatic and Symptomatic stages

Diseases	Japan		Asia
	Screening* (Fukui + pilot)	Symptomatic** (Shimane)	
Organic acidemias			
1. MMA	9	40	142
2. PPA	16	12	22
3. MCD	4	15	26
4. GA1	3-	6	13
5. HMG	3	5	3
6. MCC def	-	4	4
7. 3KT def	1	1	14
8. IVA	-	1	2
9. Other OA		12	16
Fatty acid disorders			
1. VLCAD def	4	12	3
2. GA2 (MAD)	3	9	2
3. MCAD def	3	5	3
4. CPT2 def	2	3	2
5. SC def	2	5	1
6. MTP def	-	3	-
7. SCAD def	1	2	1
8. CPT1 def	3	2	2
9. SCHAD def	1	-	-
10. Other FAOD	-	-	3
Amino acidemias			
1. MSUD	-	(3)	17
2. PKU	8	(1)	10
3. ASA	1	-	-
4. Citrin def	6	-	-
Incidence Ratio	1: 9300		

* TMS screening from 1997 to 2007

** metabolic screening on symptomatic patients from 2001 to 2007 at Shimane University

3KT def: 3-ketothiolase deficiency; ASA: argininosuccinic acidemia;

Citrin def: citrin deficiency (including citrulinemia type 2)

CPT1 def and CPT2 def: carnitine palmitoyltransferase-1 and 2 deficiencies, respectively; GA1: glutaric acidemia type 1; GA2: glutaric acidemia type 2;

HMG: 3-OH-3-methylglutaric acidemia; IVA: isovaleric acidemia;

MCC def: methylcrotonyl-CoA carboxylase deficiency;

MCD: multiple carboxylase deficiency;

MMA: methylmalonic academia;

MSUD: maple syrup urine disease;

MTP def: mitochondrial trifunctional protein deficiency;

PKU: phenylketonuria; PPA: propionic acidemia;

SC def: systemic carnitine deficiency;

VLCAD def: MCAD def, and SCAD def, very-long-, medium-, and short-chain acyl-CoA dehydrogenases, respectively;

SCHAD def: short-chain 3-OH-acyl-CoA dehydrogenase deficiency;

Other OA means other organic acidemias including 5-oxoprolinemia, alkaptonuria, or glycerolemia.

for screening of new targeted disorders. (iv) Medical support for patients in treatment and availability of new information. (v) Restructuring of the screening system, including regionalisation of screening laboratory.

Results of Pilot Screening using MS/MS

We have carried out pilot screening using MS/MS in 6 laboratories, which are located in Sapporo, Tokyo, Fukui, Osaka, Shimane, and Kumamoto. A total number of 606,380 babies between 1997 and 2007 were screened, and 65 cases were detected: 33 cases of organic acidemias, 18 fatty acid oxidation disorders, and 14 amino acidemias (Table 3). The overall incidence in Japan was thus calculated to be 1 in 9330 which is lower than that of Europe or USA (about 1 in 4000 to 5000).

Comparison of Disease Proportions between Symptomatic and Asymptomatic Cases in Japan and other Asian Countries

We have had collaborative studies with some hospitals in other Asian countries. We are working closely with Dr Yang YL from Peking University and Dr Gu XF from Shanghai Xiao Tong University in China, Dr Nguyen Thu Nhan from Hanoi Medical University in Vietnam, Dr Wasant P from Mahidol University in Thailand, and Dr Verma IC from Sur Ganguaram Hospital in India. We analysed cases of organic acidemias, acylcarnitine deficiencies or amino acidemias who became "symptomatic" using GC/MS or MS/MS. The results are listed in Table 3. In "symptomatic" cases detected in the laboratory at Shimane University, some differences in the disease incidence were observed between Japan and other Asian countries. 3-Ketothiolase deficiency, 5-oxoprolinemia (data not shown), PKU, or MSUD were more commonly detected in other Asian countries than in Japan. On the other hand, urea cycle defects and multiple carboxylase deficiency were more likely to be detected in Japan.

It was also found that the disease incidence between symptomatic and asymptomatic cases in MS/MS screening were different. In MS/MS screening, the most common organic acidemia was propionic acidemia, followed by methylmalonic acidemia, whereas in symptomatic cases, methylmalonic acidemia is the most common, followed by multiple carboxylase deficiency and propionic acidemia. Using MS/MS screening, it was found that the incidence of Japanese patients with propionic acidemia was at least 10 times higher than previously reported, and it was likely that many Japanese children with propionic acidemia detected in MS/MS screening had no or mild symptoms (the latter needing some intervention at least in childhood).[4] Further study of ethnic diversity in the severity of propionic academia should be carried out.

Table 4. Effectiveness of MS/MS Screening

Disease	Pre-symptomatic (Pilot screening)	Symptomatic cases (Shimane)
Number of cases	54	153
Organic acidemia	39	120
Normal	31 (79%)	3832%
Handicapped	6 (15%)	5445%
Death	2 (5%)	2823%
Fatty acid disorder	15	33
Normal	15 (100%)	1752%
Handicapped	-	618%
Death	-	1030%

Beneficial Effect of MS/MS Screening

The outcomes of Japanese patients detected in the symptomatic and pre-symptomatic (MS/MS screening) stages were compared. As shown in Table 4, in the case of organic acidemias, favourable outcomes were achieved in 83% of the symptomatic and 33% of the MS/MS screening groups respectively. In the case of fatty acid disorders, all 15 cases detected in MS/MS screening had no impairment, whereas in the "symptomatic" group, only 52% of cases had normal intellectual developments. It is encouraging that the outcomes of children with certain disorders detected through newborn screening may be significantly more favourable. On the other hand, we should note that the MS/MS screening detects not only typical cases but also milder cases. Hence, the clinical benefits of screening should be further determined carefully, with more patients studied.

Diagnostic Supports for MS/MS Screening

Basically, analysis of acylcarnitines and amino acids in blood filter paper is just "screening". There may be a considerable number of false positive or false negative cases. This implies a reliable diagnostic support system is needed. Therefore, we are developing a support network of GC/MS analysis and enzyme evaluation.

For example, an elevation of C3 (propionylcarnitine) suggests not only propionic acidemia but also methylmalonic acidemia. Urinary organic acid analysis is indispensable for differential diagnosis. In Shimane University, an original system of automated GC/MS data processing and diagnosis was established in collaboration with Shimadzu Cooperation, Kyoto, Japan.[5] In this system, metabolic profiles from the GC/MS data, and "suspected" or "suspicious" disease names were listed automatically in a minute. GC/MS data processing became extremely fast and simple with this system.

Enzyme evaluation for diagnostic support for screening has also been developed. The group in Hiroshima University developed a simple enzyme assay sytem, using peripheral lymphocytes and high performance liquid chromatography (HPLC) to determine enzyme activity.[6] Sample to be collected requires only 5 ml heparinised blood. For example, in very-long-chain- acyl-CoA dehydrogenase (VLCAD) deficiency, after palmitate (C16) is used for sample incubation as substrate, its product, C16:1, is measured by HPLC. The amount of C16:1 produced represents the activity of VLCAD.

We are also introducing another method of enzyme evaluation for beta-oxidation, named "in vitro probe assay".[7-9] In this method, palmitate (C16) or octanoate (C8) is added to the culture medium as substrate, and the cells are incubated for 72 or 96 hours. After that, acylcarnitines in the medium are analysed using MS/MS. If long-chain fatty acid oxidation is blocked, C16 acylcarnitine is increased after palmitate loading as shown in Figure 1. If medium-chain fatty acid oxidation is blocked, C8- and longer chain acylcarnitines are increased after loading with both palmitate and octanoate. Hence, fatty acid oxidation defects are clearly detected with cultured cells.

Relocation of Screening Laboratories in Japan

Currently, the population of Japan is 127 million, and the number of births per year is about 1.1 million as mentioned above. There are 47 provinces, and screening laboratories are now located in each province. There are over 50

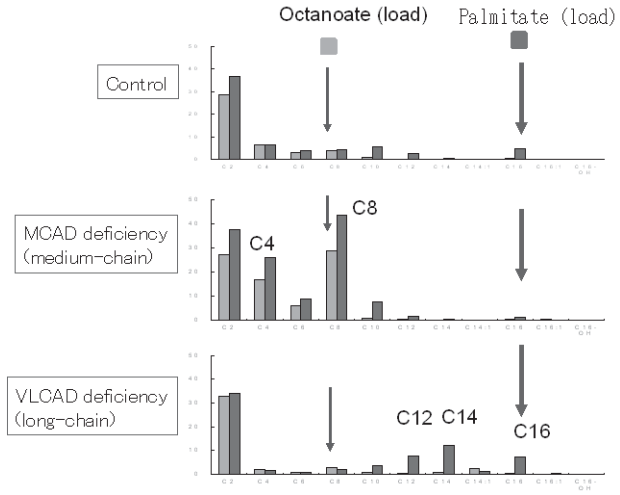

Fig. 1. Results of vitro probe assay for evaluation of fatty acid oxidation. A: acylcarnitine profile of normal control. B: medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. C: very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency. In MCAD deficiency, C8, C6 and C4 acylcarnitines are increased with both palmitate and octanoate loading. In VLCAD deficiency, C12, C14, and C16-acylcarnitines are increased with palmitate loading, although no abnormality is seen with octanoate loading. Hence, beta-oxidation capacity can be evaluated sensitively.

screening laboratories, and the number of screening in each province varies roughly from 5000 to 100,000 births per year. Furthermore, the birth rate is constantly declining. Relocating the screening laboratories should be considered with the introduction of MS/MS for neonatal screening to be cost-effective.

The rough estimated cost of the MS/MS screening, which includes the costs of reagents, amortised cost of instruments, and labour charge is as follows. If 50,000 samples a year are analysed in a laboratory, the core cost of each sample is calculated to be about 6 dollars. If 30,000 samples are analysed, the cost is about 9 dollars, whereas if only 10,000 samples are analysed, that will be over 25 dollars. Hence, we should consider the economies of scale in use of MS/MS screening. We should also consider that in the future, screening laboratories should be relocated and merged for a more efficient screening network. The number of screening laboratories should be reduced from 50 to 20 or 25 such that at least 30,000 samples are analysed in each laboratory.

Conclusions

Our current activity for restructuring neonatal screening after MS/MS was introduced are summarised as follows: (i) Medical support system: we recently published a guidebook for new targeted diseases detectable in MS/MS screening, and considered the setting up of consultation centres; (ii) Diagnostic support system: a network of several laboratories in which GC/MS, enzyme determination or DNA tests are available, is being considered; (iii) Reliable and quick result reporting system: Screening laboratories should be relocated to analyse at least 30,000 samples per year in each laboratory; (iv) Continuing system for clinical follow-up and feedback to patients: We are consolidating the particulars of the patients detected through neonatal screening in the National Centre for Child Health and Development of Japan, with all their information treated with strict confidentiality. Periodical publications are used as a means of communication between doctors, researchers and patients, and to provide more appropriate and up-to-date information for patients; (v) Others: we should ensure the quality of measurements and intensify efforts to solve ethical issues related to neonatal screening.

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Clinical Applications of Molecular Genetics: The Model of Congenital Adrenal Hyperplasia

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Abstract

Spectacular advances in molecular genetics have enabled the molecular characterisation of many genetic disorders. The clinical applications include: (i) identification of pre-symptomatic and symptomatic affected individuals (monogenic diseases), allowing for early treatment and prevention of complications, (ii) carrier testing for genetic counselling, (iii) pharmacogenetic testing to guide medical treatment, and (iv) susceptibility testing (in polygenic diseases) to determine the risk of developing future disease. Using the model of congenital adrenal hyperplasia (CAH), direct mutational analysis can be applied to: (i) confirm the diagnosis when hormone assays have been equivocal, which would allow for early treatment and prevention of adrenal crisis, (ii) prenatal diagnosis and prenatal treatment in affected females to prevent or reduce prenatal virilisation, (iii) heterozygote carrier identification for genetic counselling, (iv) novel therapeutic applications to optimise treatment, including adjusting the steroid dose based on consistent genotype-phenotype correlations, so as to reduce the incidence of growth-inhibiting effects of steroid excess. However, molecular analysis can occasionally be complicated by multiple mutations on one allele, which may potentially affect genotype-phenotype correlations. Hence, molecular genetic analysis of CAH may eventually be adopted as a second tier confirmation of the disease, but is unlikely to replace the current first tier screening assays of precursor steroid metabolites proximal to the enzyme deficiency.

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Key words: Clinical applications, Congenital adrenal hyperplasia

Introduction

Spectacular advances in genetics have enabled the molecular characterisation of many genetic disorders, and an improved understanding of disease pathogenesis has resulted in significant clinical applications. The early days before the 1960s, was the era of phenotype recognition, identification and classification. However, with the advent of new molecular technologies in the 1980s, the gene can now be amplified and examined, and this heralds the era of genotype identification.

After amplification by polymerase chain reaction (PCR), genotyping can be performed by a variety of techniques, including restriction digests, the use of DNA probes and sequencing. However, large scale association studies by genotyping many single nucleotide polymorphisms (SNPs) in individuals with well characterised phenotypes, are now promising methods of identifying the cause of many complex diseases. For diseases with established genetic causation, sequencing the entire gene will be time consuming and will not be cost effective. In these diseases, a good strategy would be to screen for known common mutations of the gene in question, followed by sequencing of the entire gene if no mutations are detected.

Although genetic testing studies the genome directly, the sensitivity (or percentage of positive tests among subjects who will develop the disease) is not necessarily high.[1] Heterogeneity (the concept that more than one gene can cause a given disease) and the location of promoters or other gene-controlling elements outside the portion of the gene that is tested, are some reasons to explain why DNA tests may fail to identify affected individuals, thus accounting for false negatives.

With regards to specificity (the percentage of negative test results among subjects who will not develop the disease), a diagnosis is not always made by the presence of a DNA change.[1] Some gene changes are harmless variants, and mutations in a single gene can sometimes cause several different diseases. Hence, the interpretation of many genetic

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tests can be complex, because of several factors which include: (i) the effect of a given mutation which may be modified by other genes and the environment; (ii) different changes in a given gene may have different results; (iii) intermediate alleles may cause disease in only a fraction of cases; (iv) other genes, the environment and individual factors such as age and gender can affect penetrance so that 2 individuals with the exact same change may have different clinical presentations; and (v) a person with a 'disease causing mutation' may appear unaffected.[1]

Nonetheless, the completion of the Human Genome Project has now provided a reference of the entire human genetic instruction book. It is clear that changes in genetic code can result in changes in the encoded amino acids. If these changes are functionally significant, they may affect the proteins, enzymes and receptors, with resultant clinical pathology and significant morbidity and mortality. Increasingly, the complexities of gene regulation, gene to gene interaction and gene expression are just being unravelled, and many clinical applications have arisen.

Clinical Applications of Molecular Genetics

Clinical applications of molecular genetics include the following:

1. Diagnostic testing: Molecular genetics can provide a genetic diagnosis and confirm an existing disorder.
2. Predictive testing: This determines the presence of a genetic condition for pre-symptomatic late onset disorders, for example in neurodegenerative disorders like X-linked adrenoleukodystrophy and Huntington's chorea. The tests predict the future, so that individuals can be better prepared. However, predictive testing raises its own ethical issues in relation to emotional trauma, depression and discrimination, as a consequence of knowing that disease will eventually develop.

   Nonetheless, in both diagnostic and predictive testing, the identification of affected individuals may allow for early treatment, prognostication and the prevention of complications.
3. Carrier testing: In carrier testing, heterozygote carriers for X-linked and autosomal recessive disorders are identified so as to determine the probability of the birth of a normal or affected child. This would aid in reproductive choices. If the risk is unacceptable, couples may decide not to marry or to have children, or plan to terminate the pregnancy, if the foetus is affected. Other couples who are at risk of having a child with a serious abnormality may seek in-vitro fertilisation. However, the caveat is that carrier testing by genetic studies is only informative if the mutations have been fully identified in the propositus.
4. Prenatal testing: In prenatal testing, DNA is extracted from the chorionic villous sample between 10 to 13 weeks gestation, with a risk to the foetus of 1% to 2%. The foetus can be genotyped to predict risk of disease, and genetic counselling can be provided.
5. Pharmacogenetic testing: Pharmacogenetics is a study of how genetic polymorphisms can influence drug metabolism. The genotype can then be used to predict an individual's response to a specific drug, and the dosage can be modified accordingly.
6. Susceptibility testing: In susceptibility testing, the individual susceptibility to develop common polygenic disorders such as heart disease, diabetes and cancer is determined. At-risk individuals can be identified for primary prevention (diet/exercise) and secondary prevention (pharmacologic intervention).

The Model of Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia refers to any of several autosomal recessive diseases resulting from mutations of genes for enzymes mediating the biochemical steps of cortisol production from cholesterol by the adrenal glands. The most common form, 21-hydroxylase deficiency, can present with 3 phenotypes, based on the mutation which determines the degree of enzyme deficiency: (i) The Classical Salt Waster will present with a salt-losing adrenal crisis, and affected females present with ambiguous genitalia. (ii) In the Classical Simple Viriliser, affected females will have prenatal virilisation without salt loss, while affected males will present with pseudoprecocious puberty. (iii) The Non-classical CAH presents later with hirsutism, acne and irregular menstruation.

Likewise in the second most common form of CAH, 11-β hydroxylase deficiency, there is prenatal virilisation in affected females, but the hallmark is hypertension and hypokalemic alkalosis as a result of accumulation of precursor metabolites with mineralocorticoid activity, such as 11-deoxycortisol and deoxycorticosterone.[2] However, it is well recognised that hypertension may be absent or intermittent in the first few years, resulting in misdiagnosis.[3]

Clinical Applications of Genotyping for Congenital Adrenal Hyperplasia

Although hormonal screening can diagnose CAH effectively, genotyping can be useful in these circumstances:

1. Hormonal assays can diagnose CAH, but there may be false positives with mildly elevated serum 17-hydroxyprogesterone (17-OHP) levels associated with low birth weight, prematurity or the presence of stress-inducing illness which stimulates the adrenal production of steroids, including serum 17OHP.[4] In contrast, direct

mutational analysis is not affected by these factors, and can also be applied to confirm the diagnosis when hormone results are equivocal.

2. If steroid treatment has been started in a child suspected of CAH before adequate diagnostic blood tests have been performed, genotyping can confirm the diagnosis without stopping treatment.
3. Genotyping may also help to differentiate 11-β hydroxylase deficiency from the simple virilising form of 21-hydroxylase deficiency. In both these conditions, there is no salt loss. Unfortunately, the hallmark hypertension in 11-β hydroxylase is variable, and the hormonal profile can be identical to 21-hydroxylase deficiency (low serum cortisol levels, elevated plasma ACTH, serum androstenedione, serum testosterone levels, with modest increases in serum 17-hydroxyprogesterone), making them indistinguishable. Hence genotyping provides an alternative, fast and accurate diagnosis of both 21-hydroxylase and 11-β hydroxylase deficiency. It is also important to make a diagnosis of 11-β hydroxylase deficiency so that fludrocortisone is not used for treatment. Whereas the hypertension in 21-hydroxylase deficiency will respond to a reduction in glucocorticoid dose (hypertension arising from presumed overdose of steroids), the treatment of hypertension in 11-β hydroxylase deficiency, conversely, necessitates an increase in glucocorticoid dose with the addition of spironolactone or amiloride. Even more importantly, undetected hypertension is potentially life-threatening, and may lead to fatal vascular accidents observed even in mildly virilised patients.
4. Generally, consistent genotype-phenotype correlations in CAH allow for prognostication, which can aid management in the field of pharmacogenetics. Conventional treatment for CAH with the replacement of glucocorticoid and mineralocorticoid is never perfect, as it is impossible to exactly mimic physiological secretion of cortisol and aldosterone.

   With pharmacogenetics, it is potentially possible to adjust the glucocorticoid dose based on the genotype-phenotype correlations. The more severe genotypes may require a higher dose of glucocorticoid, and the milder genotypes may require lower doses, so as to avoid steroid toxicity and yet avoid hyperandrogenism.[5]

   In addition, there are new treatment modalities which may improve growth potential in CAH due to severe mutations, which are currently being researched:

a. Peripheral blockade of androgen action and estrogen production, which can be achieved with an androgen receptor antagonist (flutamide) and an aromatase inhibitor (testolactone), which blocks the conversion of androgens to estrogens and allows for the use of a lower glucocorticoid dose.[6]
b. Prophylactic bilateral adrenalectomy has been proposed for those with severe CAH with non-functional *CYP21* genes. In the null mutation of CAH, the adrenal gland is not functional and over-produces androgens. Hence, a bilateral adrenalectomy will eliminate excess androgens and avoid the risk of overtreatment with glucocorticoids.[7]

5. In carrier detection, if the index case is genotyped, the heterozygote carrier status of parents and siblings can be accurately determined. Genotyping has the advantage in carrier detection because hormonal assays for carriers are not always accurate, and there is considerable overlap in serum 17OHP for normals and carriers, even after a synacthen stimulation test. Knowing the parental carrier status enables accurate prediction of recurrence risks for future pregnancies.
6. In prenatal diagnosis, genetic studies of the DNA from chorionic villous cells are generally preferred over hormonal studies of the amniotic fluid. Following prenatal diagnosis, CAH is an example of an inborn error of metabolism which can be treated prenatally. The aims of prenatal diagnosis and prenatal treatment are to effectively prevent virilisation of an affected female foetus, to avoid gender confusion and sex mis-assignment, to avoid genital surgery and psychological trauma and the burden of genital ambiguity to the family. More significantly, with prenatal diagnosis, early treatment can be commenced so as to prevent a potentially life threatening adrenal crisis in the neonate.

From our studies on the *CYP21* gene in Singapore, the relatively low carrier frequency of 1.7% (1 in 60) does not warrant screening by genetic analysis.[8] In addition, molecular analysis can occasionally be complicated by multiple mutations in one allele, which may potentially affect genotype-phenotype correlations. Hence, molecular genetic analysis of CAH may eventually be adopted as a second tier confirmation of the diagnosis, but is unlikely to replace the current first tier screening assays to quantify precursor steroid metabolites.

In conclusion, while neonatal screening of metabolites can diagnose disease, molecular genetics can confirm the diagnosis of some diseases, allow for specific genetic counseling with regard to heterozygote detection, prenatal diagnosis and treatment. Having screened and detected disease, genotyping may then help in optimising therapy to minimise morbidity, and to improve the quality of life, as illustrated in the model of CAH.

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Considerations in Choosing Screening Conditions: One (US) Approach

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Abstract

The lack of a national policy on newborn screening (NBS) in the United States has resulted in 51 state-specific NBS policies (including 50 states and the District of Columbia). In 2000, a working group of the American Academy of Pediatrics provided a national NBS blueprint for the future. Using this guidance, the Health Resources and Services Administration contracted with the American College of Medical Genetics to: (i) develop a decision-making algorithm for states to use in selecting conditions for screening panels, and (ii) recommend a panel of tests to guide states in their screening requirements. This report outlines and summarises the processes and outcomes leading to the current NBS recommendations in the United States.

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Key words: Newborn screening, Paediatrics, Screening

Introduction

Newborn screening (NBS) in the US is a state public health responsibility and a unifying national NBS policy does not currently exist. As a result, US NBS expansion has developed sporadically through the years, sometimes as a result of new scientific findings (i.e. testing and treatment advances), sometimes from financial incentives (i.e. federal grants), and sometimes as a result of consumer advocacy and accompanying political pressures. Consumer advocates directly influenced early screening efforts and they continue their influence today through advocacy organisations and individual lobbying efforts.[1]

All states and the District of Columbia have legislation that results in required NBS.[2] In most states, NBS oversight is a responsibility of the state health department. Some state laws specify conditions that must be screened and may provide limited support funding. However, decisions about addition or deletion of NBS tests and other critical issues, including how financing will occur, are usually assigned to the state health officer or his/her designee. Many NBS programmes have an advisory committee (or in some cases subspecialty committees such as metabolic, haematology, endocrinology) that provides input into programme operations. In such cases, proposed programme changes are usually evaluated by this committee (in consultation with NBS programme personnel) and recommendations are made to the appropriate policy maker(s). Despite recent changes (discussed later) most NBS programmes still lack formal procedures for adding or subtracting tests, and thus testing panels continue to vary. Funding issues play a major role in policy decisions and start-up funding for new processes is particularly problematic, often requiring specific legislative action. Once a screening change is initiated, it can usually be sustained by a screening fee. It is increasingly popular to add a surcharge to an existing fee rather than to increase an existing fee, which may require a rule or statute change.

Several reports have significantly impacted US NBS policies through the years. The World Health Organization's (WHO) first Scientific Group meeting on "Screening for Inborn Errors of Metabolism" suggested screening criteria that have formed the basis for screening policy through the years. In considering, "whether and how newborn screening programmes could improve the health of mankind," they suggested dividing screening conditions into 3 groups: "(i) conditions for which there is a well-defined screening test and a fairly uniform policy of management once the disease has been identified; (ii) conditions in which the abnormal gene can regularly be identified but in which the condition only becomes symptomatic in a specific environment; and (iii) a miscellaneous category which includes conditions for which more information is needed before they will fit

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easily into a routine screening programme."[3] The Wilson and Jungner companion report attempted to chart the public health course for "bringing to treatment those with previously undetected disease" while "avoiding harm to those persons not in need of treatment." It recommended criteria for population screening and observed that early phenyketonuria (PKU) screeners had done a poor job of documenting successes.[4] These population screening criteria were immediately embraced as useful for newborn screening, and programmes worldwide began to use them in deciding about screening panels.

Frankenburg revisited these screening principles in discussing how physicians might best decide on diseases for private practice screening programmes. He noted that, "…if the decision to screen is based only on the availability of a test or the apparent importance of a test, great harm can be done, both in the waste of valuable medical resources and in direct harm to the persons screened." He concluded that, "The availability of a suitable screening test does not justify screening for a disease unless the disease is important, relatively prevalent, and amenable to early treatment. Screening … cannot be justified unless there is an acceptable, reliable, and valid test … at a reasonable cost."[5]

The US National Academy of Sciences also issued a report on genetic screening to, "… review current screening practices…identify the problems and difficulties and give some procedural guidance, in order to minimise the shortcomings and maximise the effectiveness of future genetic screening programmes."[6] While providing some useful national guidance with its 21 recommendations in 5 categories, it did little to change the use of Wilson and Jungner criteria in screening panel decisions.

In 1999, the Maternal and Child Health Bureau of the US Health Resources and Services Administration (MCHB/HRSA), in response to increasing congressional NBS interest fuelled by consumer advocacy, contracted with the American Academy of Pediatrics (AAP) for a NBS Task Force to: (i) review the issues facing state NBS systems, and (ii) to make recommendations. The collective report of its 5 multi-disciplinary work groups provided national guidance for US NBS in the 21st century. The Task Force's foremost concerns were of unequal availability of state screening services, lack of national policy defining the parameters for state-required NBS, and programme finances. Their "agenda for action" identified a need for partnerships between the public health system(s), health professionals and consumers to: define federal and state responsibilities, model NBS regulations, define minimum system standards, model guidelines and protocols for professionals, model systems of care from infancy to adulthood, design strategies for informing and involving families and the public, and fund demonstration projects to evaluate technology, quality assurance, and health outcomes.[7]

The AAP NBS report provided a publication to which various agencies could turn for guidance and documentation of improvement needs. Shortly after its publication, an Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children (ACHDGDNC – now called the Advisory Committee on Heritable Disorders in Newborns and Children) was created to advise the Secretary of Health and Human Services (HHS) on NBS issues (among others). In response to the AAP report, MCHB/HRSA contracted with the American College of Medical Genetics (ACMG) "to undertake the scientific and medical analysis of conditions being considered for newborn screening and develop the criteria for assessing and evaluating conditions." Specifically to: "(i) recommend a uniform screening panel for all US NBS programmes, and (ii) develop a model decision-matrix for use in state NBS programme considering expansion."[8]

Method

Expanded details of the methodology employed by ACMG have been reported.[9] A "NBS Steering Committee" of stakeholder partners was first assembled for project planning and oversight. Next, a multi-disciplinary "NBS Expert Group" was chosen and 2 work groups were created: "NBS Conditions and Criteria" and "Diagnosis and Follow-up." All participants were selected to bring strong scientific and clinical perspectives to discussions of the issues with attention paid to professional, cultural, ethnic, and geographic diversity. To vet various ideas and potential project outputs, a limited number of experts (outside reviewers) were selected for input throughout the project deliberations.[9]

Input into the project included expert reviews of the scientific literature, invited presentations from national and international experts, comments from the public and professional organisations, and meetings of both the NBS Expert Group and the various working groups. Eighty-four conditions amenable to NBS were selected for review. Infectious diseases were deferred for consideration in another forum leaving 81 conditions for final consideration. To guide the process, a group of basic principles about newborn screening was developed by the NBS Expert Group. The NBS Conditions and Criteria Working Group developed evaluation criteria for a quantitative assessment tool (Table 1). This tool was used to evaluate the known information about each condition by polling interested individuals and recognised experts. The data obtained were statistically evaluated and the poll responses were compared to the scientific literature. A core panel of required and secondary conditions was developed

Table 1. Quantitative Instrument used to Gather Information on Current Knowledge about Conditions under Consideration by the ACMG Expert Group[9]

Criteria	Categories	Score
Incidence of condition	>1:5,000	100
	>1:25,000	75
	>1:50,000	50
	>1:75,000	25
	<1:100,000	0
Signs and symptoms clinically identifiable in the first 48 hours	Never	100
	<25% of cases	75
	<50% of cases	50
	<75% of cases	25
	Always	0
Burden of disease (Natural history if left untreated)	Profound	100
	Severe	75
	Moderate	50
	Mild	25
	Minimal	0
Does a sensitive AND specific screening test exist?	Yes	200
	No	0
Test characteristics (Yes = apply score; No = zero)	Doable in neonatal blood spots OR by a single in-nursery physical method.	100
	High throughput (>200/employee).	50
	Analytical cost <$1/test / condition.	50
	Multiple analytes relevant to one condition are detected in same run.	50
	Other conditions identified by same analytes.	50
	Multiple conditions detected by same test (multiplex platform).	200
Cost of treatment	Inexpensive	50
	Expensive (>$50,000/ patient/year)	0

Criteria	Categories		Score
Availability of treatment	Treatment exists and is widely available in most communities.		50
	Treatment exists but availability limited.		25
	No treatment available or necessary.		0
Potential efficacy of existing treatment	Prevents all negative consequences.		200
	Prevents most negative consequences.		100
	Prevents some negative consequences.		50
	Treatment efficacy not proven.		0
Benefits of early intervention (INDIVIDUAL OUTCOME)	Clear	Scientific evidence that early intervention from screening optimises outcome	200
	Some		100
	No		0
Benefits of early identification (FAMILY AND SOCIETY)	Clear	Evidence that early identification provides clear benefits to family and society	100
	Some		50
	No		0
Early diagnosis and treatment prevent mortality		Yes	100
		No	0
Availability of diagnostic confirmation	Providers are widely available.		100
	Limited availability of providers.		50
	Available only in a few centres.		0
Acute management	Providers are widely available.		100
	Limited availability of providers.		50
	Available only in a few centres.		0
Simplicity of therapy	Managed at primary care or family level.		200
	Requires periodic specialist involvement.		100
	Requires regular specialist involvement		0
Total	Maximum score available		2100

as a recommendation for implementation by all US NBS programmes.[9] The completed report was provided to MCHG/HRSA and subsequently to the ACHDGDNC.

Results

The ACMG NBS Expert Group produced a list of 29 core conditions that should be included in all required NBS panels. Additionally, 25 "secondary" conditions (potentially identified incidental to screening for the core panel) were listed as optionally required (see Table 2). These secondary conditions lacked sufficiently high scores for inclusion in the core group, usually because of a lack of efficacious treatment, and represent a de facto research agenda. Included in the report was the recommendation for full optimisation of screening technologies (i.e. full scan interpretations for tandem mass spectrometry) and reporting of all significant findings. Also included in the secondary conditions were screening for kinase- and epimerase-deficient forms of galactosemia and other significant haemoglobinopathies, all currently detectable with current testing procedures. Arguments supporting these decisions are documented in the full ACMG report. Details of supporting studies and findings were also reported in a companion special issue of the journal, Pediatrics (see supplement to Pediatrics, vol. 117, 2006). The final report was approved by the ACHDGDNC and sent to the Secretary of HHS for review and further action.

Table 2. Conditions Recommended for Inclusion in US Newborn Screening Programmes

29 core conditions	**25 secondary conditions**
MS/MS Detectable Organic Acid Disorders	
Isovaleric Acidemia	Methylmalonic Acidemia
Glutaric Acidemia Type I	Malonic Acidemia
3-Hydroxy 3-Methyl Glutaric Aciduria	Isobutyryl-CoA Dehydrogenase Deficiency
Multiple Carboxylase Deficiency	2-Methyl 3-Hydroxy Butyric Aciduria
Methylmalonic Acidemia (Mutase Deficiency)	2-Methylbutyrly-CoA Dehydrogenase Deficiency
3-Methylcrotonyl-CoA Carboxylase Deficiency	3-Methylglutaconic Aciduria
Methylmalonic Acidemia (Cbl A,B)	
Propionic Acidemia	
Beta-Ketothiolase Deficiency	
MS/MS Detectable Fatty Acid Oxidation Disorders	
Medium-Chain Acyl-CoA Dehydrogenase Deficiency	Short-Chain Acyl-CoA Dehydrogenase Deficiency
Very Long-Chain Acyl-CoA Dehydrogenase Deficiency	Glutaric Acidemia Type II
Long-Chain L-3-Hydroxy Acyl-CoA Dehydrogenase Deficiency	Medium/Short Chain L-3-Hydroxy Acyl-CoA Dehydrogenase Deficiency
Trifunctional Protein Deficiency	Medium-Chain Ketoacyl-CoA Thiolase Deficiency
Carnitine Uptake Defect	Carnitine Palmitoyltransferase II Deficiency
	Carnitine Acylcarnitine Translocase Deficiency
	Carnitine Palmitoyltranferase I Deficiency (liver)
	Dienoly-CoA Reductase Deficiency
MS/MS Detectable Amino Acid Disorders	
Phenylketonuria	Benign Hyperphenylalaninemia
Maple Syrup Urine Disease	Tyrosinemia Type II
Homocystinuria	Tyrosinemia Type III
Citrullinemia	Defects of Biopterin Cofactor Biosynthesis
Argininosuccinic Acidemia	Defects of Biopterin Cofactor Regeneration
Tyrosinemia Type I	Argininemia
	Hypermethioninemia
	Citrullinemia Type II
Hemoglobinopathies	
Sickle Cell Anemia (SS-Disease)	Variant Hemoglobinopathies
Sickle-C Disease (SC-Disease)	
S-Beta Thalassemia	
Other	
Transferase Deficient Galactosemia (Classical)	Galactokinase Deficiency
Primary Congenital Hypothyroidism	Galactoepimerase Deficiency
21-Hydroxylase Deficient Congenital Adrenal Hyperplasia	
Biotinidase Deficiency	
Hearing Screening	
Cystic Fibrosis	

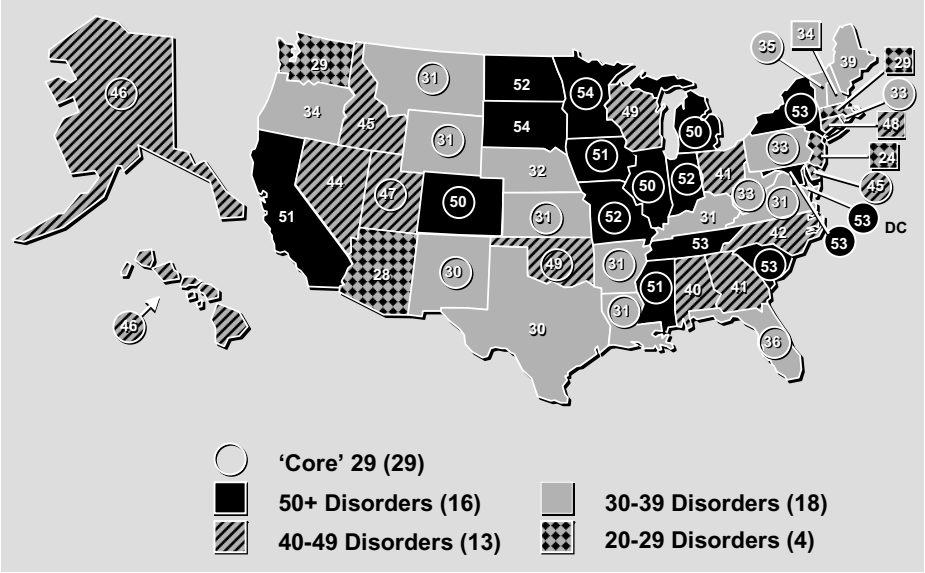

Fig. 1. Conditions required to be screened in various US jurisdictions – 12 January 2009. Some conditions may be required but not yet implemented pending funding or other issues. Available at: http://genes-r-us.uthscsa.edu/nbsdisorders.doc. Accessed 12 January 2009).

Discussion

In response to public pressure from consumer advocates, improved screening technologies, evolving medical information, the national guidance provided by the ACMG report, and the subsequent ACHDGDNC actions, almost all US NBS programmes have recently undergone some form of expansion.[10] Figure 1 provides a summary of the current status of NBS in the various states. The numbers listed refer only to state required screening conditions. In some cases, a requirement may exist but may not yet have been implemented, usually because of financial or training/technology issues. For example, Massachusetts, with 29 conditions on the map, actually provides optional screening for 11 other conditions and lists 21 others as likely to be identified due to the screening methods used. When a number is circled on the map, it indicates that screening for the "core 29" conditions is currently required on all newborns. In cases where there is no circle, it is usually the result of technical wording in the state requirement for hearing screening, which sometimes fails to require screening on all newborns. A few states have not yet implemented screening for cystic fibrosis and some do not yet require screening for tyrosinemia type I.

During the coming year, it is anticipated that all programmes will require screening for essentially all of the "core 29" conditions. Some controversies still exist regarding the secondary 25 conditions and some programmes include conditions outside of the 54 core/secondary list. For example, 2 programmes screen for toxoplasmosis, 1 programme screens for G6PD deficiency, etc. One programme includes screening for Krabbe disease and one has just completed its first year-long trial of screening for severe combined immunodeficiency disorder.[10] The ACHDGDNC has recently published its process for considering other conditions for addition to the core group of recommended screening conditions and has now begun to accept nominations.[11] Reports on the outcome of nominations are available at: http://www.hrsa.gov/heritabledisorderscommittee/reports/default.htm.

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Diagnosis and Management Support for an Expanded Newborn Screening Programme

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Abstract

The introduction of tandem mass spectrometry technology expands newborn screening and permits early diagnosis of inborn errors of metabolism. Through measurement of a number of acyl carnitines, amino acids and associated ratios, infants at risk of inborn errors of metabolism can be detected. However the increasing availability of the technology places new challenges to areas with established programmes, as well as those without existing newborn screening programmes. Once the technical aspects of tandem mass spectrometry operation are overcome, the initial challenge lies in determination of whether a borderline result is abnormal. Participation in quality assurance and international collaborative programmes is critical to optimise sensitivity and specificity. Some conditions are readily detected, others are more problematic. All positive results must be confirmed with formal testing but the tests required will vary with the disorder. Even after confirmed diagnosis, the significance of the diagnosis for that child may not be clear, as mild forms of disorders, previously thought to be rare, are being recognised by newborn screening programmes. Parents should be provided with easy to understand written information and a management plan. Education of health professionals who may not be familiar with these conditions raises another challenge. Treatment should be supervised by an expert centre and outcome data must be collected to determine the effectiveness of the screening programme in each area.

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Key words: Management, Mass spectrometry, Newborn screening

The introduction of tandem mass spectrometry (MSMS) into front line newborn screening brings with it a series of challenges, not least of which is determining the significance of determining whether a borderline result is normal or abnormal. Education of treating health professionals and affected families about unfamiliar disorders also pose significant challenges.

Building on experience from earlier newborn screening programmes, such as those for congenital hypothyroidism and cystic fibrosis, factors critical for programme success have been determined and distilled into 4 major principles (Fig. 1) A high quality laboratory is the essential backbone of a successful tandem mass spectrometry programme and this point cannot be emphasised too strongly.

Tandem mass spectrometry screening measures 2 main groups of analytes: amino acids and acyl carnitine species for the detection of aminoacidopathies, organic acidurias and fatty acid oxidation defects respectively. Calculation of specific amino acid and acyl carnitine ratios increases the specificity of the results generated by the testing programme and increases confidence in the results. Our programme uses 13 acyl carnitine stable isotope and 9 amino acid internal standards, increasing the ratios that can be calculated. The optimum time for diagnosis of inborn errors of metabolism is around 48 hours of age, but diagnosis is possible (with reduced sensitivity) between 24 and 72 hours.

Current experience suggests that the diagnosis of phenylketonuria and some of the urea cycle defects (citrullinemia and argininosuccinic aciduria) is straightforward using MSMS. More problematic ones are maple syrup urine disease, homocystinuria, particularly the B12 responsive forms. In addition, larger programmes such as the Bavarian programme (personal communication – Professor A Roscher) suggests that only two-thirds of the cases of non-ketotic hyperglycinemia will be detected. Importantly, tyrosinemia type 1 will not be detected by neonatal testing unless a second tier method to detect succinyl acetone is used, although the other forms of tyrosinemia are clearly evident.

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- Laboratory excellence makes the life of the clinician easier
- A good data management system is critical
- Data analysis software is essential
- Referral to an external expert laboratory for samples with results of unknown/uncertain significance

Fig. 1. Principles for a successful programme.

Once elevated metabolites are recognised in a screening sample, the disorder must be confirmed by formal testing. In general, the infant should be recalled as a matter of urgency. Suspected urea cycle defects and maple syrup urine disease are relative emergencies because of the risk of cerebral oedema with metabolic decompensation. We have found that how that first information is given to the family influences future interactions and recommend great care be taken with delivering the results. The process of notification of abnormal screening results to the family will vary according to local practice: in some situations, contact is by a counsellor or physician attached to the newborn screening service. In others, an intermediary such as a paediatrician, midwife or general practitioner delivers the news. In the latter situation, we have found it to be critically important to provide written information on the disorder, as well as the results, to the person who will be contacting the family. As they are unlikely to have dealt with this disorder previously, this information should be faxed immediately to provide a factual basis for the discussion. In 21st century, most parents in Australia present for review having already gained information from the internet.

For aminoacidopathies, blood and urine should be collected for formal amino acid analysis. Other tests may be indicated, depending on the abnormality, e.g. plasma total homocysteine for homocystinuria, plasma liver function tests, coagulation profile and urine succinyl acetone for elevated tyrosine, plasma ammonium and urine orotic acid for urea cycle defects.

Confirmatory testing for organic acidurias and fatty oxidation defect will depend on the disorder suspected, but, in general, urine organic acids, plasma carnitine and repeat blood spot testing are performed. Caution must be taken in the interpretation of acyl carnitine profiles outside the optimum screening window as these can be normal, even in affected infants, in the long chain fatty acid oxidation defects.

Even once the disorder is confirmed, the significance of the diagnosis for that family may not be known. Mild cases of conditions such as very long chain acyl CoA dehydrogenase (vLCAD) deficiency and isovaleric acidemia are increasingly being recognised by newborn screening. The significance of conditions such as short chain acyl CoA dehydrogenase (SCAD) deficiency, short branched chain acyl CoA deydrogenase deficiency and 3 methyl-crotonyl Co A dehydrogenase deficiency are currently being hotly debated among the metabolic community, with the majority opinion in 2008 that these are benign conditions.

Counselling in such situations must be skilled, to ensure that parents are informed, but not unduly alarmed, and understand the reasons the diagnosis has been raised as a possibility, the limitations of current knowledge and the management plan for future intercurrent infections. It is helpful to discuss ambiguous situations and results with a colleague or supervisor.

Parents should be provided with written information, preferably in their local language. For fatty acid oxidation defects, definition of safe fasting times, description of the signs and symptoms that should raise concern and a written, detailed, management plan for sick days is mandatory. We have written fact sheets for the more commonly seen disorders, such as MCAD deficiency.[1] Information available on the internet, even from parent support group sites, is of variable quality and may be unduly technical or alarming. If available for a particular topic, the information provided by GeneClinics (www.geneclinics.org) stands out by being comprehensive and well written.

Treatment should be supervised by an expert in the area of inborn errors of metabolism and begun as early as possible. The offending toxin should be removed from the diet. In general, if the infant is metabolically unstable or the disorder is significant, protein is excluded from the diet until the target is reached, e.g. phenylalanine below 600 μmol/L or ammonia is normal. Vitamin therapies may be indicated. Calorie supplementation is critical to prevent or reverse catabolism. Support of vulnerable or damaged organs may be required.

Laboratory experience reduces "noise" in the screening programme, as does participation in international collaborative efforts such as the US-based Region 4 Genetics Laboratory collaborative quality improvement project.

Above all, outcomes must be audited to determine the effectiveness of the screening programme in your local setting.

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My Early Experiences in Establishing Neonatal Screening and the Reason for Regional Meetings of the International Society for Neonatal Screening

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Abstract

When I started the neonatal screening in Japan, I could not obtain the necessary information for establishing the national screening system in my own country. Thus, around 1970, I visited H. Bickel, R. Guthrie and several other experts in the field of the neonatal screening in USA and Europe. Through their help, I could learn: (i) the philosophy of the world of screening, (ii) the way to improve the basic techniques in this field and (iii) the way to improve the level of screening. On the other hand, I realised that in some countries, people received imprecise information from non-authoritative sources. I also realised that it was difficult for people in developing countries to meet experts of other countries. Therefore, when I was appointed the first president of International Society of Neonatal Screening (ISNS), I proposed to have the regional meetings held in many areas. In this report, I explained how we were asked to establish the national screening system in Japan through the support of experts around the world. I hope that people will understand the reason why I proposed that regional meetings of the ISNS be held in various locations.

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Key words: Improvement, Precise information, Screening system

In 1966, I had started a regional neonatal screening system for Tokyo based on the Guthrie method. By the end of 1970, several laboratories in Japan had also started screening in their areas. As the head of the Division for Mental Retardation at the National Institute of Mental Health in Japan, I believed that we had to establish a screening system which would give all the babies in Japan the privilege to be screened. This belief in the need for a national screening system in Japan was shared by many colleagues and it encouraged me to take steps to establish such a system.[1]

However, I soon realised the inadequacy of my knowledge to pioneer a national screening system. So, I tried to meet world renowned screening professionals and learn from them the way to establish a nationwide screening system. In 1971 summer, I received a special travel grant from my government to visit other screening centres. This was the first and a really important opportunity for me as I could learn all about neonatal screening.

The main purpose of this trip was to visit Professor Horst Bickel (Fig. 1), Director of the Medical School in Heidelberg, West Germany. Over a period of 3 hours, he very kindly shared with me his expertise and experience and allowed me to discuss issues related to neonatal screening. He told me that he already knew some screening techniques for the early detection of phenylketonuria (PKU) patients but when he had learned the Guthrie method, he recognised that he should cooperate with Guthrie to further develop this method all around the world. Also, he emphasised that neonatal screening should be recognised as a public health initiative and hence should be funded by the government. He also shared his lack of success in establishing a national neonatal screening programme for inborn errors of metabolism in West Germany in spite of many representations. Prof Bickel however encouraged me to establish a national programme in Japan and promised personal and international support. Subsequently, he also responded to us several times when we needed his help.

In the beginning of 1972, I was invited to present a paper reporting on the status of screening in Japan at the "International Symposium on Laboratory Screening Techniques for Inborn errors of Metabolism (IEM) in Newborn and Selected High-risk Infants" that was held in Warsaw. My participation at this meeting of a select group

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Fig. 1. Horst and Barbara Bickel, 1975.

Fig. 2. R. Guthrie in a training Course in Tokyo, 1976.

Fig. 3. Harvey and Barbara Levy in USA,1974.

was facilitated by Horst Bickel and Robert Guthrie. At this meeting, I was able to meet and establish contacts with the screening fraternity of the USA. These included Robert Guthrie from Buffalo, Harvey Levy from Boston, Kenneth Shaw from Los Angeles, Catlin Brandon from Oregon, William Murphy, an associate of Robert Guthrie and Rudolf Hormuth. I also was fortunate to meet with Ronald Gitzelman from Switzerland, Otto Thalhammer from Austria, Barbara Cabalska from Poland, Bohunka Blehova from Czechoslovakia, Nina Carson from Ireland, and Arthur Veale from New Zealand. I also had the opportunities to ask many questions and receive a variety of perspectives on some difficult issues.

From personal conversations with Guthrie (Fig. 2), I learned many more important things about neonatal screening. Therefore, immediately after my return to Japan, I had a discussion with several Japanese pioneers of neonatal screening. We invited Guthrie to Japan in March 1974 as an official guest. His many lectures and extended discussions remained of great importance to many of us.

In the September 1974, I visited Robert Guthrie and 4 laboratories in Boston, Oregon and Los Angeles, where there were important co-workers of Guthrie who were developing a multiple screening system. I spent several days in Buffalo to learn the details of the Guthrie method. During my stay in his laboratory, I learned about the making of good bacterial inhibition assay (BIA) plates and ensuring an accurate recognition of abnormal samples. I realised that my knowledge of the Guthrie method was not correct and, I decided in my mind that before we started the national screening system with its many regional screening laboratories, we should invite Guthrie to come again and teach the technicians, the precise preparation and accurate reading of the BIA plates. The government thus managed to organise a final training course for the screening laboratories in all areas of Japan at the end of December 1976, just 1 year before the initiation of the nationwide screening system.[1] Also, I had learnt the necessity of quality control for the Guthrie method as Robert Guthrie had already started to distribute the standards made in his laboratory. I thought that this was the most important step of quality control for the Guthrie method.

In Boston, I visited Harvey Levy (Fig. 3), the expert on the various kinds of IEMs who taught me the crucial technical points and the necessity of early treatment of the different types of IEMs. After I met him, I was convinced to start screening in Japan for as many diseases as we could.

In Los Angeles, I met Richard Koch, Professor of Paediatrics and a specialist in the treatment of PKU and other IEMs and the consultant for the screening system in California. I have also learnt from both Koch and Kenneth Shaw, a chemist who started the Guthrie method in California. At that time, there were many private laboratories for PKU screening in California and they worried about the poor quality of PKU screening in some laboratories. Hence, we discussed about the way to control the quality of the screening process. After this discussion, I considered the establishment of a national quality control system. Also, in LA, I had a chance to meet Robert Phillips, who in the beginning of 1970 developed the Punch index machine when Guthrie and his co-workers decided to start the multiple screening system. Most laboratories in this group used 4 BIA plates for one sample. This machine was very useful for preventing the mistake of not placing the disc in the exact position of each BIA plate. I stayed at his laboratories, learned how to use it and finally concluded that the machine was very reliable. When we started nationwide screening, many laboratories used this kind of machine.

Based on the support from these people, Japan could start the national screening system in 1977. Our federal government supported all regional governments so that all screening laboratories could use the standard prescribed programme. All screening laboratories were covered by the quality control system. Many people, including myself, who were consultants to the screening laboratories were

well trained. After we had started the nationwide screening, the Japanese Society For Mass-Screening was established with about 500 members initially.[1]

Had we not received any support from many of the experts mentioned in this article, we might have started Neonatal Screening in Japan with many weak technical points or incorrect procedures based on inaccurate information. I am very grateful to Horst Bickel, Robert Guthrie, Harvey Levy and many others who had helped us tremendously. Based on their support, we were able to start the nationwide screening well without making any serious mistakes.[2-4]

I think it will be very important for people who work in the screening laboratories to participate in international meetings, meet experts from different countries and learn from them how to carry out their duties and responsibilities well. Besides Japan, many people in other countries working in screening laboratories also faced difficulty in attending such meetings held overseas.

Therefore, when I was asked to serve as the president of the International Society for Neonatal Screening (ISNS), my first idea was: "Establishment of regional meeting in various parts of the world, so that many people will be able to meet excellent people in the field of screening." When we had the first business meeting of the ISNS in Brazil in November 1988 I proposed this idea. This was agreed by all and hence we decided to organise regional meetings.[1,2] In the Asia-Pacific region, the first regional meeting was held in Sapporo, Japan in June 1993. Since then, 4 more such meetings have been conducted in the Asia-Pacific region, namely in Hong Kong, Thailand, Philippines and China. Besides these 4 countries, the regional meetings were also held in Europe and South America.[4]

I sincerely hope that many neonatal healthcare co-workers in the Asia-Pacific region will have the chance to attend excellent international lectures conducted by the experts. Should they have the opportunities to interact with these overseas experts, it will be definitely a beneficial experience for them.

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Newborn Screening for all Identifiable Disorders with Tandem Mass Spectrometry is Cost Effective: Supporting Arguments

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Abstract

Tandem mass spectrometry (MS/MS) has become increasingly popular as the preferred technology for detecting inborn errors of metabolism in newborn screening (NBS) programmes. Its sensitivity and specificity for detecting numerous metabolic conditions is well-documented. As a NBS technology, there are continuing questions about whether MS/MS should be utilised to the fullest when such usage may mean detecting and reporting analytical findings that could lead to differentiating and diagnosing for which treatment efficacy may not yet be proven. As part of a friendly debate to educate conference attendees on both sides of a somewhat controversial issue, 2 papers awere presented giving information supporting or questioning the cost effectiveness of full scan usage and reporting when using MS/MS in NBS. Reported here are some of the supporting arguments.

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Key words: Cost effectiveness, Newborn screening, Tandem mass spectrometry

Introduction

Tandem mass spectrometry (MS/MS) was first proposed as a possible newborn screening (NBS) technology in the early 1990s.[1] Not only was the feasibility for detecting large numbers of metabolic disorders from NBS dried blood spots recognised, but also that disorders that did not yet have efficacious treatments might be detected in newborns. The relative rarity of metabolic diseases makes defining their natural history a challenge, and NBS for new diseases often uncovers disease variations not previously recognised. Thus, there were also questions about the reliability of detecting some disease markers early in life and how their concentrations (and detectability) might vary over time (from birth to time of specimen collection). On the other hand, NBS is a rare opportunity to increase knowledge about disorders including methods for early detection and treatment/management that can lead to improved outcomes. The net result is a continuing debate about which conditions to include in screening panels, how these policy decisions should be made, and whether such screening is cost effective. [2]

The arguments about MS/MS detectable conditions are particularly complex since the technology allows for multiplex testing (simultaneous detection of large numbers of conditions). In addition to analysing for profiles of detectable ions in the full scan mode, the technology can be restricted to look for specific target ions [commonly referred to as selective reaction monitoring (SRM) or multiple reaction monitoring (MRM)]. Adding to the confusion is the fact that some metabolic disorders may have the same MS/MS detectable markers. This can result in clinically significant disorders other than the primary screening target being revealed during the differential diagnosis of a targeted disorder. Thus, major policy decisions include whether to screen for and report all MS/MS detectable disorders. If all detectable disorders are not included in screening, what should happen if information about non-targeted disorders is obtained incidental to confirming the presence of a targeted disorder?[3]

The question proposed in this debate is whether NBS for *all* detectable conditions using MS/MS is cost effective. The 2 sides of the question can be seen simplistically from 2 comments previously published. Dr. Wilcken, a physician who presents the other side of the cost-effectiveness

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argument in this journal, has noted that, "The problem of costs and benefits is difficult, and a 'reasonable balance' rather than positive cost/benefit ratio seems desirable."[4] On the other hand, Dr. Howse, a consumer advocate, has stated the position of the March of Dimes that, "… a currently available test should be abandoned for a newer one, if the latter achieves a greater precision and offers a shorter turnaround time, no matter what the cost differential. … The primary consideration should be the health of the infant."[5] So, cost effectiveness appears at the outset to be based on the perspective from which it is viewed – professional or parent. That is, many physicians who must react to screening results by spending time, energy, and resources to try to make an accurate diagnosis may have a different perspective from many parents who may feel entitled to know everything possible about their newborn's health. In this report, we try to focus on the cost effectiveness studies previously reported as one important consideration impacting NBS policy decisions about MS/MS screening.

The economic arguments are challenging and often vary depending on individual perspectives since there are large knowledge gaps in considering rare diseases and subjective considerations must be included. Economic arguments can be used to compare health outcomes, costs of interventions, and averted costs, but they include information that may not be certain. For example, information about disorder prevalence, severity, testing quality (sensitivity and specificity), treatment benefits (decreased mortality and morbidity), and potential harms (recall rate and false positive findings) may be included in costing calculations. Cost-effectiveness usually considers health outcomes in terms of quality adjusted life years (QALYs). That is, a year of life is adjusted for its quality with 1 year of perfect health defined as 1.0 QALY. A year of ill health is discounted based on health outcome projections and experiences. Cost-benefit analyses usually seek to translate health outcomes into money. Economists generally agree that a cost-effective investment exists if the cost for a QALY is $50,000 or less.[6] For a more detailed look at the economic arguments in NBS, readers are referred to published reports by persons with specific health economics expertise.[7,8]

Method

The primary method of obtaining information for this report was through the use of various search engines on the Internet. The terms used included various combinations of 'cost effectiveness,' 'cost benefit,' 'newborn screening,' and 'tandem mass spectrometry.' As articles were located, their references were scanned for additional information. Various parent advocacy organisations were also contacted for stories and information from their members on personal experiences in jurisdictions where MS/MS screening capabilities were not fully utilised and *all* detectable conditions were not included in screening. While many poignant stories were obtained describing adverse affects when MS/MS screening was limited by MRM techniques, these stories were used primarily as illustrative examples during the oral presentation of this report and are not repeated here because of their anecdotal nature and space limitations.

Results

Because NBS using MS/MS technology is relatively new and the economic arguments are complex, published cost studies are limited and do not generally include all detectable conditions. Therefore, the arguments favouring MS/MS full scan screening must be constructed from combinations of individual and multiple cost studies (and their appropriate extrapolations). Because NBS for medium chain acyl-coA-dehydrogenase deficiency (MCADD) has the longest history, several MCADD costing studies exist, some with combinations of other disorders. Early MS/MS cost effectiveness reports date to 2002. In that year, 2 cost studies reported the benefits of MS/MS NBS. The first reviewed the impact of comprehensive MS/MS screening on a large California, USA health maintenance organisation. In the base scenario, the cost for comprehensive MS/MS screening was $5,827/QALY; in the least favourable scenario, it was $11,419/QALY, and in the most favourable scenario, it was $736/QALY. All of these amounts were well within the favourable cost effective range previously noted (≤$50,000). Cost savings occurred primarily in the first years of life due to decreased hospital stays, with lower cost saving in later years as a result of higher follow-up costs due to increased life expectancy. Cost savings were dependent on a low recall rate and subsequent low number of false positive findings.[9]

The other 2002 cost study was conducted in Wisconsin, USA. There, an incremental cost-effectiveness analysis looked at the differences in screening for MCADD alone and in combination with 13 other detectable disorders. Screening for MCADD alone was calculated to cost $41,862/QALY in the worst case scenario and $6,008 in the most realistic case scenario, both illustrating cost effectiveness. The incremental costs of simultaneously detecting the 13 other disorders with MS/MS NBS also gave an acceptable cost effectiveness ratio of $15,252/QALY (assuming that the incremental cost of screening remained under $13.05 per test).[10] A study in Pennsylvania, USA at about the same time also concluded that MCADD screening was cost effective. Their base-case analysis (including start-up costs) over the first 20 years of life, showed a screening cost of $5,600/QALY (95% CI: <0-$17,100/QALY) with cost effectiveness improving to

approximately $100/QALY (95% CI: <0-$6900/QALY) when the life span was expanded to 70 years.[11]

A 2005 Finnish study examining the costs, effects, and ethical consequences for NBS decision making found that MS/MS screening for several metabolic conditions was cost effective when compared to other health interventions already in use (despite the fact that the incidence of PKU is known to be extremely low in Finland. This study of 5 disorders, including 4 detectable by MS/MS [MCADD, long chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD), phenylketonuria (PKU) and glutaric aciduria type 1 (GA-1)] found a range of cost effectiveness from €5,500/QALY gained to €25,500/QALY. Calculations and modelling included considerations for adding additional conditions. Avoidance of a single severe handicap as a result of screening was found to decrease the worst case scenario to €18,000, making it cost effective.[12]

In another European study, medical records on all clinically diagnosed MCADD patients in the Netherlands born between 1985 and 2003 were compared to experiences of patients found through NBS. The incremental cost-effectiveness ratio (ICER) was calculated using life years (LYs) as the outcome measure by combining the 2 cohorts in a decision model with second-order Monte Carlo simulation. The resulting ICER was $1653 per LY gained. A sensitivity analysis gave an ICER of $14,839 to $4345 per LY gained. All calculated ICER/LY were well within acceptable range for cost effectiveness.[13]

Perhaps most pertinent to the question at hand is a more recent costs effectiveness study in California, USA. Costs were extrapolated from a NBS pilot program considering *all* identifiable conditions detectable by MS/MS. Cost-effectiveness, benefit/cost, and cost-utility analyses were conducted using a base-case set of assumptions, which were varied to examine more-favourable and less-favourable assumptions. MS/MS screening was estimated to identify 83 affected newborns annually from 540,000 screened and, when all programme costs and savings were calculated, NBS was estimated to save approximately $1.5 million ($3.4 million savings in the best case scenario and $3.8 million additional costs in the worst case scenario) annually. A more rigorous cost effectiveness calculation with sophisticated economic modelling found that NBS with MS/MS was cost effective with a cost of $1,628/QALY in the best case scenario and $14,922/QALY in the worst case scenario.[14]

Discussion

As noted by the American College of Medical Genetics' Expert Group, MS/MS screening for a core set of conditions usually results in screening for a much wider range of conditions since metabolic disease indicators are often not specific to a particular disorder. In the case of the ACMG 'core 29' conditions, several additional conditions, perhaps as many as 8, would likely be detected incidentally as part of the differential diagnosis if MRM screening were used. Further, full scale MS/MS profiling, in addition to being the most efficient use of the screening technology, offers better quality for the MS/MS analytical screening procedure. That is, full scale MS/MS testing allows better detection of spurious signals and/or reagent contaminants. Use of full MS/MS profiles also enhances clinical interpretation by revealing anomalies in associated compounds or internal standards against which excesses or deficiencies can be better interpreted.[3] All of these considerations play a role in cost effectiveness considerations.

Some have argued that increasing the conditions screened by MS/MS increases the psychological stress to parents resulting from increased patient recall to resolve presumptive findings. Indeed, a 2003 study of child outcomes and parental stress in New England, USA found that recall with subsequent false positive findings caused increased parental stress and parent-child dysfunction. This study also confirmed that screening and early diagnosis with MS/MS resulted in fewer and shorter hospitalisations, fewer medical problems, higher developmental scores, and reduced stress to parents in the screened cohort.[15] A separate study in Ohio, USA using questionnaires to assess parent's knowledge about screening, parental stress, and parental attitudes confirmed the New England findings, and also found that the majority of responders favoured expanded testing to greater numbers of conditions even if screening resulted in a recall with subsequent false positive findings.[16]

In conclusion, there is significant cost effectiveness data justifying screening for MCADD. Likewise, there is a growing literature suggesting that MS/MS full scanning techniques used to identify all detectable conditions of clinical significance are also cost effective. As noted by others,[12] a cost effectiveness analysis is only a technical aid that may lead to different screening policy decisions depending on available resources and value judgments. Cost effectiveness is usually not the sole basis for decision-making since the values of individuals, families, healthcare personnel and health decision-makers are different. Ultimately, the decision to screen for all MS/MS detectable conditions or to limit the screening panel must meet both the needs of the professional screening community and families.

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Newborn Screening for all Identifiable Disorders with Tandem Mass Spectrometry is Cost Effective: The Negative Case

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Abstract

Tandem mass spectrometry has become increasingly popular as the preferred technology for detecting inborn errors of metabolism in newborn screening programmes. Its sensitivity and specificity for detecting numerous inborn errors has been well documented. However, there are continuing questions about whether the technology should be used to the fullest when such usage may mean detecting and reporting analytical findings that could lead to diagnosing conditions for which clinical outcome is unclear and treatment may not be needed, or treatment efficacy may not yet be proven and cost effectiveness is unlikely. As part of a friendly debate to educate conference attendees on both sides of somewhat controversial issues, these 2 papers at the conference presented some of the information supporting or questioning the cost effectiveness of full scan usage and reporting in tandem mass spectrometry newborn screening. Reported here are some of the questioning arguments.

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Key words: Benign disorder, Clinical outcome, Treatment

The introduction of tandem mass spectrometry (MSMS) was indeed a great leap forward for newborn screening, and after over a decade of experience of this technique, evidence about its performance is accumulating. The most important information needed about a newborn screening programme is the clinical effectiveness of early diagnosis, an estimation of the possible harms that may be involved to families (mainly due to false positive, and perhaps false negative results, and stigmatisation) and some estimation of the financial costs.

Cost effectiveness compares costs and outcomes of 2 different actions – in this case, screening or not screening. For screening, it is of major interest, because costs and outcomes are multiplied across large populations. Cost more than outcome often affects government decisions.[1] But there are problems in its assessment. The cost of the intervention, screening, can be measured reasonably accurately; the effect of the intervention on the health of the population can also be measured, at least in the short term. But the increase in health outcome is sometimes the avoidance of death. It is not always clear how to combine these 2 sets of data.

There have been over a dozen studies of the cost aspects of tandem mass spectrometry. The majority of these have relied on many unproven assumptions rather than actual screening data, and over half have focussed primarily on medium-chain acyl CoA dehydrogenase (MCAD) deficiency.[2-12] Almost all these concluded positively, usually that screening uses "more resources…but attains better health outcomes"[3] or similar findings. Two studies[13,14] used actual newborn screening data and compared this with data from unscreened comparable populations, both coming to positive conclusions. In our Australian study,[13] we measured the within-laboratory cost of screening, including depreciation of instrumentation, costs of follow-up for true- and false-positive cases, and all costs of treatment and healthcare in the first 4 years of life. The actual costs per screened patient were more than those per patient for the non-screened, but the life-years gained and deaths averted altered the balance in favour of screening. The missing cases (those never diagnosed) among the non-screened complicate the analysis. The current weight of evidence, however, is in favour of screening being cost effective. So am I changing sides in this debate? Not really.

Let us examine again the motion: "That newborn screening with tandem mass spectrometry *for all identifiable disorders* is cost effective". The studies referred to above cannot

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address that question for many reasons:

- There is a lack of data on the outcome of clinically-diagnosed cases for many of the disorders;
- Far more cases are found by newborn screening than by clinical detection;
- Some of these cases are "extra" cases of a disorder that usually but not always produces symptoms, but some are cases of disorders which now appear benign, or nearly so.

And then there are other reasons why screening might not be cost effective:

- There is a risk of litigation in relation to missed cases, or healthy children who have received unnecessary treatment;
- There is an extra burden expected for laboratories and clinicians to deal with the cases of all sorts, including the false-positives.

Let us examine some of these points:

In many countries and states, there is a lack of systematic data on the unscreened population. The costs of not screening might be smaller than estimated, as there will have been unknown numbers of deaths, and additionally many affected patients with less serious conditions might never receive any healthcare.

It is certain that more cases are found by screening. We do have good data on the unscreened population in Australia: for MCAD deficiency, there are more than twice as many cases found by screening.[15] Our later data suggest that the ratio for other fatty acid oxidation disorders is more than 4 times as many, and for some organic acid disorders – eg 3-methylcrotonyl CoA carboxylase deficiency, also a similar increment. Going hand-in-hand with this, we may be finding the wrong sorts of cases. Several disorders detectable by current MSMS strategies were rarely found before screening occurred, and appear benign. Short-chain acyl-CoA dehydrogenase deficiency is a case in point and there are several others. Many of the extra cases may receive life-long treatment where none was needed[16] which will increase costs. The treatment could have adverse effects – also an increase in costs which may be hard to quantify. For MCAD deficiency, we know this is a potentially fatal condition. But screening identifies fewer patients with the common (northern European) mutation, c.985G, and several (8 in our first 50 cases) with a mutation known to be "mild", c199C, which has never been found in patients with symptomatic MCAD deficiency.[17] It would be a bold physician who would assert that such cases were at no risk ever, so they too will almost certainly be "extra" patients.

Will there be a risk of much extra litigation? If a disorder in the long term is accepted as benign, could physicians be sued for unnecessary treatment? Certainly if a very restrictive diet is prescribed unnecessarily this could be the case.[16] What if a case is missed? Homocystinuria is an example where we know that no pyridoxine-responsive case has ever been found by newborn screening, but non-responsive cases may also be missed. Those detected benefit greatly, but this will not be a comfort to the parents of patients missed by the screening and subsequently handicapped. All of screening is a balance between sensitivity and specificity – danger of missing cases versus the adverse effects, including cost, of false positive results.

The motion specifies, "all identifiable disorders". So do we want to include, for example, the isolated hypermethioninaemias, most examples of which appear entirely benign? We can easily detect histidinaemia in the same laboratory run as routine MSMS screening. This disorder we certainly know we do not want to detect, as it has clearly been shown to be a benign quirk. Do we currently know what disorders need treatment and might benefit from early diagnosis? I have recently addressed this question in relation to dietetic management.[16] The studies cited earlier have been largely theoretical, with many doubtful assumptions. Our own study did not show that screening was less costly than not screening, (although it suggested a balance in favour of screening, when including averted deaths) and no study has yet looked at "screening for all identifiable disorders".

I believe that MSMS screening can be cost effective under some circumstances, but not if there is screening for all identifiable disorders. We certainly need to consider screening for all disorders for which there is credible evidence of a substantial risk of an adverse outcome unless there is early detection and effective treatment. But we should not institute screening for disorders where there is insufficient evidence of adverse clinical outcomes, and no rational intervention proposed or needed. That can only lead to unnecessary distress and increased costs. Because of this, I suggest that the motion, "that newborn screening with tandem mass spectrometry for all identifiable disorders is cost effective" cannot be sustained.

Disclaimer: This was a debate. The views presented here do not necessarily represent the views of the authors or those of the US Health Resources and Services Administration, or the New South Wales Newborn Screening Programme administration.

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Issues on Universal Screening for Galactosemia

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Abstract

Galactosemia is an inborn error of galactose metabolism, caused by an abnormality in the conversion of galactose and uridine diphosphoglucose to glucose-1-phosphate and uridine diphosphogalactose through the action of 3 sequential enzymes: galactokinase (GALK), galactose-1-phosphate uridyltransferase (GALT), and uridine phosphogalactose 4-epimerase (GALE). The advent of newborn screening brought hope with early diagnosis and prompt treatment. Newborn screening advocates have pushed for inclusion of galactosemia in the newborn screening panel. However, reports of complications despite early treatment have questioned the merits of universal screening. This paper presents issues in favour and against universal newborn screening for galactosemia.

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Keywords: Galactokinase (GALK), Galactose-1-phosphate uridyltransferase (GALT), Uridine phosphogalactose 4-epimerase (GALE)

Introduction

Galactosemia is an inborn error of galactose metabolism, caused by an abnormality in 3 sequential enzymes involved in galactose metabolism namely: galactokinase (GALK), galactose-1-phosphate uridyltransferase (GALT), and uridine diphosphate galactose 4-epimerase (GALE). These enzymes allow the subsequent conversion of galactose into galactose-1-phosphate (GALK), of galactose-1-phosphate and uridine diphosphate-glucose (UDP-glucose) into glucose 1-phosphate and UDP-galactose (GALT), and the interconversion of UDP-glucose and UDP-galactose (GALE).[1] The biochemical consequences of this genetic disorder are abnormally high concentrations of galactose and its metabolites in body tissues and fluids. It is a serious disorder with significant mortality/morbidity should its diagnosis be missed, with mortality understood to be preventable by newborn screening.[2]

There are 3 types of galactosemia based on the deficient enzyme: GALT deficiency (type I), GALK deficiency (type II), and GALE deficiency (type III).[2] Type I (classical galactosemia), the most common type and most severe form, may lead to life threatening complications if not treated promptly with a low galactose diet within a few days after birth.[3] In the first few weeks of life, it may present as poor feeding and weight loss, vomiting, diarrhoea, lethargy and hypotonia, signs and symptoms of liver dysfunction, bleeding tendencies, cataracts and septicemia.[2] Affected patients are also at increased risk of delayed development, speech difficulties and mental retardation and female patients may experience reproductive problems caused by ovarian failure.[3] The main clinical feature of Type II is cataracts that are usually bilateral and detectable in the early weeks of life and even at birth and in some reports in foetus at 20 weeks' gestation. Type III galactosemia, the most rare type, have an enzyme defect principally in their erythrocytes and have normal growth and development[2] but some patients may present with cataracts, delayed growth and development, mental retardation, liver disease and kidney problems.[3]

Frequency depends on the genetic mix of the population with an overall incidence of 1 in 30,000 to 60,000 for classic galactosemia; less than 1 in 100,000 for type II, and type III appears to be very rare.[3] The incidence of complete absence of epimerase activity was found to be 1:23,000 in Japan.[4]

Issues in Favour of Universal Screening

Newborn screening, if performed in the first 1 to 2 days of life, provides an opportunity for diagnosis either before or just as the infant presents with symptoms. This early

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diagnosis can lead to an early diet shift to a soy based formula that reduces permanent damage from the immediate impact of high doses of galactose, such as life threatening liver failure and its complication, by cutting short the duration of exposure to the offending metabolites. In a 10 year period, statistical mortality was reportedly reduced more than ten-fold (from 4.6 to 0.3) in galactosemia children as a result of newborn screening.[5]

Some infants may avoid brain damage from the early high doses of galactose, with the result of a normal IQ outcome with no ataxia. If a child with a severe IQ loss, yielding an IQ of 60, does not die, the galactosemia model predicts US$1,022,000 in additional non-medical and indirect costs.[6] Cost of care of a child with mental retardation may reach up to US$1,014,000.[7]

Newborn screening is expected to reduce the cost of clinical identification. Currently, most galactosemic infants are hospitalised in neonatal intensive care units with an expected reduced cost per stay of US$12,000 per child.[5]

Early screening is a cost effective means of reducing infant death. It is a cost-saving intervention which results in both better health outcomes and less total spending, including medical care and other direct costs of care, as well as costs associated with the intervention. From the societal perspective, economic benefits include averted indirect costs or productivity losses from premature mortality or disability.[8]

Costs for screening of galactosemia alone may outweigh its benefits but the addition of galactosemia on an existing newborn screening infrastructure for congenital hypothyroidism, for example, results in net benefits of US$4.58M with a benefit:cost ratio of 2.0 (Table 1).[9,10]

Despite arguments that the costs of screening for galactosemia alone or in certain combinations with other conditions outweigh the benefits, screening is expected to reduce the cost of clinical identification. Early commencement of treatment does not necessarily prevent complications like neurological defects especially affecting language and ovarian failure,[11] but early diagnosis and intervention can limit early mortality and morbidity from the disease, minimise the magnitude or severity of complications, help prevent disability, and improve health related quality of life. According to cost calculations from the Washington State newborn screening programme,[5] minor neural damage that reduces IQ ultimately reduces the function of the individual in all areas of life. Without retardation, loss of IQ generates a loss of productivity, which was valued at US$14,500 per IQ point (year 2000 dollars). Thus, even when the difference in IQ is as small as a few IQ points, a financial loss is incurred for the individual and society. Likewise, they calculate that ovarian failure will require hormone replacement therapy at a cost of US$360 per year and will mean that the girl will be unable to bear children. A cost for the latter was assigned at US$21,000. Cataract surgery costs were also estimated at US$3500 by the Washington report. In the long run, therefore, funding comprehensive newborn screening programmes may save money for society.

Issues Against Universal Screening

To understand the economics of universal screening for this disease, it is important to decipher the expenditure and gains within the concept of the screening system. On the one hand, expenditure is incurred right from the start of planning and organisation, when stakeholders have to be informed, the strategy defined and consensus attained. Then, the process of educating of professionals and the public starts, together with the implementation of the whole screening system, including issues of testing, quality assurance, counselling and patient tracking/confirmation. For confirmed cases of galactosemia,[5] the costs for patient monitoring and subsequent management must be considered, including training families for food selection and treatment if the child eats inappropriately. If galactosemia screening is added to a cord blood screening programme, then there is a significant cost to change the programme to one of blood collection on filter paper, since cord specimens are unsatisfactory for this testing. These costs include such items as filter paper card development and distribution, training in collection technique, specimen transport, and assignment of collection responsibilities.

Table 1. Cost-benefit Analysis of GAL and in Combination with Other Disorders[9]

Condition(s)	Total costs*	Total benefits*	Net benefits*	Benefit:Cost ratio
Gal	$ 1.12 M	$ 0.21 M	$ (0.9 M)	(0.2)
Gal + CH	$ 4.80 M	$ 9.37 M	$ 4.58 M	2.0
Gal + CH + CAH	$ 10.91 M	$ 13.12 M	$ 2.22 M	1.2
Gal + CH + CAH + PKU	$ 23.96 M	$ 19.68 M	$ (4.27 M)	(0.8)
Gal + CH + CAH + G6PD	$ 15.89 M	$ 33.80 M	$ 17.91 M	2.1
Gal + CH + CAH + G6PD + PKU	$ 28.94 M	$ 40.36 M	$ 11.42 M	1.4

* computations in US dollars

The expenditure arm of the equation does not stop at that point. Long-term medical outcome costs must also be included. That is, despite early intervention, some galactosemia patients may still suffer from long-term sequelae in the form of developmental delay and cataracts, and 90% of surviving females experience ovarian failure which requires hormonal replacement. There are also costs associated with care giving since caring for a galactosemia child may result in expenses to the family in terms of medical visits, time away from work, etc. Additionally, activities such as food sorting itself can be a time consuming and costly process.

In the case of screening for galactosemia, it should be noted that the incidence of the disease may vary significantly from population to population. For example, the classical type of galactosemia has been found to be low among the Chinese. In Taiwan, it was found to be 1 in 419,286 (personal communication – Hsiao KJ). Additionally, the availability of clinical expertise varies in different countries. The problem with cost-benefit analyses in galactosemia screening is that there are few published reports. Costing reports that are published must make assumptions based on available data relating to incidence, sensitivity and specificity of tests, treatment accessibility, cost and compliance, the value of improved or saved lives and costs for lost productivity. All these considerations vary in different populations, making cost analyses difficult to compare.

In a recent US cost-utility analysis of many newborn screening strategies,[12] the cost-effectiveness of each component of a multi-test newborn screening programme was studied and one of the diseases emphasised was galactosemia. In this study, a detailed economical decision model was used, drawing on sources that included cohort studies, government reports, secondary analyses and others. Using this model, data were extracted to ascertain the probabilities of sequelae for each of the conditions screened, their quality-adjusted survival rates, estimated prevalence, costs for treatment, life expectancy as a result of disability and sensitivity and specificity of screening tests for individual conditions. In addition, the quality-adjusted life years (QALYs), discounted costs and incremental cost-effectiveness ratios were measured. This study found that the incremental cost for adding a screening condition to an ongoing filter paper newborn screening system was negative for all diseases except galactosemia and congenital adrenal hyperplasia. By definition, a negative incremental cost means that screening for these 2 conditions would not save money over not screening. For galactosemia screening, it was estimated that the cost was US$94,000 for each QALY.

Conclusions

It is apparent that many issues have to be considered in implementing a newborn screening programme for galactosemia. The tools and results of cost-effectiveness and cost-utility analyses are important considerations in this process, as well as the impact of societal costs and considerations that may vary among different countries. As a result, each healthcare system must evaluate its priorities according to its own calculations of expenditures, gains and other social factors.

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Neuro-developmental Deficits in Early-treated Congenital Hypothyroidism

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Abstract

This paper summarises the current evidence on neuro-developmental deficits in the early (< 1 month of age) treated congenital hypothyroid and the influencing factors. A literature search revealed only few citations that compared outcome with matched controls. In all but one, the median age of treatment onset was >2 weeks. Mean Global IQ scores are about 10 points lower and remain identifiable in adulthood. Verbal and performance scores are usually similar. Deficits persisting into adolescence and adulthood involve the visuomotor, memory, attention and posture domains. Lower academic performance is common in the early years. Prenatal factors associated with a worse prognosis are aetiology (dysgenesis), low birth weight, associated complications and severity of hypothyroidism. Postnatal factors are age at onset of treatment (>1 month), lower thyroxine dose at onset (<8 mcg/kg/day), late normalisation of thyroid function (>2 weeks after treatment), and a lower socio economic family status. The author proposes the evaluation of a multi centre cohort with a median age of treatment onset <1 week, TSH normalisation by <3 weeks with treatment thyroxine levels maintained in the 3rd quartile for age. The outcome of this cohort should indicate if current targets in management need to be revised.

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Background

About half a century ago, it was documented that severe neuro-developmental deficits were present in children with hypothyroidism started on treatment after 7 months of age.[1] An IQ above 90 was obtained in only 10 of 22 children treated before that age. Almost a decade and half later, it was reported that if treatment was started before 3 months of age, 85% had an IQ >85.[2] Newborn screening for congenital hypothyroidism was thus initiated about 35 years ago with the primary goal of preventing the severe deficits found in treated hypothyroids. The outcome of the earliest cohort of patients diagnosed by screening was reported by Quebec Network who found that none had an IQ <85 and that the mean IQ at 12 and 18 months of age were 113 and 105 respectively.[3] At 18 months of age the mean was significantly lower (105) than that of the controls (111).

Since then there has been a constant stream of publications documenting the practically normal development of hypothyroids detected by screening and treated within 1-3 months of age. In this group of babies, IQs though in the normal range are lower than the population mean; in addition specific learning disabilities have been reported. Data also suggest that epidemiological characteristics and treatment onset and goals do influence the outcome. This paper summarises the current evidence on developmental deficits in the early (<1 month of age) treated congenital hypothyroid and the influencing factors.

Methods

A literature search yielded many citations describing the outcome of babies treated at less than 1 month of age. The majority were descriptions of the short term outcome of small cohorts. Only 8 of these were described as clinical trials with a control arm of healthy children against which the outcomes were compared. Only 1 study was randomised. There was also only 1 among all the citations, that reported a median age of 2 weeks for treatment onset.

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Results

In the only RCT, 31 children from Oregon, USA with severe and moderate congenital hypothyroidism and 3 different initial thyroxine doses had their neuro-developmental outcomes compared.[4] The age at evaluation was wide, ranging from 2 to 8 years. Also studied was the effect of the time taken to normalise thyroid function. Higher (50 mcg) and lower (37.5 mcg) dose groups were separated by about 11 points on the full scale IQ. Verbal, performance and achievements scores however did not differ. Subjects taking longer than 2 weeks to normalise thyroid function had significantly lower cognitive, attention and achievement scores.

Connelly and associates from Victoria, Australia were among the earliest to report on the long term outcome of a large cohort with age matched controls.[5] Their cohort of about 152 cases had median age of onset of treatment of 14 days. At 2, 5 and 8 years, their mean composite scores were 8.5 to 10.2 points lower than the 60 age matched controls. Very low scores at the same ages were 10.1%, 3.9% and 6.8% respectively. A lower socio economic status and maternal education of less than 10 years in school were associated with about a 10 point lowering of verbal but not performance scores.

The outcome at adulthood of hypothyroids in the Netherlands has been documented.[6] Their median age at treatment onset was 28 days and they were divided into 3 severity groups based on the screening T4 value. In those with severe hypothyroidism, the full scale, Verbal and performance intelligence quotients were 91.3, 92.9 and 90.4 respectively. In those with moderate disease, the respective scores were 99.1, 97.8 and 101.3 respectively. The scores were 101.3, 101.8 and 100.4 respectively in those affected mildly. The severely, moderately and the mildly affected had from the Movement Assessment Battery for Children, motor scores of 9.8, 4.3, and 6.7 respectively. In the controls, it was 3.2. Their conclusion was that Global IQ and motor scores correlated with severity rather than the age of commencement of therapy.

Similar results were obtained from the 49 Norwegian cases of the 1979-81 cohort who were assessed at the mean age of 20 years and compared with 41 sibling controls of mean age 21 years.[7] The cases had lower scores than controls: Global IQ 102.4(13) and 111.4(13). Secondary schooling was not completed in 24% of cases and 6% of controls. Motor outcome was associated with severity of the hypothyroidism while verbal and arithmetic scores correlated with initial thyroxine dose and second year T4 levels and, arithmetic score with only second year T4 levels.

The subgroup of severe hypothyroids from the Dutch 1992-1993 cohort with median age of treatment at 20 days had at 10 years of age , significantly (P <0.05) lower scores (Full scale 93.7, Verbal 94.9 and Performance 93.9) than the normative population.[8] Moderate and mild hypothyroids and the normative population had similar scores. The 1981-1982 cohort with a median age of 28 days at the onset of treatment showed a similar outcome with that of the 1991-1992 cohort. Reducing the age of onset of treatment also appeared not to have a benefit in the severely affected hypothyroid.

The effect of initial and post initial treatment factors on outcome at 5 to 7 years was recently reported. The cohort comprised 45 Dutch patients (19 severe and 26 mild hypothyroids) and 37 controls. The global IQ was similar between the groups. However, the visual-motor scores and verbal scores were lower.[9] The TRF scores (teacher report form) were also higher. The authors concluded that suboptimal initial and post initial treatment would lead to abnormalities in IQ and in behavioural problems. They recommended that TSH concentrations be maintained within the normal range.

A Toronto cohort of 83 hypothyroids in grade 3 when compared to 80 classmates scored lower in reading, comprehension and arithmetic.[10] They, however, did not differ on word recognition, writing or spelling. By Grade 6 the differences were not present. However, cognitive problems in memory, attention and visual-spatial processing areas have persisted into adolescence. The impact of these deficits on future educational accomplishments needs further investigation. A subgroup of this cohort comprising 42 cases was compared at 6 to 9 years of age to their 42 unaffected siblings.[11] The cases scored lower by about 8-6 points on the McCarthy/WISC-R. Factors contributing to the effect were only aetiology (dysgenesis vs. dyshormonogenesis) and starting dose (above or below 8.2 mcg/kg/day).

Deficits in postural control have also been observed in those where TSH normalisation took more than 3 months.[12] In a Japanese cohort of 129 cases, the intellectual outcome was similar except in a subgroup of unfavourable cases (LBW, complications and a high TSH) whose scores were less than 100.[13] A French cohort of 131 cases from 1979-94 and 30 controls were studied to determine the importance of age of treatment versus the initial dose of thyroxine.[14] Treatment onset before 15 days produced global IQ scores of 119 (1.8). Below and beyond 3 weeks the scores were 117.1 (1.2) and 107.7 (2.4). Initial doses above and below 6 mcg/kg/day were associated with performance scores of 117.3 (1.8) and 112.8 (1.2).

Summary

Newborns treated early in infancy for hypothyroidism still appear to have mild cognitive and learning deficits.

The factors predicting deficits include those originating prenatally – aetiology (dysgenesis), low birth weight, associated complications and severity of hypothyroidism. The postnatal factors are age of treatment onset >1 month, low thyroxine dose at onset, late (>2 weeks), normalisation of the TSH/fT4 and lower socio economic strata.

It is now time to ascertain if this gap between the healthy child and the early treated hypothyroid can be narrowed further. This will require the establishment of a large multi national collaborative in which congenital hypothyroids are treated with a target of median age of treatment onset <1 week, TSH normalisation by <3 weeks and thyroxine levels maintained in the 3rd quartile. The outcome of this cohort should indicate if current treatment targets need to be revised.

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Eliminating Iodine Deficiency: Obstacles and Their Removal

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Abstract

Iodine deficiency remains a global concern for developing countries and some industrialised countries. Iodine deficiency is the most common cause of preventable mental retardation, posing a threat to the social and economic development of countries. Initiatives were developed and instituted to accelerate progress to achieve the goal of universal salt iodisation (USI). However, these efforts were not successful in eliminating iodine deficiency disorders (IDD) in some countries. Every year, 50 million children are born without the protection that iodine offers to the growing brain and body and about 18 million suffer some significant degree of mental impairment. The World Health Organization (WHO), United Nations Children's Fund (UNICEF) and non-governmental organisations assist to ensure that populations at risk have access to iodised salt. This paper will review the highlights of iodine deficiency and present the experiences in the various countries in Asia, i.e. assessments of the situation, action plans, and obstacles to implementation.

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Key words: Iodine deficiency, Iodised salt coverage, Mean urinary iodine excretion (UIE), Supplementation, Total goitre prevalence (TGP), Universal salt iodisation (USI)

Introduction

Iodine is an essential nutrient for normal body function and the thyroid gland, which is essential for normal growth and development. Deficiency of this nutrient leads to development of thyroid gland enlargement (goitre) and iodine deficiency disorders (IDD). The most serious effect is mental retardation with a loss of 13.5 IQ points. The primary cause of iodine deficiency is a low dietary supply of iodine aggravated by intake of natural goitrogens in staple foods like cassava, and exacerbated by deficiencies of selenium, iron, and vitamin A.[1]

Urinary iodine excretion (UIE) is the most appropriate outcome indicator for iodine deficiency under field conditions at the district level.[2] Severity of iodine deficiency can be assessed using mean UIE (μg/L) based on the set criteria by the WHO/UNICEF/ International Council for the Control of Iodine Deficiency Disorders (ICCIDD): mean UIE >100 (no IDD), 50 to 99 (mild IDD), 20 to 49 (moderate IDD), <20 (severe IDD).[3]

Strategies

The following strategies have been used to prevent and control iodine deficiency:

(i) USI and (ii) supplementation with iodised oil.

Universal Salt Iodisation (USI). USI is the iodisation of all salt for human (food industry and household) and livestock consumption. It is safe, effective, and cost beneficial. Other fortification vehicles that can be used are bread, water, milk, and complementary food. In some regions where iodisation of salt may not be feasible, particularly in remote areas, supplementation with iodised oil is a good alternative. Another way is by giving potassium iodide (KI) or potassium iodate (KI03) as drops or tablets in drinking water.[1] Global progress towards USI, which was rapid during the 1990s, has slowed down over the past decade. The latest survey showed that only 70% of households worldwide, only 54% in South Asia, and 85% in the East Asia and the Pacific have access to iodised salt. These findings argue for renewed efforts to reach the remaining one-third of the global population not covered by iodised salt.[4]

Supplementation. A single dose of iodised vegetable oil delivers 200 to 480 mg of iodine that provides sufficient coverage for 6 to 12 months (orally) or longer (intramuscular administration).[1] Table 1 shows the recommendation of the WHO for the daily and annual

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Table 1. WHO-recommended Dosages of Daily and Annual Iodine Supplementation[5]

Population group	Daily dose of iodine supplement (μg/day)	Single annual dose of iodised oil supplement (mg/year)
Pregnant women	250	400
Lactating women	250	400
Women of reproductive age (15-49 years)	150	400
Children< 2 years [a,b]	90	200

[a] For children 0 to 6 months of age, iodine supplementation should be given through breast milk. This implies that the child is exclusively breastfed and that the lactating mother received iodine supplementation as indicated above.

[b] These figures for iodine supplements are given in situations where complementary food fortified with iodine is not available, in which case iodine supplementation is required for children 7 to 24 months of age.

dosages for iodine supplementation for different age groups.[5]

WHO, in collaboration with UNICEF and the ICCIDD, helps raise awareness of the importance of IDD; ensures scientific consensus and information on standards for levels of salt iodisation, the safety of iodised salt in pregnancy, and indicators for monitoring and evaluation; and provides technical and financial support for many steps of the process. Other international agencies involved are the World Food Program (WFP), Micronutrient Initiative (MI), Kiwanis International, United States Agency for International Development (USAID), Canadian International Development Agency (CIDA), Australian Government Overseas Aid Program (AUSAID), and the Gates Foundation.[1]

Current Situation

Table 2 shows the current situation in countries in Asia. In Australia, there has been a reduction in the mean UIE due to replacement of iodine-containing sanitizers with chlorine-containing sanitizers in the dairy industry and non-use of iodised salt in the preparation and manufacture of food by manufacturers.[6]

In Bangladesh, legislation contributed to the reduction in the total goitre prevalence (TGP); however, the following problems continue to exist: (i) illegal smuggling of relatively cheap non-iodised salt from India and Myanmar; (ii) poor quality salt production with substantial amount of mud and other insolvable compounds; (iii) lack of microcredit / loans for farmers to purchase polyethylene sheets used to separate crude salt from mud and other impurities; (iv) small domestic salt factories are not registered/licensed and have not complied with salt iodisation; (v) handicapped law enforcement; and (vi) significant portion of households do not perceive importance of iodised salt.[7]

The reduction in iodised salt coverage in India is due to (i) insufficient availability of adequately iodised salt, especially in the rural areas; (ii) low awareness; and (iii) poor enforcement of legislation against the sale of non-iodised salt.[7]

The problems in Indonesia are due to (i) a diverse cultural pattern that dictates various preferences for types of food and salt; (ii) inadequate public awareness; (iii) existence of local salt of differing quality produced by poor farmers; (iv) inadequate and high cost of iodised salt; (v) limited or absent supply of potassium iodate (KIO3); (vi) inadequate monitoring of iodine content in salt; and (vii) poor enforcement of regulations mandating iodisation of salt.[7]

The increase of goitre cases in New Zealand is attributed to (i) the decline in the use of table salt in response to public health recommendation to decrease sodium intake; (ii) replacement of iodophors by less expensive detergent-based sanitizers in the dairy industry; (iii) little awareness among the public; and (iv) absence of legislation.[8]

In Pakistan, the high incidence of goitre is due to (i) absence of legislation; (ii) low enforcement of provincial regulations; (iii) negative propaganda; (iv) availability of non-iodised salt in the market; (v) poor monitoring of consumption of iodised salt; (vi) lack of interest and motivation among salt producers due to low demand and cost implications; (vii) USI programme and IDD control not integrated within the health service delivery; and (viii) lack of awareness and demand for iodised salt.[7]

The increase in goitre cases in the Philippines has been attributed to (i) inadequate capacity of the Department of Health programme managers in monitoring and quality controls; (ii) poor monitoring and enforcement of the law; (iii) limited financial resources for small scale salt producers; and (iv) lack of awareness of households on the health benefits of iodised salt.[7]

Despite complaints of the lack of political will, China was successful in reducing the incidence of goitre and increasing the UIE.[9]

Challenges

Countries can achieve and maintain optimal iodine

Table 2. Current Situation in the Asia Pacific Region in Terms of Total Goitre Prevalence (TGP), Mean Urinary Iodine Excretion (UIE), Iodised Salt Coverage and Policies

Country	TGP (%)	Mean UIE (μg/L)	Iodised Salt Coverage (%)	Legislation
Australia	–	>200 (1992)[10] 104 (2003-2004)[6]	10 (2001)[11]	–
Bangladesh	47.1 (1993)[7] 17.8 (1999)[7]	123 (1999)[7]	70 (2003)[7]	1989 – Mandatory iodisation of edible salt[7] 1994 – Salt Bi-Law[7]
China	20.4 (1995)[9] 5 (2005)[9]	164 (1995)[9] 246 (2005)[9]	39.9 (1995)[99] 94.9 (2005)	–
India	17.9 (1960-1990)[7]	85.5-123.3 (2001-2002)[7]	50 (1998-1999) 36.7 (2002-2003)[7]	1997 – Nationwide ban on storage and sale of common salt for human consumption[7] 2000 – lift of ban except in 2 districts[7] 2005 – re-instatement of ban[7]
Indonesia	29 (1982)[7] 11 (2003)[7]	229 (2003)[7]	58 (1996)[7] 73.2 (2003)[7]	Kepres 69/1994 (Presidential Decree)[7]
New Zealand	1 (1953)[12] 30 (2003)[12]	60-76 (1992-1993)[12] 67 (2002)[12]	83 (2000-2006)[13]	–
Pakistan	84.9 (1993)[7]	16 (1993)[7]	17 (1995)[7]	–
Philippines	3.5 (1987)[14] 6.7 (1993)[14]	71 (1998)[14] 201 (2003)[7]	9.7 (1998)[14] 56.4 (2003)[7]	1987 - RA 3720 (Cosmetics Act)[7] 1995 - RA 8172 (Asin Law)[7] 2000 – RA 8976 (Philippine Food Fortification Law)[7]

nutrition by ensuring adequate availability of iodised salt to meet total consumer requirements, by monitoring iodine content at production and consumer level, by increasing awareness about the effects of iodine deficiency, and by increasing public demand for iodised salt.[1] Where salt iodisation has yet to occur, the main challenges are: (i) political commitment; (ii) salt iodisation legislation; (iii) iodisation of salt licks for animals; (iv) iodisation of processed food; (v) effective enforcement and monitoring; (vi) small scale producers are not supported and organized to ensure that their products are iodised; and (vii) consumer awareness and demand for iodised salt.[15]

Pregnant and lactating women and infants are the most susceptible groups to IDD. However, most of the surveys are done on children or general adult population because monitoring of iodine status of the susceptible groups is difficult since there are no established reference criteria for UIE. Optimal indicators, the mean UIE and the correct timing when to screen, are yet to be identified. Approaches to salt fortification and supplementation using iodised oil are different and bioavailability of iodine in iodised oils and optimum dose are yet to be determined. Iodised salt may not provide enough iodine to meet a child's needs during complementary feeding, therefore, research must be done.[1]

The key to a successful national programme towards USI is constant monitoring of iodine content in household and retail salt. To ensure easy monitoring there is a need to develop rapid test kits (RTK).[1]

The push from WHO for a reduction in population salt intake to reduce the risk for hypertension is another factor that contribute to poor iodised salt intake. It is suggested that other vehicles other than salt must be used. There is also a low consumer demand for iodised salt particularly among lower income consumers. There is a need for effective health promotion to ensure use of iodised salt and need for effective strategies to empower consumer organisations to work with government and industry to ensure quality assurance and monitoring of USI.[1]

The Wheel Model for IDD Elimination Programme

This model represents the continuous "feed back" process involved in the national IDD programme. A continuous series of steps are required to achieve success: (i) assessment of the IDD problem; (ii) communication of the problem to the public and the politicians in terms they can understand; (iii) development of a plan including the salt industry, the education system, the media, the public health professionals, and the consumers; (iv) political decision, which includes the allocation of the necessary funds from the government resources within the country supplemented by external funds from bilateral and/or multilateral sources; (v) implementation, including the organization of the supply of iodised salt, iodised oil (if necessary), appropriate training, and education programmes; and (vi) monitoring and evaluation of the programme which requires process indicators on delivery of iodised salt, checking its iodine content in the factory, on arrival at the destination and in the

home and determination of iodine in the population by measurement of urine iodine.[16]

The final responsibility for the national programme rests on national governments and the role of the international agencies is to work with national governments to assist them with appropriate advice, funding, and training.[16]

There is a need for further research on: (i) iodine nutrition in pregnancy and infancy; (ii) adverse effects on cognitive development; (iii) safe upper limit of iodine intake; (iv) laboratory methods for monitoring salt iodine content; and (v) metabolic interactions of iodine and other micronutrient deficiencies.[1]

Specific issues include: (i) optimal indicators to assess iodine nutrition during pregnancy, lactation, and infancy need to be identified; (ii) use of newborn serum thyroid stimulating hormone (TSH) concentration as an indicator of iodine status in pregnancy needs further validation; (iii) effects of mild to moderate IDD during pregnancy on the cognition of the offspring; (iv) validation of evidence from China's IDD control programme suggesting that a median UI of 240 μg/L is associated with an increased prevalence of subclinical hypothyroidism; (v) large scale trials looking at the correlation between community iodine intake and autoimmune thyroid disease or papillary thyroid cancer are needed; (vi) rapid test kits (RTK) for iodine in salt need to be improved; and (vii) the effects of Vitamin A, iron, zinc and selenium deficiencies in the setting of iodine deficiency should be investigated.[1]

Conclusions

Iodine deficiency is still a problem in the Asia Pacific as shown with the high prevalence in the region. The eradication of iodine deficiency must be a national goal and countries must not remain complacent with decreasing goitre percentage or increasing UIE. There must be continuous evaluation of all problems including iodine supplementation for pregnant women. Other vehicles for iodine besides salt (e.g water and rice) must be seriously studied. Rapid tests that can measure quantitatively the iodine levels are critical particularly for remote areas. Lastly, there must be a single method for detecting iodine levels so that global comparisons can be more accurate.

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Outcome of Early Cochlear Implantation

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Abstract

Introduction: Universal newborn hearing screening facilitates early detection of congenital hearing loss. A child found to have severe to profound hearing loss may require a cochlear implant to access sounds in the speech frequency range. Materials and Methods: This retrospective study compared the speech perception outcomes of children implanted at 2 years and below (C1) with those implanted later (C3). Baseline and post-implant speech perception scores were recorded using IT-MAIS, TACL-R or PPVT. The percentage of improvement was calculated for each group and statistical significance was determined using the Student's *t*-test. Results: The median follow-up period for C1 (n = 29) and C3 (n = 29) was 29 months (range, 6 to 29 months) and 20 months (range, 5 to 32 months) respectively, which was not statistically significant. Although both groups recorded post-implant improvement of speech reception scores, the difference in the degrees of improvement was statistically significant (*P* = 0.034). Conclusion: More rapid development of speech perceptive skills was achieved in children who were implanted early. Early implantation therefore, enables children to develop good core listening skills and to potentially develop spoken language at a young age. This enhances successful integration into mainstream pre-schools which provide the environment for the early nurturing of social and cognitive skills.

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Key words: Cochlea, Cochlear implant, Deafness, Hair cells, Hearing loss

Introduction

In Singapore, about 4 in 1000 babies are born with hearing loss.[1] Childhood hearing screening in Singapore was traditionally conducted at the primary healthcare level when the child was due for vaccination and subjective free-field methods of screening were generally used. This resulted in congenital deafness being detected and intervened relatively late, at a mean age of 20.8 months (range, 0 to 86) and 42.2 months (range, 1 to 120) respectively.[2] Since 2001, a national universal newborn hearing screening (UNHS) programme has been implemented, providing the opportunity for early detection and intervention of congenital hearing loss. At about the same time, the Listen and Talk Programme was launched at the Singapore General Hospital.[1] This Programme provided cochlear implants for selected children with severe to profound hearing loss, and offered a hospital-based auditory-verbal therapy habilitation service. This paper studied children managed by the Programme, comparing those who had received cochlear implants early with those who were implanted later.

Materials and Methods

Our database on children who had received cochlear implants was reviewed and the speech receptive scores were analysed. The children studied were categorised as early implantees (C1) if they were aged 2 years and below or late implantees (C3) if they were above this age. Children lacking in family support and motivation were excluded.

In our Centre, the post-implant audiological and auditory-verbal interventions received by the 2 groups are similar. Depending on the age of the child, the appropriate testing tool was used to assess speech reception ability for each child. The IT-MAIS (*Infant Toddler Meaningful Auditory Integration Scale*), TACL-R (*Test for Auditory Comprehension of Language Revised Edition*) and PPVT (*Peabody Picture Vocabulary Test 3rd Edition*) were used and the tests were administered by Audiologists or Auditory-Verbal Therapists. The results of all the 3 tests were converted to age-equivalent percentage scores. As far as possible, each test was administered by the same assessor to minimise inter-rater variation. Final assessments were made only after a stable MAP had been achieved.

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The base-line and final post-implant percentage scores for each child were obtained and the percentage of improvement was determined. Statistical significance was determined using the Student's t-test.

Results

Children in C1 (n = 29) and C3 (n = 29) have median ages of 19 months (range, 13 to 24 months) and 57 months (range, 29 to 165 months) respectively. The final assessment was done at a median of 29 months(range, 6 to 29 months) and 20 months (range, 5 to 32 months) post-implant for C1 and C3 respectively, which was not statistically significant. The sex, race, implant model and processing strategies used were also comparable between the 2 groups (Table 1). Although both groups recorded an improvement of speech reception scores after implantation, early implantees experienced a statistically significantly higher rate of post-implantation speech reception improvement compared to late implantees (P = 0.034) (Fig. 1).

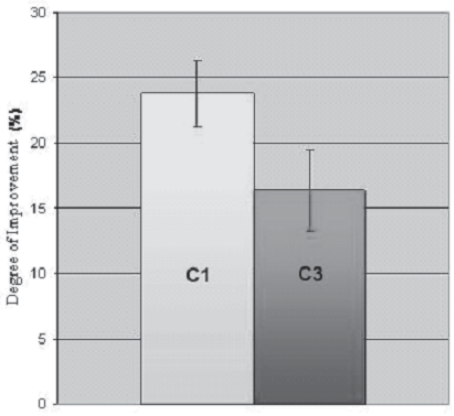

Fig. 1. Chart comparing the mean percentage improvement of post-implant speech perception scores in early (C1) and late (C3) implantees.

Discussion

UNHS provides an opportunity for early detection and intervention for congenital hearing loss. Children with significant cochlear hair cell loss may have hearing loss in the speech frequencies that could not be adequately amplified by hearing aids. These children are potential candidates for cochlear implants which by stimulating the auditory neural elements directly, allows early auditory access to the whole speech frequency range. It is well established that auditory stimulation is a prerequisite for appropriate auditory development.[3] Providing a child with an implant at an early age decreases the effects that auditory deprivation can have on the development of speech and language skills and maximises the amount of auditory information available to the child during this critical period.[4] The argument for early implantation has been supported by the results of animal studies. Hsu et al[5] found that electrical stimulation of the inner ear was more effective in younger rats than in older rats in eliciting gene expression associated with development of a functional network in the auditory pathways. Leake et al[6] observed that neuronal survival in the spiral ganglion was enhanced when electrical stimulation was applied shortly after deafening and that spiral ganglion cell size was larger in stimulated than in non-stimulated ears.

Table 1. The Number (percentage) of Subjects for Each Characteristic in the Young (C1) and Older (C3) Implantee Groups

Group	C1	C3
Sex		
Male	17 (59%)	14(48%)
Female	12 (41%)	15(52%)
Race		
Chinese	22(77%)	24(83%)
Malay	3(9%)	2(7%)
Indian	2(7%)	2(7%)
Others	2(7%)	1(3%)
Model of implant		
Nucleus freedom	27(93%)	25(86%)
Nucleus CI24RCS	2(7%)	4(14%)
Processing strategy		
ACE	28(97%)	28(97%)
SPEAK	1(3%)	1(3%)

The present study demonstrated an improvement in speech perception scores in both young and older aged groups, although a statistically significantly higher rate of post-implantation improvement was found in the younger group. This was consistent with the observations made by Manrique et al,[7] who reported that a higher level of performance was attained at a faster rate by children implanted early compared to those who were implanted later.

An area of continuous interest is whether the advantages of earlier implantation will be maintained over a relatively long time course. It is possible that the children who receive an implant later may eventually catch up to this group in terms of language skills. At least one previous study found that children implanted between 2 and 4 years of age did not differ among themselves in language performance measured at ages 8 and 9 years.[8] Nevertheless, a distinct advantage for earlier implantation is that these children are able to achieve good core listening skills at a younger age, providing the a foundation of skills for these children to be fully and satisfactorily integrated into an oral social environment.[9] With the potential of achieving spoken language competency

early, it facilitates successful integration into mainstream pre-schools which provides the environment for the nurturing of social and cognitive skills.[7]

With UNHS, it becomes feasible to diagnose congenital hearing loss by 3 months and start intervention by 6 months of age, which are the recommendations of the American Academy of Pediatric Joint Committee on Infant Hearing.[10] Intervention should include hearing amplification with the use of appropriate hearing aids, although the amount of benefit depends on the degree of existing residual hearing. In children who did not receive significant hearing amplification from hearing aids, Nicholas & Geer[11] found that the amount of pre-implant intervention with a hearing aid did not affect spoken language outcomes at the age of 3.5 years. Rather, it was cochlear implantation at a younger age that served to reduce the gap between a deaf child's chronological age and his or her language level. Therefore, early cochlear implantation should be considered for children who need them. What then, is the optimal age for early implantation?

The US Food and Drugs Administration approves the use of cochlear implant only in children aged 12 months of age and above. In recent years, emphasis on the importance of early implantation has lead to growing interest in implanting children below the age of 12 months.[12,13] It is however, cautioned that cochlear implantation in young infants may not necessarily be the best practice.[14,15] Pre-operatively, besides a higher possibility of misdiagnosing the degree of hearing loss, maturation of the central pathways within the first few months of life may unexpectedly improve the patient's hearing performance.[11,14] Intra-operatively, the higher surgical and anaesthetic risks encountered in surgeries on infants are well known, although these risks can potentially be lessened by availability of relevant expertise.[13] Post-operatively, the auditory performance of implanted young infants can be difficult to reliably assess, although the use of intra-operative NRT nowadays has alleviated this problem to some extent.[14] More importantly, the benefits of cochlear implantation in children less than 12 months of age compared to those aged say between 12 to 18 months, are still unclear. Speech perception results as reported by Holt et al[15] found no advantage for these children compared to those implanted at 13 to 24 months of age. Therefore, it has been rightly pointed out that before advocating cochlear implantation in children less than 12 months of age as standard practice, the expected benefits derived from such practice should be further studied.[11,14,15] Nevertheless, a notable exception is in post-meningitic deafened infants with signs of cochlear obliteration. In these children, very early implantation can enhance successful insertion of the implant electrode with better outcomes.

Conclusion

UNHS provides the opportunity for early detection and intervention of congenital hearing loss. In severe to profound deafness, children who received their cochlear implants at the age of 2 years or younger experienced faster rate of improvement compared to those implanted later. Although it may be possible that children who receive an implant later may eventually catch up with this group in terms of language skills, a definite advantage for earlier implantation is that these children are able to potentially achieve good core listening skills and develop spoken language at a much younger age. This facilitates successful integration into mainstream pre-schools which provide the environment for the early nurturing of social and cognitive skills.

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Impact of the National Hearing Screening Programme in China

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Abstract

China has a large population with different levels of medical care among the eastern, central and western areas. The national universal newborn hearing screening (UNHS) programme was initiated in 1999 and then progressively implemented nationwide. A "National UNHS Experts Group" was set up, formulating the national UNHS administration rules and technological specifications. 3 March had been named as national "ear-care day" since 2000 and such social activities help make deafness prevention work more widely accepted. UNHS in China presently has 3 phases due to disparities in economic development. 1) Implementation in stages: in economically under-developed areas. 2) Implementation completed: in the coastal cities. 3) Beyond basic UNHS: i) Development of a completed UNHS system including follow-up and quality control based on the neonatal disease screening system, ii) Exploration of a new public health care programme: simultaneous screening of newborn hearing and ocular disease, iii) Carrying out of a multi-centre study on high-risk factors and GJB2 gene mutations in newborn with non-syndromic hearing impairment. The incidence of newborn bilateral hearing loss was 2.22 per 1000, and 2.74 per 1000 for unilateral hearing loss. Though UNHS have been carried out widely in the eastern parts of China, there are difficulties for its implementation in the western regions. Economic development and technical expertise are the main restricting factors.

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Key words: Hearing, Newborn, Screening

Introduction

China has a large population, with great differences in economic development and levels of medical care within the eastern, central and western areas. There are 1.3 billion people or 25% of the world's population, of whom almost 40% are urban and 60% rural. There are about 16 million babies born every year, with 88.4% of babies being delivered in hospitals in 2006.[1] The eastern, central and western areas respectively contributed 72.5%, 16.8% and 10.7% to industry revenue.[2] The hearing impaired population was 20.57 million in 2007. The number of children with hearing impairment increased by 20,000 to 30,000 per year.[3] The incidence of newborn bilateral hearing loss was 2.22 per 1000, and 2.74 per 1000 for unilateral hearing loss.[4]

Development of UNHS Programme in China

i) Promotion of Newborn Hearing Loss (NHL) Services as National Policy

Neonatal hearing screening began in 1999. Demanded as a routine service in the national maternal and child health care system in 2001, the government issued a new management guideline in 2003, organised a "National UNHS Experts Group", compiled the national unified training book and formulated the national neonatal hearing screening administration rules and technological specifications in 2004. March 3rd has been named as national "ear-care day" since 2000. Local health service organisations are encouraged to offer service models and framework tailored to different local conditions. NHS programmes in rural areas were also emphasised at the same time.

ii) UNHS Technology and Clinical Programmes

The national UNHS programme was established according to the situation in China and the Year 2000 Position Statement by the Joint Committee on Infant Hearing (JCIH) included 2 aspects: i) hospital-based UNHS,[4] ii) community and children healthcare system-based inspection programme (0 to 6 years).[6]

iii) UNHS Technologies

OAE and AABR are common technologies for UNHS

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which can be combined in different ways. Screening protocols in China are as follows: (i) two-stage OAE/ AABR programme: OAE is used first, and if the baby fails , AABR was used before discharge; (ii) AABR screening alone; (iii) OAE is used in the well-baby nurseries alone, and both OAE and AABR are used in neonatal intensive care units (NICU). The third is the most frequently used protocol.[4,6]

iv) Diagnosis, Follow-up and Intervention after Screening

This is the protocol created for early hearing loss intervention and treatment

- Severe to profound sensorineural hearing loss (SNHL): Fit hearing aids at 4 months. Suggest a cochlear implant at 1 year of age if necessary.
- Moderate SNHL: Fit hearing aids at 6months of age.
- Mild and some moderate SNHL: Follow-up till 8 to 10 months of age, fit hearing aids if permanent SNHL was diagnosed. Family-based rehabilitation: speaking in a louder voice.
- Conducted HL: follow-up, medical or surgical treatment if necessary.

Continued Surveillance for Children

The follow-up rate for hearing impaired children is a hot topic in many countries.[4-7] The follow-up rate was about 40% to 80% in eastern coastal cities and 30% to 50% in western cities.[4] Follow-up centres were set up in many hospitals as part of the UNHS programmes.

Impact of the National Hearing Programme

There are 3 stages in the national UNHS programme due to different local situations.

First stage: hospital-based implementation in mid-western underdeveloped areas. OAE or AABR was used for screening in obstetric wards, with diagnosis in the ENT department. Initiation of UNHS programme is easy in this stage, but the follow-up rate is low and screening in NICU is difficult.

Second stage: completed implementation of integrated programmes. The UNHS programme was run with the support of the national maternal and childcare systems. This system included 3 grades: province, district and county. From the upper to the basic level, technological support was provided gradually. Generally there is 1 diagnostic and rehabilitation centre in each city, which is also charged with staff training and quality control. The health bureau of the city coordinated the UNHS programme. Screening was conducted in different hospitals, diagnosis made in approved centres, and rehabilitation carried out in appointed organisations.

Third stage: beyond newborn hearing screening

- Periodic hearing screenings for 0 to 6 year old children: In Beijing, there are 54 hospitals and 237 community service centres which carry out UNHS programmes (2007), and the coverage rate was 63.2%.[8]
- Hearing screening synchronised with newborn screening programme: Developing follow-up and quality controls in conjunction with newborn disease screening programmes. Hearing screening can be conducted by newborn disease screening staff.
- Simultaneous screening programmes for hearing and ocular diseases:[9] The UNHS was simultaneously conducted with newborn ocular disease screening programme. Well newborns were screened 2 to 7 days after birth (this included testing reaction to light, red reflex and external ocular examination). Those with abnormalities were subjected to a diagnostic examination (external ocular examination with a hand-held slit-lamp, red reflex and mydriatic examination). Newborns in NICU were subjected to screening 5 to 14 days after birth and they, together with those with high risk factors, received a comprehensive examination for screening and diagnostic purpose. In 15,398 cases who underwent simultaneous screening, the incidence was 3.11 per 1000 (48/15,398) for bilateral congenital SNHL and 2.27 per 1000 (35/15,398) for unilateral SNHL. Four cases of congenital SNHL were complicated with newborn eye diseases, including 1 case of profound SNHL (bilateral) and auditory neuropathy accompanied by congenital cataract (bilateral), 1 case of mild SNHL (bilateral) complicated with membrana pupillaris perseverans (left), 1 case of mild SNHL (bilateral) with retina vein dilatation (bilateral), and 1 case of mild SNHL (right) with accompanying bilateral persistent hyaloid artery. In all the 15,398 newborns, 12 different eye diseases were detected (involving 1266 cases) with a prevalence of 82.2 per 1000 newborns.
- A case-control multi-centre study on high-risk factors for NHL in 7 cities of Shandong Province.[10] Three hundred and thirty one cases (including 177 cases of bilateral HL and 154 cases of unilateral HL) that fully satisfied the matching requirements were included as subjects. Among 39 factors studied, bilateral NHL had 3 independent risk factors: parity (OR = 16.29), neonatal diseases (OR = 34.97), family history of congenital hearing loss (OR = 69.49) and 1 protective factor, birth weight (OR = 0.24). Unilateral NHL had 2 independent risk factors: parity (OR = 9.789) and a family history of congenital hearing loss (OR = 4.234).
- Mutations of GJB2 gene in infants with non-syndromic hearing impairment.[11] In 20 infants with severe to profound bilateral SNHL confirmed by UNHS, PCR and sequencing technique were used to analyse the

coding region of the GJB2 gene. Three infants (15%) were identified as 235delC 235delC homozygotes, 1 infant was identified as 235delC 299-300delAT compound heterozygote, 1 as 235delC heterozygote, 1 as 235delC 605ins46 compound heterozygote with 605ins46 mutation. 605ins46 is a novel mutation reported in Chinese for the first time.

Challenge and Opportunity

i) Interdisciplinary collaboration needs to be improved: The UNHS programme has 3 phases: screening, diagnosis and long-term hearing rehabilitation which involve different academic branches and different administrative areas. The development of these 3 phases was not balanced among different areas.

ii) Specialty education and professional training need to be reinforced. At present, audiology clinics are commonly conducted in ENT departments. Speech pathology clinics are common conducted in paediatric departments or rehabilitation centres.

iii) The National UNHS information system need to be further improved.

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Early Intervention For Hearing Impairment: Appropriate, Accessible and Affordable

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Abstract

Before the onset of universal newborn hearing screening, children with hearing loss are not identified until they fail to meet important speech and language milestones at 2 years old and beyond. With the current widespread implementation of universal newborn hearing screening programmes, more infants with hearing loss can now be identified in the first few weeks of life and be fitted with amplification within the first few months. This presentation aims to discuss the adverse effects of hearing loss in a child's development. More importantly, it will highlight the value of early identification and early intervention and how these can maximise a child's healthy development of speech, language, academic, emotional and psychosocial skills, thereby facilitating his/her successful integration into mainstream society. In Singapore, universal newborn hearing screening is in place in major hospitals and polyclinics with childbirth services, making it accessible to all families with newborn babies. There are also a number of early intervention programmes that provide rehabilitation services focusing on the development of communication skills of children with hearing impairment. With the availability of services and abundant government support, any child with hearing loss should be identified as soon as possible and provided with early, appropriate intervention. A hearing impairment is said to be an "invisible disability," yet it is the most common major birth defect. In Singapore, one in 1000 babies are born with severe to profound hearing loss and about 5 in 1000 with lesser degrees of hearing loss. Several surveys indicate that between 1 and 3 percent of all children suffer from hearing loss.

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Key words: Early intervention, Hearing impairment

The Importance of Early Detection and Intervention

Universal newborn hearing screening (UNHS) is defined as the use of an objective measurement of the auditory system to identify infants at risk for hearing loss. Before the advent of UNHS programmes, children with hearing loss were not identified until they failed to meet important speech and language milestones after 2 years of age, with many children who were deaf or hard of hearing not being identified until they entered school at 5 or 6 years old. Studies have demonstrated that when hearing loss of any degree including mild bilateral or unilateral hearing loss is not adequately diagnosed and addressed, the hearing loss can adversely affect the speech, language, academic, emotional and psychosocial development of young children.

But when early identification and intervention occur, hearing-impaired children make dramatic progress, are more successful in school and become more productive members of society. The earlier intervention and rehabilitation begin, the more dramatic the benefits.[1] According to Flexer, early identification and intervention is critical because the neuroplasticity of the brain is greatest in the first three and half years of life and so the younger the infant, the greater the neuroplasticity.[2] Rapid infant growth requires prompt intervention, typically including amplification or cochlear implants, and a programme to promote auditory skill development. The child is able to develop his/her auditory, speech and language skills during the linguistically formative years along with his/her normally hearing peers. Therefore, the identification of newborn hearing loss should be considered a neurodevelopmental emergency.

Impact of Early Intervention

In a study by Yoshinaga-Itano, children whose hearing loss were identified by 6 months of age and who received intervention shortly after were found to have better language quotients than those whose hearing losses were identified after 6 months of age.[3] In another study, it was found that early identification of hearing loss is not only related to better language development, but also related to better personal-social development, better self-description and

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self evaluation.[4] Better language development is also related to higher mastery and motivation of the child.

The developmental outcomes of children with hearing loss born in Colorado hospitals with and without UNHS programme were compared by Yoshinaga-Itano et al and they found out that children in the screened group had better receptive and expressive language quotients, more different consonants in the spontaneous phonetic repertoire, better speech intelligibility, and larger expressive vocabulary inventories.[5] These findings are encouraging and suggest that early identification and subsequent intervention is associated with improved language development in deaf and hard of hearing children. Hence, best standard practice dictates that all infants with hearing loss be identified by 3 months of age and receive early intervention by 6 months. UNHS would be an excellent vehicle for attaining this goal.

Early Hearing Detection and Intervention In Singapore

The birthing facility is the most efficient and cost-effective environment for newborn hearing screening. The infant is readily available and qualified personnel are available to provide screening. The purpose of UNHS is to provide early hearing detection and intervention to infants in an attempt to minimise speech and language delays. This is the first in the early hearing detection and intervention process; other important steps are audiological evaluation to confirm hearing loss and early intervention services.

In Singapore, UNHS has been established in all hospitals with delivery facilities for the past few years. Data gathered from the Singapore General Hospital, National University Hospital and KK Women's and Children's Hospital for the period 1 April 2002 to 31 March 2004 gave an overall prevalence of 4 in 1000 babies having hearing loss, with 64 being severe or profound. The median age of diagnosis was 2.7 months.[6]

Intervention Services in Singapore

There are a number of early intervention services in Singapore which accommodate infants and very young children with newly diagnosed hearing loss. These services offer 3 different types of communication approaches.

1. Auditory-Verbal Therapy (AVT)

Auditory-Verbal Therapy is an approach that utilises special techniques and strategies to enable children with hearing impairment to develop spoken language primarily through listening. With an emphasis on early detection of hearing loss, early fitting of hearing aids or cochlear implants, ongoing diagnostic therapy and a partnership between the family and professionals, AVT can provide opportunities for any children with hearing loss to process spoken language and to talk. The goal is for the child to function independently in as normal a learning environment as possible. Mainstream schools are usually recommended for the hearing-impaired child's education and socialisation.

2. Natural Auditory Oral (NAO) Approach

The NAO follows the pattern of language learning of children with normal hearing and lays heavy emphasis on the development of a child's listening skills through appropriate amplification. This results in talking children who become linguistically independent adults.

3. Total Communication (TC) Approach

The TC approach is a philosophy involving the collaboration of oral, manual and auditory modes of communication which include speech, speech reading, sign system, gestures, reading, writing, visual symbols and effective use of residual hearing.

Although these are different communication approaches, all have the same goal of maximising a child's hearing potential, developing communication skills, and integrating him/her successfully into the mainstream as a productive member of society.

Affordability of Early Hearing and Detection and Intervention

A nominal fee is charged for every UNHS test. For early intervention programmes, subsidies are available in government as well as non-government institutions and voluntary welfare organisations. Whatever amount is spent for the early detection and intervention of children with hearing loss is a small price to pay compared to the costs to parents, guardians, school systems and society for those children who go undiagnosed and untreated who often leave school with language levels too low to fare well in society. Because of this, many cannot find employment and end up receiving disability support.[7]

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A Multidisciplinary Approach to Paediatric Hearing Loss: Programme at the Centre for Hearing Intervention and Language Development, National University Hospital, Singapore

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Abstract

The objective is to describe the multidisciplinary management programme at the National University Hospital (NUH) in Singapore for children with hearing impairment (HI). Over 99.95% of babies born at NUH have hearing tested with both otoacoustic emission and automated auditory brainstem response tests by 6 weeks of age. The referral rate to Otolaryngology is 0.5%. Acquired causes of congenital HI are decreasing. Thirty percent of patients at NUH with idiopathic congenital sensorineural HI have DFNB1/ GJB6 Connexin 26 HI. CT scan or MRI imaging has a higher diagnostic yield when there is unilateral, fluctuating or non-Connexin 26 related HI. Routine electrocardiogram and Opthalmology evaluations will exclude associations of fatal cardiac rhythm anomaly and retinopathy. Other investigations are directed by history and clinical examination. There is now a very wide range of increasingly sophisticated medication, neuro-otologic external, middle and inner ear surgery, hearing aids, middle ear implants and cochlear implants available to improve hearing. A multidisciplinary team from neonatology, paediatrics, otolaryngology, audiology, auditory verbal and speech therapy, ophthalmology, radiology, and psychology working closely with the child, family and schools is needed to develop a cost-effective and comprehensive management programme for paediatric HI.

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Keywords: Hearing Impairment, Management

Introduction

Congenital hearing impairment (HI) occurs in approximately 4 per 1000 newborns worldwide. If intervention is delayed, the child with HI will have delayed speech and language acquisition, impaired cognition, and difficulty integrating into mainstream society socially and at work. I was asked to present on the multidisciplinary management programme at the National University Hospital (NUH) in Singapore currently available for children with HI.

Detection of Paediatric Hearing Loss

Universal Newborn Hearing Screening (UNHS) is performed on approximately 99.95% of newborns delivered at NUH within 6 weeks of life.[1] Both Otoacoustic Emission (OAE) and Automated Auditory Brainstem Response (ABR) hearing tests are administered by colleagues from the Neonatology Department. Those failing UNHS screening are referred to Otolaryngology. Paediatric otolaryngologists first exclude ear conditions that need medical or surgical interventions. Paediatric audiologists then perform tympanogram, OAE, ABR and steady state evoked potential (SSEP) hearing tests. Hearing aids can be fitted as early as 1.5 months if appropriate. The referral rate to ENT from UNHS was 0.5% (33/6836) for newborns between 2003 and 2005. Among these 33 newborns, 4 had false-positive HI, 3 defaulted follow-up and 26 had HI.

Age-appropriate hearing tests include behavioural observation audiometry for children less than 3 years old, play audiometry for children 3 to 5 years old, and pure tone audiometry from 5 years old. ABR is used in neonates and for children unable to cooperate with subjective testing when awake.

Missing a Diagnosis of Hearing Impairment

HI is easily missed. A child with mild-moderate HI can still hear door bells and telephones, and can use visual and other sensory cues to aid in understanding what caregivers are saying. For normal development of speech and language however, near perfect hearing is needed. Many consonants of speech are heard only at 20 to 30 dB levels. Even mild HI may therefore result in speech, language, cognition and behavioural disorders. Only a formal hearing test can detect

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mild HI accurately. HI is easily missed with informal testing in a non-sound proof room. Hearing tests need to be repeated if there is any suspicion of HI. A genetic or congenital HI may pass UNHS but manifest later in childhood, or progressively deteriorate or fluctuate.

In Central Auditory Processing Disorder (CAPD), hearing tests are normal, but decoding of auditory information is impaired. Auditory sound information may not be optimally heard by the child, especially in challenging situations of background noise, poor room acoustics, rapid speech or long sentences. Special speech lists testing are needed for diagnosis. The centre started comprehensive CAPD evaluation and tailored CAPD management in Singapore between 2004 and 2005. We have found that definitions of norms used in the West may not be appropriate for Singapore, so we are developing Singapore-specific CAPD normative data for Singaporean children.[2] After the medical evaluation by paediatric otolaryngologists, paediatric audiologists sub-specialising in CAPD administer the CAPD tests, and a speech language therapist sub-specialising in CAPD works with the family and child on a tailored programme of rehabilitation strategies.

Excluding an Infectious Cause of Deafness

In NUH, every newborn is screened for hypothyroidism at birth. Infectious causes of HI due to TORCHES (Toxoplasmosis, Rubella, Cytomegalovirus, Herpes, Syphilis) infections are decreasing. Toxoplasmosis and Herpes Simplex infections are also uncommon with improved hygiene and standards of living. The cost-effectiveness of universal neonatal screening for Cytomegalovirus (CMV) is currently controversial. However, a diagnosis of congenital CMV can only be made if tested for within the first month of life, and CMV accounts for 10% to 35% of congenital HI in the West. HI in congenital CMV affects 40% to 60% of symptomatic infants and 7% to15% of asymptomatic infants. Both HI and neurological deterioration can progress undetected in asymptomatic CMV. HI due to CMV can develop months or even years after birth.[3] Early ganciclovir therapy may reverse neuro-developmental delay and HI.[4]

Excluding a Genetic Cause of Deafness

Genetic testing for congenital HI is cost-effective, and is now performed worldwide.[5] Seventy-five percent of genetic HI is non-syndromic, and 75% of non-syndromic HI is autosomal recessive in inheritance. Babies with genetic HI therefore look normal, are well except for HI, and are born to hearing parents. It is thus easy to miss a diagnosis of genetic HI unless a blood test of DNA is done. The most common genetic mutation responsible for non-syndromic, autosomal recessive HI is a DFNB1/ GJB2 mutation that results in an abnormal Connexin 26 gap junction protein required for potassium recycling in the cochlear. Connexin 26 HI alone accounts for 20% to 60% of idiopathic congenital sensorineural HI in the West.[6,7] In a recently concluded ASTAR-BMRC funded study evaluating idiopathic HI, Connexin 26 HI was responsible for 3 out of 10 cases of idiopathic congenital sensorineural HI seen at NUH.[8] It is thus now the first test in our evaluation of the etiology of idiopathic congenital bilateral sensorineural HI. Singapore's most prevalent connexion 26 mutation is the missense mutation V37I, unlike the 35delG and 235delC nonsense mutations common in the West and China respectively. Connexin 26 HI ranges from mild to profound in severity and can progress with time. No A1555G mitochondrial mutations which increase the risk of aminoglycoside-induced HI, nor Connexin 30 (DFNB2/ GJB6) mutations were identified in our Singapore population. Genetic testing for connexin 26, connexin 30 and A1555G mitochondria congenital HI was made available in 2004 as a diagnostic service in NUH at the Molecular Diagnostic Laboratory.

Excluding Other Causes of Deafness

Urinary blood and protein analysis may alert us to the renal dysfunction associated with HI in Alport's syndrome. Electrocardiogram is a simple and inexpensive investigation to exclude the prolonged QT interval associated with Jervell Lange Nielsen (JLN) syndrome, especially important if there is a family history of sudden death or syncope. Eye examination is especially important in those with severe-profound HI as 50% of these children may have concomitant visual problems, example like in Usher's Syndrome. Early retina treatment can prevent or delay the blindness. CMV and Toxoplasmosis retinitis can also be identified. Co-management with geneticists and multidisciplinary paediatric specialists are important in children with syndromes affecting multiple systems. Children with cleft palates and craniofacial anomalies are co-managed with plastic, maxillofacial and orthodontic surgeons. Syndromic HI may have mixed conductive and sensorineural HI. If conservative management fails, conductive HI may benefit from insertion of ventilation middle ear tubes, repair of obvious or occult cleft palates, replacement of middle ear ossicles, and mastoid surgery to remove cholesteatoma tumour and chronic mastoid infection.

Radiological imaging gives higher diagnostic yield than genetic tests in children with unilateral or fluctuating HI.[9] Enlarged vestibular aqueduct (EVA), cochlear and semicircular canal anomaly in the temporal bone can be detected on computer tomography (CT) scan. In a recently completed study on patients with EVA seen at NUH, our patients had larger overall mean diameter of EVA, and higher association with Mondini's cochlear compared to those in reported literature.[10] Most had late diagnosis and

severe-profound bilateral hearing loss that needed hearing aids or cochlear implants. If EVA is diagnosed, genetic testing for Pendrin is useful to exclude Pendred disease in which there is EVA, possible cochlear malformation, and thyroid dysfunction. Children with EVA should avoid sudden increase in intracranial pressure that may cause deterioration in hearing (examples are head trauma, diving and contact sports). Early steroid therapy may reverse sudden deterioration in hearing in EVA. Magnetic resonance imaging (MRI) can detect an absent or hypoplastic cochlear nerve, cochlear fibrous ossification and brain pathology better than the CT scan.

Other investigations ordered are guided by history, examination, and careful follow-up of the child. For example, post-lingual progressive HI may be associated with autoimmune problems, and HI will be associated with branchial fistulas and renal dysfunction in Branchio-Oto-Renal Syndrome.

Hearing and Speech Rehabilitation

Children are fitted with appropriate hearing aids (HA) as early as 6 weeks old by our paediatric audiologists. A wide range of sophisticated digital HAs with greatly improved sound quality and cosmesis is now available. Behind-the-ear HAs are usually used for growing children as they require frequent change of ear moulds. When HAs fail to give optimal hearing outcomes, a similarly wide range of hearing implants and middle ear reconstruction surgery is now available. Malformed ears can be managed with silicon prosthetic ears, and reconstruction of the auricles. HI due to ear canal atresia and congenital anomaly of the middle ear bones can be managed with bone-anchored hearing aid, canalplasty, ossiculoplasty and middle ear implant surgery. The centre performed Asia's first Vibrant Soundbridge middle ear implant (MEI) on the incus for adult presbyacusis in 2006, and expanded MEI use to round and oval window applications for mixed or conductive HI in 2007. In 2008, the centre performed the first totally implantable Carina MEI in Singapore for moderate congenital sensorineural HI in an adult who was not satisfied with HAs. Severe to profound bilateral HI may require cochlear implants. The centre pioneered simultaneous bilateral cochlear implantation in 2006 in Singapore. Bilateral hearing can translate to better speech and language acquisition outcomes, ease of listening in background-noise situations, and improved localisation of sound. The selection of an appropriate candidate is made only after thorough medical, audiology and auditory verbal rehabilitation assessments, with pros and cons fully discussed with the family.

Auditory Verbal Therapy (AVT) rehabilitation at our centre is done by Speech Language Therapists who further undergo AVT training. The aim is to integrate the child with HI into mainstream society, without the need for lip reading and signing to communicate if possible. Rehabilitation is tailored to the child and family, with some patients performing better with some cued speech, especially if they have additional problems like autism and cognitive delay. Children may also have speech language impairment and other language problems coexisting with HI that needs to be identified and co-managed with other therapists. A holistic and coordinated rehabilitation programme tailored to the child is ideal. It often involves the otolaryngologist, speech and language therapist, physiotherapist, occupational therapist, specific paediatric medical specialist, psychologist, medical social worker and teachers from the child's school (main-stream, Canossian School or School for the Deaf).

Conclusion

A multidisciplinary team from neonatology, paediatrics, paediatric otolaryngology, paediatric audiology, speech language and auditory verbal therapy, child development, genetics, ophthalmology, radiology, medical social work, and psychology working closely together with the child, family and schools is needed to develop a cost-effective and comprehensive management programme for paediatric HI.

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Current Understanding of Auditory Neuropathy

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Abstract

Auditory neuropathy is defined by the presence of normal evoked otoacoustic emissions (OAE) and absent or abnormal auditory brainstem responses (ABR). The sites of lesion could be at the cochlear inner hair cells, spiral ganglion cells of the cochlea, synapse between the inner hair cells and auditory nerve, or the auditory nerve itself. Genetic, infectious or neonatal/perinatal insults are the 3 most commonly identified underlying causes. Children usually present with delay in speech and language development while adult patients present with hearing loss and disproportionately poor speech discrimination for the degree of hearing loss. Although cochlear implant is the treatment of choice, current evidence show that it benefits only those patients with endocochlear lesions, but not those with cochlear nerve deficiency or central nervous system disorders. As auditory neuropathy is a disorder with potential long-term impact on a child's development, early hearing screen using both OAE and ABR should be carried out on all newborns and infants to allow early detection and intervention.

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Key words: Auditory brainstem responses, Cochlear implant, Otoacoustic emissions

Introduction and Definition

Until a decade ago, hearing loss was generally classified as either (i) conductive, due to pathologies in the middle ear and/or blockage to the external auditory canal, or (ii) sensorineural, due to abnormalities in the cochlea including the outer hair cells and the auditory nerve. In 1995, a new category of hearing loss termed auditory neuropathy (AN) was first reported based on a longitudinal study of 10 patients with absence of or grossly abnormal auditory brainstem responses (ABR), but preservation of otoacoustic emissions (OAE) and cochlear microphonics.[1] Eight of these patients subsequently developed clinical evidence of peripheral nerve neuropathy. As the ABR evaluates neural function of the auditory pathway (which includes the inner hair cell and spiral ganglion of the cochlea, the VIII nerve and brain stem), an absent or abnormal ABR indicates abnormalities in any part of this auditory neural pathway. The OAE evaluates the outer hair cell (OHC) function within the cochlea. The presence of a response to OAE indicates normal cochlear OHC function. The cochlear microphonic is a receptor potential displayed in the ABR, produced by polarisation & depolarisation of cochlear hair cells. The response is pre-neural with no latency delay on the ABR. The presence of cochlear microphonics in the ABR reflects the integrity of OHC. Based on these hearing test findings and the clinical features of this first reported series of patients, their investigators proposed the term "auditory neuropathy" for patients with this type of hearing impairment as they opined that it was due to a disorder of the auditory nerve with preservation of the cochlear hair cell function.[1,2]

Sites of Lesion

Subsequent studies in humans and animal models with characteristic electrophysiological features of AN, however, showed that various sites of the auditory pathway could be affected. These include the cochlear inner hair cells, spiral ganglion cells of the cochlea, synapse between the inner hair cells and auditory nerve, or the auditory nerve itself due to either a reduction of neural elements or disruption in the temporal integrity of the neural signals.[3-9] All these varieties share a relatively spared receptor function and an impaired neural response with diminished ability to follow fast temporal changes in the stimulus.[2] To reflect their concern that the term AN may be anatomically inaccurate for such a heterogeneous sites of hearing impairment, some

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investigators have proposed "auditory neuropathy/dys-synchrony (AN/AD)" or "auditory synaptopathy (AS)" in place of "auditory neuropathy." [8,10-14]

Epidemiology

The actual prevalence of AN/AD is unknown as reports on population studies are lacking. Based on the result of a universal newborn hearing screening of 14,807 infants born in a large Singapore hospital where the prevalence of hearing loss was found to be 3.5 per 1000 infants (n = 52), the prevalence of AN/AD was reported to be 0.6 per 1000 (n = 9) infants.[11] Most of the studies reported on the prevalence of AN/AD were on patients with hearing loss. In children with hearing loss, the prevalence of AN/AD varied from 2.4% in school children with hearing loss[12] to 8.44% among profoundly hearing impaired children.[13] In adults, the prevalence of AN/AD was reported to be 1 in 183 adults with neural sensory hearing loss.[14]

Aetiologies

The etiologies of AN/AD have been identified to be due to numerous disorders. The most common ones can be classified primarily into one of 3 main groups: genetic, infectious or neonatal/perinatal insults.[10] Mutations of several genes which are important for the inner hair cell, spiral ganglion or peripheral nerve function have been found to be associated with AN in families with this type of hearing loss.[15-17] Perinatal hypoxia,[18] neonatal hyperbilirubinemia[6,8,10,19,20] and prematurity[4,10] have been identified to be the most common neonatal/perinatal insults associated with the AN/AD.

Clinical Presentation

Children with AN/AD usually presents with delay in speech and language development.[21] Adult patients with AN/AD often complain that they can hear sounds but cannot understand speech. Difficulty in perception of speech both in quiet and background noise is a consistent feature in patients with AN/AD type of hearing loss.[2,22] In children with AN/AD, a high proportion (about 50%) show little or no ability to understand speech even in favourable (quiet) listening conditions.[23-25] Unlike the case of cochlear loss/damage which results in hearing loss of severity consistent with pure-tone audiometry, patients with AN/AD present with hearing loss with speech discrimination worse than that predicted by pure-tone audiometry. This is because AN/AD affects the timing of neural activity in the auditory pathway and disrupts the aspects of auditory perception based on temporal cues.

The clinical course of patients with AN/AD is variable, ranging from fluctuating hearing loss in some,[26-28] improvement over time in others,[29] or remain unchanged in others.[30] Transient hearing loss has been reported mainly in some children during febrile episodes.[27,28] Furthermore, infants with low birth weight (LBW) were identified to be a significant predictor associated with the subset of AN/AD which recovers over time, thus suggesting that in LBW infants the AN/AD may be due to a delay maturation of the auditory pathway.[29]

Investigation

As AN/AD is a disorder with potential long-term impact on a child's development, early hearing screen using both OAE and ABR should be carried out on all newborns and infants to allow early detection and intervention. In children and adults complaining of difficulty in understanding speech, both hearing tests should be similarly carried out. All patients with AN/AD have recordable OAEs and absent or abnormal ABR. Their hearing thresholds for pure-tone detection can range from normal to profound level. Majority of AN/AD patients (>90%) are bilateral.[9]

Once a patient is identified to have a recordable OAE but absence of or abnormal ABR, a thorough clinical evaluation, including history and neurological examination, should be carried out to exclude central nervous system (CNS) involvement before a definite diagnosis of AN/AD is made. Furthermore, other electrophysiological test should be carried out to locate the site of the pathology accounting for these electrophysiological changes. These should include testing for middle ear function, acoustic reflex studies, and electrocochleargraphy.[31] It is also crucial to obtain brain MRI with contrast enhancement in all patients with electrophysiological findings characteristic of AN/AD to exclude possible CNS lesions[26] and cochlea nerve deficiency.[32] This is because pathologies affecting the central auditory pathway up to the level of the brainstem can produce similar electrophysiological changes. The term "AN/AD" is anatomically appropriate only for lesions in spiral ganglion cells or their axons, or of the 8th nerve, but not lesions in the brainstem and brain.[33] It is important to differentiate between them as the management and outcome differ.[26,34,35]

Management

Cochlear implants is the treatment of choice[36,37] for patients with AN/AD in the absence of cochlear nerve deficiency and higher cortical deficiency.[32] Conventional amplification using hearing aids are rarely beneficial. Electric-ABR (EABR) testing at the time of implant surgery or in the immediate postoperative period has been shown to help predict success to cochlear implant in patients with AN/AD. Patients with robust brainstem potentials on EABR testing, thus suggesting normal cochlear nerve function, have good outcomes to cochlear implants.[38] As spontaneous recovery of useful hearing has been reported in subsets of children with AN/AD associated with prematurity and

hyperbilirubinemia,[29,30] serial clinical and audiometric evaluations should be carried out in young children before cochlear implantation be considered.

Although many investigators have reported significant improvement of speech perception in children with AN/AD following cochlear implants,[26,39] others found that the these children tend to be at the low end of post-implant performance range in speech perception when compared with children with sensorineural hearing loss.[32,37] Concomitant non-auditory factor such as CNS abnormalities could be one possible explanation for this poorer performance as many of these children were graduates of neonatal intensive care units. Hypoxia, prematurity and hyperbilirubinemia with resultant CNS damage are common problems present in this group of high risk infants.

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Establishing a Universal Newborn Hearing Screening Programme

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Abstract

As congenital hearing impairment has a worldwide incidence of 4 to 5 per 1000 babies and is thus one of the most common congenital problems seen today, universal newborn screening has a crucial role to play in its early detection and intervention. It provides the opportunity for better outcomes and normal language development. Prior to embarking on a screening programme, the newborn population and the current health care system should be analysed to select the best method of coverage. The screening tool and protocol, communication of results, as well as the follow-up measures should be clearly determined and tested. The multidisciplinary team required should be provided with the necessary information. Parents need to be educated about the importance of early hearing screening. Data management and surveillance should be established in a systematic manner. The costs of the programme should be carefully anticipated and funding sources determined. Finally, support for the programme should be sought from governmental or public health bodies, to ensure the success of the programme. Legislation can be considered if necessary.

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Key words: Congenital, Hearing, Newborn, Screening

Congenital hearing impairment is one of the most common congenital problems seen today, with a worldwide incidence of 4 to 5 per 1000 babies.[1,2] Out of 1000 babies, 1.7 have severe or profound hearing loss.[2] By comparison, congenital hypothyroidism, which has a longer history of newborn screening, has a lower incidence of 1 in 3000 babies.[3] Targeted hearing screening of babies with risk factors for hearing impairment failed to detect up to 50% of affected babies without any identifiable risk factors.[4] More than 90% of affected children have parents with normal hearing.[4] Without universal newborn screening, hearing impairment was often diagnosed very late. In Singapore, students in special schools for hearing-impaired children were diagnosed at a mean age of 20.8 months (range, 0 to 86) and received intervention at a mean age of 42.2 months (range, 1 to 120).[5] Late diagnosis of congenital hearing impairment can result in significant delays in language and reading. In the United States, children diagnosed with severe to profound hearing impairment in the pre-UNHS era, completed 12th grade with a 3rd to 4th grade reading level and a language level corresponding to an 8 or 9 year old child.[6] Children who were diagnosed and provided with intervention before 6 months had significantly better outcomes and the opportunity for normal language development at 5 years.[7,8] Although babies with significant bilateral hearing loss are those for whom early intervention is most urgent, it is also important to detect those with unilateral hearing loss, as they are 10 times more likely to be retained at least 1 grade compared to their unaffected peers.[9] Even children with mild hearing loss had poorer communication scores than their peers at 3rd grade.[10]

Universal Newborn Hearing Screening (UNHS) is the first crucial phase of a 3-prong approach for Early Hearing Detection and Intervention (EHDI). The other 2 phases are the diagnostic audiological services and the interventional services for infants with confirmed hearing loss. In order to maximise the outcome of infants who are deaf or significantly hearing impaired, the 2007 position statement by the Joint Committee on Infant Hearing[11] recommended that

i) all infants should be screened for hearing loss no later than 1 month of age,

ii) infants who do not pass the hearing screen should undergo comprehensive audiologic evaluation no later than 3 months of age, and

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iii) infants with confirmed hearing loss should receive appropriate intervention no later than 6 months after birth.

Several areas need to be carefully considered before embarking on a UNHS programme:

i) *The newborn population.* The size, nature and geographical distribution of the population to be screened will make a huge impact on the programme. The larger the population to be screened, the larger will be the network of screeners who need to be trained, the greater the role of the coordinating office and the need for good data management systems and the greater the economics of scale. A wide geographic distribution may create difficulties with recall of patients to a central area. This can affect the choice of the screening protocol or create the need for screeners in a community or rural setting.

ii) *The existing healthcare system*. Newborns are best screened in the birth hospital, prior to discharge, thus ensuring the best coverage. Current workflow and discharge practices need to be studied to determine the most appropriate interval between birth and screening. A protocol or mechanism should be developed for all infants who are born outside of a hospital or if hospital screening is missed. Working together with the current screening or immunisation programmes can also be a useful strategy, especially for out-of-hospital births. Screening for newborn hearing can be performed simultaneously with screening for congenital hypothyroidism/inborn errors of metabolism or with neonatal immunisation and reported together, as long as it is carried out by 1 month of age. If a second screen after discharge needs to be done in widely scattered communities, this can be timed together with the mother's or infant's postnatal visit.

iii) *Education*. Before the screening is carried out, parents need to be educated about the need, importance and ease of hearing screening, the implications of the results and the impact of undiagnosed hearing loss. Common concerns in the individual community should be addressed. This information should also be made available to all medical staff involved in infant care to ensure consistency. They should understand the implications of a failed screen and the need to be evaluated audiologically to exclude or confirm hearing loss. This information could be provided during routine maternal antenatal care.

iv) *The screening tool*. Both the automated auditory brain stem response (ABR) and the otoacoustic emission (OAE) have been successfully used for the UNHS.[10] The OAE is the simpler and shorter of the 2 tests, requiring approximately 10 minutes to perform. However it requires a fairly quiet room, is affected by debris in the ear and only reflects the status of the peripheral auditory system up to the outer cochlear hair cells.[10] The ABR takes approximately 15 to 20 minutes per test, is more tolerant of environmental noise and is less affected by debris in the ears. Unlike the OAE, it reflects the status of the peripheral auditory system, the 8th cranial or vestibulocochlear nerve and the brainsterm auditory pathway. Thus while both can detect sensorineural hearing loss, the OAE will not detect auditory neuropathy or dyssynchrony (AN/D). As the infants who received intensive care are particularly at risk of AN/D, automated ABR is the only screening test that should be used in this population.[10] In terms of costs, the equipment for the AABR is generally more expensive that the OAE. As a result, pre-discharge screening costs vary depending on the equipment used (ABR, US$32.81, 2-step ABR/AOE US$33.05, OAE US$26.89).[11]

v) *The screening and follow-up protocol.* Many programmes use a 2-step protocol using either OAE or ABR. An infant who does not pass the first screen can be rescreened with the same test either before hospital discharge or soon after discharge. A combination of OAE followed by automated ABR for rescreening can also be used. However, the infant who does not pass the automated ABR should not be rescreened with the OAE, which may miss an AN/S. Referral rates for audiological assessment differ significantly among the programmes (ABR, 3.2%, 2-step OAE/ABR 4.6%, OAE 6.4%: $P < 0.01$).[11] Thus, limited audiological services or access may require the use of costlier equipment with a lower false positive rate (0.2[12] to 0.9%[13] for the 2-step ABR, 1.7%[14] for the 2-step OAE). A mechanism for onward referral for audiological investigations in infants who do not pass the screening should be established. The protocol should be tested in a pilot project, which can reveal barriers to a smooth screening process before the full programme is established. Infants who are readmitted in the first month of life for conditions associated with hearing loss should be re-screened before hospital discharge.[15] As hearing loss can develop later in life, infants who pass the newborn hearing screen should have regular surveillance of developmental milestones, auditory skills and parental concerns. Those with risk factors for hearing loss should be referred for an audiological assessment at least once by 24 to 36 months of age.[15]

vi) *Staff.* A multidisciplinary team should be involved in the establishment of the UNHS, including

neonatologists/paediatricians, otolaryngologists, audiologists, nursing staff and screeners. A formal training programme should be established for hearing screeners, as well as regular recertification. There should be a designated coordinator or coordinating office to be responsible for the direction of the programme, maintenance of equipment, adherence to established standards, follow-up of infants who either need rescreening, audiological confirmation of hearing loss or intervention services, data collection, communication with parents, other staff members and members of the infant's medical home, family support, etc.

vii) *Communication of results.* Communicating and recording screening results needs to be conducted in a systematic manner, preferably verbally to parents after the screen, together with a written record of the result in both hospital and child's medical records. Parents should also receive either verbal or written education about the importance of follow up, if rescreening or audiological investigations are required. The follow up medical home care team should also be informed of the results, so that appropriate follow-up can be ensured.

viii) *Data management, follow-up and surveillance.* Commercial data management systems are available to manage the large amounts of data that will emerge from the screening programme. They can also be used to identify those who require or have missed screening, rescreening, or audiological investigations and for quality control.

ix) *Cost of the programme.* The factors that affect cost include the screening protocol and equipment, the number of infants who need to be screened, the number of staff required and the cost of their services etc. These costs must be balanced against the societal cost of hearing loss, which include the cost of special education and training and the loss of potential and lifetime earnings of an hearing impaired individual. Options for payment include insurance, self-pay, public sponsorship (full or partial), etc.

x) *Support for the programme.* Public support from governmental and/or public health bodies would assist greatly in both the establishment, execution and funding of the UNHS. Some countries have used legislation to ensure that every infant has a hearing screen.

Despite the obvious challenges in the establishment of the UNHS, there is no doubt of its critical role in reducing the devastating effect of significant congenital, pre-lingual bilateral hearing loss. However, because of the need of sound audiological confirmatory testing and long-term intervention, it should be developed in tandem with these services.

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Spectrum of Inherited Metabolic Disorders in Malaysia

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Abstract

Issues pertaining to the diagnosis and management of inborn errors of metabolism (IEM) in Malaysia included low awareness of atypical and variable presentations in IEMs leading to delayed diagnosis or treatment, absence of reliable population data on IEMs and involvement of multiple siblings in the same family due to consanguinity. The importance of careful family history taking and genetic counselling are emphasised. Selected testing of ill infants and children for IEM yielded a positive 2% (264/13,500) results for IEMs in Malaysia. Out of the 264 patients, the spectrum of IEMs in Malaysia included organic acidurias (98), aminoacidopathies (78), urea cycle defects (54), neurotransmitter conditions (12) and lysosomal disorders, mainly mucopolysaccharidosis (14). Confirmatory studies of IEMs are an important aspect of management of IEMs. There is a need for more metabolic specialists and funding for diagnosis and treatment of IEMs in Malaysia. Long-term care issues and cost-effectiveness of IEM therapy, supportive and preventive aspects will need further studies in Malaysia.

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Key words: Inborn errors of metabolism, Newborn screening

Introduction

Inborn errors of metabolism or inherited metabolic disorders (IEM) are genetic enzyme defects that cause abnormal function of biochemical pathways.[1] When enzyme activity is reduced, the substrate accumulates, causing secondary metabolic effects and deficiency of an essential product of a metabolic pathway, leading to "acute metabolic crisis". Individual case of IEM is rare but collectively, birth prevalence is estimated to be 1 in 3000 to 5000 in the general population. There are a number of anecdotal reports on IEMs in Malaysia.[2-4] However, the actual number of patients with IEMs from population-based studies is not known. In addition, many IEMs which involves "large molecules" or storage disorders with progressive psychomotor regression are often not diagnosed.

Early recognition and treatment of an IEM are important. The child with IEM often deteriorates suddenly and progresses rapidly with severe permanent brain damage. Treatment is effective if started early and the earlier return of metabolic stability correlates well with long-term prognosis and prevents learning handicap. Establishing an accurate diagnosis facilitates genetic counselling for the family, usually before the next child is conceived. Prenatal diagnosis can also be offered as an alternative reproductive option if the genotype or enzyme defect is known.

Patients and Methods

We approach this paper from 2 aspects – qualitative and quantitative aspects. The first aspect relates to the clinical features of IEMs and their diverse presentation. We report on 4 different patients with their respective case histories, highlighting issues related to early diagnosis and management to ensure a better outcome, genetic counselling and long-term care issues in IEMs. For the quantitative aspect, we review the epidemiology and the spectrum of IEMs in Malaysia from the experience of the Specialised Diagnostic Centre, Institute for Medical Research Kuala Lumpur from 1999-2005 in making the diagnosis of IEMs from patients suspected to have IEMs. This will give an indication on the sensitivity of a clinical diagnosis of IEM and to serve as a direction for primary prevention of IEMs in the community.

Findings

Clinical Aspects of IEM in Malaysia

Case 1: A Chinese infant girl presented with lethargy and respiratory distress on the third day of life. The parents were unrelated and there was no significant family history. The perinatal history was uneventful. She was breast-fed and discharged well on the second day of life. Examination showed she was encephalopathic with Kussmaul respiration.

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Investigations showed hypoglycemia with severe ketoacidosis. She was resuscitated and managed in the paediatric intensive care unit as "septicaemia". All microbial cultures were negative. Urine organic acid showed methylmalonic acid >10,000 μmol/mmol, confirming the diagnosis of methylmalonic acidemia. The management included dietary restriction of protein intake via expressed breast milk with energy supplement, carnitine, metronidazole, vitamins C and B12. She was discharged well with no apparent sequelae. Genetic counselling and education of care-givers and other healthcare professionals were provided. Close liaison with the dietician and pharmacist was essential for her care.

Case 2: A 3-year-old Indian girl presented with recurrent admissions of 5 to 6 times per year for vomiting spells and "sleepiness", associated with mild viral illnesses. Her symptoms usually stop when she's put on an IV drip. She had multiple extravasation injuries. The significant dietary history included "self-selected vegetarian diet". The patient disliked "fast food meals" such as fried chicken or burgers. Her mother also admitted that she herself is a vegetarian. The parents are unrelated and the mother had no previous hospitalisation. The patient was admitted for further investigation of "cyclical" vomiting and acute encephalopathy. Investigations showed she had hyperammonemia and the diagnosis of urea cycle defect (partial ornithine transcarbamylase or OTC deficiency) was made based on the clinical picture, the amino acid profile and elevated urinary orotic acid. She was allowed to continue her "self-selected" diet with a special sick regime. She was also treated with ammonium scavenging therapy consisting of oral sodium benzoate and sodium phenylbutyrate. She did not have any more vomiting spells and no further hospital admissions were needed. She had normal developmental milestones.

During the patient's hospitalisation, the mother shared that she was pregnant. After counselling, the parents declined prenatal diagnosis for the pregnancy. There was close liaison with the obstetric team managing the mother's care during the perinatal period. A 3-kg baby boy was delivered at birth. He became symptomatic and rapidly deteriorated. He was treated aggressively in the ICU but succumbed to his illness at 12 weeks of life. The mutation in the OTC gene was successfully identified a year later and the option of prenatal diagnosis was offered to the parents for their next pregnancy.

Case 3: An 8-year-old boy, presented with acute left hemiparesis. He was extensively investigated. The family pedigree was as shown in Figure 1. His mother had short stature, hearing loss and diabetes mellitus. An aunt was also short with diabetes mellitus and an uncle died of status epilepticus. The maternal grandmother had similar medical problems as the mother. A cranial magnetic resonance (MR) imaging showed multiple transient ischaemic sites while a MR spectroscopy showed raised lactate. Mitochondrial DNA study showed: 3243 A>G mutation which confirmed the diagnosis of MELAS (mitochondrial encephalopathy, lactic acidosis and stroke-like episodes). He was treated with oral carnitine, coenzyme Q10 and advised to ensure adequate intake of energy, fluids and electrolytes and to avoid drugs that may inhibit the respiratory chain. Genetic counselling and follow-up investigation of family members were arranged.

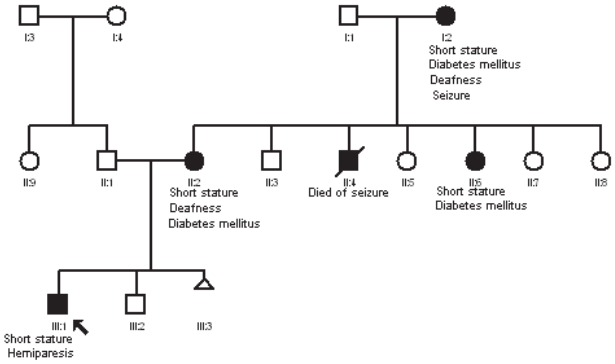

Fig. 1. Family tree of patient with MELAS (mitochondrial encephalopathy, lactic acidosis and stroke-like episodes).

Case 4: An 18-year-old boy with Sanfilippo syndrome was admitted for recurrent episodes of seizures. He presented initially with delayed milestones with coarse features at 3 years of age. Urinary screening for mucopolysaccharidosis (MPS) then was negative. He developed developmental regression but no definitive diagnosis was made despite receiving many consultations in the region. A review at 12 years of age by the metabolic service followed by an MPS enzyme study showed deficiency of sulphamidase activity consistent with mucopolysaccharidosis (MPS) type IIIA or Sanfilippo syndrome. He had many on-going issues and these included parental difficulties with medical professionals, recurrent seizures, feeding and behavioural difficulties, psychological and suicidal issues. During this admission, issues such as palliative and transition care were also discussed.

Discussion

A high index of suspicion is required to make a diagnosis of IEM in babies and children. During the neonatal period, the patients are usually well at birth but may develop symptoms the first 2 to 3 days of life after introducing milk feeding. Initial presentations are non-specific and may include poor feeding and lethargy. This progressively worsens with fulminant "septic" presentation.

In older children, the initial presentation may be acute encephalopathy, liver failure or cardiorespiratory shock. This may present for the first time at any age and be associated with a catabolic stress, intercurrent illness and fasting as well as increased protein intake during weaning or "parties". Seizures are usually late presentation of IEM. Progressive coma or central nervous system (CNS) deterioration is usually the presentation. The patient may also present with acidosis and hypoglycemia. The adage "any symptom, any organ, any age!" is important to remember in IEMs. In addition, many other common conditions in Malaysia e.g. cerebral malaria, dengue shock syndrome and acute infective hepatitis may resemble IEMs.

In an acute metabolic crisis, aggressive resuscitation and ICU care are mandatory. Working closely together with paediatric intensivists, anaesthesiologists or neonatologists, this includes correction of dehydration, shock and provision of adequate cardiorespiratory support and prevention of cerebral oedema. Empiric treatment with intravenous antibiotics, prevention of hypoglycaemia and hypothermia and correction of acidosis and hyperammonemia are required. Consultation with IEM laboratory staff and physician with metabolic expertise are essential.

Proper collection and transportation of urine and blood samples are the most important steps in the diagnosis of IEM. Samples must be obtained during the acute crisis preferably before the therapy commenced. Investigations done after metabolic stability has been achieved may give inconclusive or false negative results. Samples must be transported to the laboratory as soon as possible. The investigations of suspected acute IEM usually show one or more of these tests outcome being out of proportion to the clinical state of the child's condition. Besides the usual full blood count, blood urea and serum electrolytes, serum creatinine and liver function test, other basic investigations in suspected acute metabolic crises include blood gas and acid-base balance, plasma glucose, plasma ammonium, serum lactate and urine ketone, reducing substances & pH. Plasma amino acid and urine organic acid assays will point to common IEMs. Once metabolic stability and IEM diagnosis were made, joint management with a dietician or nutritionist experienced in IEM is mandatory. Specific dietary manipulation, example, metabolic foods for substrate limitations and specific pharmacotherapeutic agents may be required.[16,17]

Detailed family history and genetic counselling are essential. In many families, consanguinity may be an important issue. In Malaysia, it may be common to see 2 or more siblings affected by the same IEM. This may be related to acceptance of consanguineous marriages in some Asian cultures and also the delay in making a correct diagnosis before the next sibling is conceived or born. Long-term care issues in IEM include the delayed diagnosis of lysosomal conditions causing chronic neuropathic diseases and significant morbidity. Issues such as transition care for storage diseases and palliative care for neurodegenerative conditions must be discussed. Recently, many other modalities of treatment have emerged. These include stem cell transplantation and enzyme replacement therapy for lysosomal diseases. The cost-effectiveness of caring for patients with IEMs is being debated. Should there be further investments in setting up intensive care unit and training of medical practitioners in the early recognition of acute metabolic crises or will funds be better spent in an expanded newborn screening for IEMs?

Epidemiology and Spectrum of IEMs in Malaysia

Over a 6-year period from 1999 to 2005, a total of 13,400 samples were received nationwide from medical practitioners consisting mainly of paediatricians by the Specialised Diagnostic Centre, Institute for Medical Research Kuala Lumpur. These samples were obtained from infants and children who were suspected to have IEMs based on their clinical presentation such as poor feeding and vomiting, cardiorespiratory collapse, hyperammonemia, unexplained metabolic acidosis, recurrent "sepsis", acute encephalopathy and seizures. Other indications included psychomotor retardation, developmental regression and dysmorphic features. In 1998 when the service was first started, amino acid analysis of body fluid samples were processed and derivatised with PITC and 8 ul of the mixture was injected into the JASCO HPLC system using gradient pump and reversed-phase column heated at 40°C. The ultra-violet detector was set at 254 nm, and run for 80 minutes.[5] In 2003, the method was later change to fully automated amino acids analyzer (Biochrome 30+) with post-column derivatisation with ninhydrin. Plasma total homocysteine was analysed using HPLC and Bio-Rad Homocysteine kit. Patients with moderate degree of hyperphenylalaninemia and positive clinical symptoms were sent to overseas laboratory to confirm the diagnosis of pterins disorders. Random urine for organic acids was performed by GC-MS system from Hewlett Packard, USA after organic solvent extraction and derivatisation of organic acids with BSTFA.[6,7] Diagnosis of mucopolysaccharidosis was made whereby urine glycosaminoglycan was quantified using dimethyl-methylene blue and was later characterised using high resolution electrophoresis by method described by Hopwood.[8] Diagnosis of peroxisomal disorders was made by analyzing plasma samples for very long chain fatty acids (VLCFA) and phytanic acids. This was processed using a method described by Moser and Moser.[9] The specific

Table 1. Inborn Errors of Metabolism, Institute for Medical Research (1999-2005)

I	**Organic aciduria: 98**	
1.	Methylmalonic aciduria	41
2.	Glutaric aciduria type 1	19
3.	Isovaleric aciduria	13
4.	Propionic aciduria	8
5.	3-OH isobutyric aciduria	8
6.	Ethylmalonic encephalopathy	4
7.	Others	5
II	**Aminoacidopathy: 78**	
1.	Maple Syrup Urine Disease	44
2.	Non-ketotic hyperglycinemia	16
3.	Classical phenylketonuria	7
4.	Homocystinuria	6
5.	Tyrosinemia	5
III	**Urea cycle defect: 54**	
1.	Arginosuccinic aciduria	15
2.	Citrullinemia	11
3.	Partial ornithine transcarbamylase (OTC) deficiency	11
4.	Ornithine transcarbamylase deficiency	5
5.	N-acetylglutamate synthase (NAGS) deficiency	6
6.	Carbamylphosphate synthase 1 (CPS 1) deficiency	3
7.	Arginase	3
IV.	**Neurotransmitter: 12**	
1.	Tetrahydrobiopterin deficiency	9
2.	Aromatic L-amino acid decarboxylase deficiency	3
V.	**Lysosomal: 14**	
1.	Mucopolysaccharidosis	11
2.	I cell disease	3
VI.	**Others: 8**	
1.	Glutaric aciduria type II	4
2.	3-hydroxy-3-methylglutaryl (HMG) CoA lyase deficiency	3
3.	Citrin deficiency	1
	Total	**264**
	Positive IEMs: 264/13,400	**2%**

diagnosis of peroxisomal disorders could not be made as this will require enzyme assays.

The results of the analysis are shown in Table 1. An average of 2200 samples from patients were analysed a year. The yield from this cohort was 2% (264/13,400). On the basis of clinical suspicion for IEMs, the yield of 2% positive diagnosis of IEMs is a useful baseline. This is in keeping with some studies.[10,11] However, this may be an indication that some of the tests may not be required. In addition, there were 5 cases with peroxisomal disorders. This and some of the other IEMs will require confirmatory studies such as enzyme assays and molecular genetic testing. The diagnosis of fatty acid oxidation defects cannot be reliably made based on organic acid analysis. Nevertheless, the delineation of the spectrum of inherited metabolic disorders is important as it added new information to the current knowledge of IEMs in Malaysia.[12-15,17] However, further studies are required to confirm these findings. Currently, a pilot study of an expanded newborn screening programme in selected public hospitals is ongoing.

Conclusion

We reported that selective IEM testing in ill infants and children yielded 2% positive results for IEM in Malaysian paediatric practice. Issues pertaining to the diagnosis and management of IEM in Malaysia included low awareness of atypical and variable presentations in IEMs leading to delayed diagnosis or treatment, absence of reliable population data on IEMs and involvement of multiple siblings in the same family due to consanguinity. The importance of careful family history taking and genetic counselling are emphasised. Confirmatory studies of IEMs are an important aspect of management of IEMs. More resources are needed to address the morbidity and mortality related to IEM in Malaysia. Long-term care issues and cost-effectiveness of IEM therapy, supportive and preventive aspects will need further studies and debate.

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Disorders of the Carnitine Cycle and Detection by Newborn Screening

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Abstract

Carnitine is necessary for transport of long-chain fatty acids into mitochondria, to enter the β-oxidation cycle. Four carnitine cycle defects have been described. *The carnitine transporter* mediates carnitine transport across the plasma membrane. Symptoms include hypoketotic hypoglycaemia and cardiomyopathy. Some affected subjects are asymptomatic. Newborn screening detects very low levels of free carnitine in some but not all. *Carnitine palmitoyltransferase type IA* (CPTI) transports long-chain fatty acyl-CoAs across the outer mitochondrial membrane. Affected infants have hypoketotic hypoglycaemia with catabolic stress, but otherwise remain well. Newborn screening tests reveal elevated free carnitine, (elevated C0/C16+C18). Sensitivity is unclear and confirmation needs leukocyte or fibroblast assays. *Carnitine-acylcarnitine translocase* transfers fatty acylcarnitines across the inner mitochondrial membrane. The most common presentation is sudden death in the first days. *Carnitine palmitoyltransferase type II* (CPTII) converts long-chain acylcarnitines to long-chain acylCoAs for β-oxidation. Severe deficiency is lethal. Newborn screening for both disorders reveals elevated palmitoylcarnitine and enzymology or mutation analysis is needed for diagnosis. Late-onset CPTII is the most common disorder, presenting as muscle pain and rhabdomyolysis on severe exercise. All 4 disorders can be detected by newborn screening, with variable sensitivity. Late-onset CPTII probably cannot be detected. Carnitine transporter, CPTI and late-onset CPTII have proven treatment strategies.

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Key words: Carnitine transporter, Carnitine palmitoyl, Transferase, Translocase

Introduction

Carnitine is a quaternary ammonium compound which transports long-chain fatty acids into the mitochondria. It is obtained from animal protein in the diet, (the word is derived from the Latin caro, carne – flesh), and partly by de novo synthesis. Carnitine is synthesised from trimethyllysine from the S-adenosyl-methionine-mediated methylation of lysine residues in proteins. This occurs in the liver and kidney, and to a very small extent, in the brain. Other tissues are dependent on a transport system.[1] Carnitine deficiency resulting from a defect in biosynthesis has yet to be reported. Plasma carnitine is filtered by the kidney, and up to 98% is reabsorbed. To a great extent, the renal carnitine threshold determines the plasma carnitine concentration, and eventually the body stores of carnitine. It is important to note however that there is only a weak correlation between plasma and tissue carnitine levels in healthy individuals. The carnitine cycle enables the transport of long-chain fatty acids into the mitochondria to enter the β-oxidation cycle.[2] Figure 1 illustrates the steps in the cycle.

Four defects of the carnitine cycle have been described in man, all with autosomal recessive inheritance.[2]

Carnitine Transporter Defect

The carnitine transporter affects primarily the transport of carnitine across the plasma membrane and renal tubule, resulting in poor uptake of carnitine from the gut, and deficient renal reabsorption. The described defect is caused by mutations in the SLC22A5 gene which is located at 5q31 1-32 and encodes the high-affinity organic cation/carnitine transporter OCTN2.[3] Some of these mutations affect failed maturation to the plasma membrane.[4] Patients with the transporter defect may present in several ways: neonates and children may have hypoketotic hopoglycaemia; infants and children most commonly present with cardiomyopathy, which may be fatal during infancy, and skeletal myopathy

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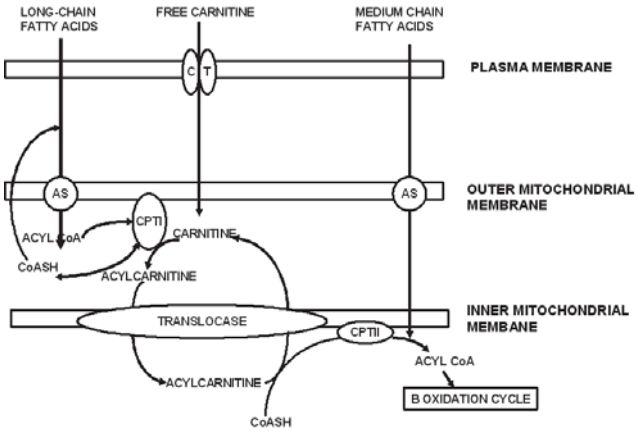

Fig. 1. The carnitine cycle: The enzymes affecting the transport of carnitine into the cell, and the carnitine cycle whereby carnitine mediates the entry into the mitochondria of long-chain fatty acyl CoAs as acylcarnitines.

CT – carnitine transporter; AS – acylCoA synthetase; CPT I – carnitine palmitoyltransferase IA; CPT II – carnitine palmitoyltranferase II; translocase – carnitine acylcarnitine translocase.

is also recorded.[4] At the other end of the spectrum, asymptomatic mothers have been found by newborn screening. Carriers may have left ventricular hypertrophy. The transporter defect can be detected by newborn screening because of low carnitine levels, usually less than 5 umol/L with an even lower level on repeat sampling. Oral carnitine therapy at a total dosage of 100 mg/kg daily, divided into 2 or 3 daily doses, usually gives a good result. In 900,000 newborns screened in the state of New South Wales, Australia (coverage over 99%) we detected 4 affected babies, and 3 mothers with less severe defects. Before screening, 3 patients had presented with cardiac failure secondary to cardiomyopathy at 18m (died before diagnosis), and 2 and 6 years. The survivors, as well as 2 affected siblings and the patients detected by screening are well on oral carnitine. We feel that newborn screening may possibly not detect all patients with this disorder, although there is as yet no definite evidence of missed cases.

Carnitine Palmitoyltransferase Deficiency Type IA (CPTI)

There are 3 isoforms of CPT I, a liver/kidney form, (IA), and isoforms that are present in heart and skeletal muscle, and in brain. Only CPT IA deficiency has been described in man. The enzyme is encoded by a gene located at 13q13.1-2. This rarely-described disorder usually presents with hypoketotic hypoglycaemic episodes mainly in the first 2 to 3 years of life. Affected subjects are well between episodes, and there appear to be no long-term sequelae. There have been reports of myopathy and a neonatal cardiac presentation, but these seem likely to have been chance associations. During attacks there are severe liver enzyme derangements and there may be renal tubular acidosis. There are 2 reports of acute maternal liver complications in pregnancy.[5] The mainstay of treatment, as for other potential hepatic presentations of fatty acid oxidation, is the avoidance of fasting, especially during intercurrent illness. The role of carnitine or of medium-chain fats is unclear.

CPT 1A can be detected by newborn screening, since carnitine levels are often elevated. In newborns, there is elevation of the ratio of free carnitine to the long chain species C16 plus C18. The sensitivity overall is not yet clear, but may be high.[7] The birth incidence is very low, except in the Hutterite community and the Inuit in Alaska, presumably due to founder effects.

Carnitine Acylcarnitine Translocase Deficiency (CACT)

The carnitine acylcarnitine translocase enzyme transfers fatty acylcarnitines into the mitochondria across the inner mitochondrial membrane, in exchange for free carnitine. CACT is coded for by the SLC25A20 gene which is located at 3p21.31, and is expressed mainly in heart, skeletal muscle and liver. The clinical expression of the disorder is usually very severe, with neonatal death in the first 1-3 days being the most common presentation.[8] Babies may have hypoketotic hypoglycaemia, seizures, hypotonia, bradycardia or other arrythmias, and cardiac failure. There is usually significant hyperammonaemia, more prominent than with other fatty acid oxidation defects during a crisis. Some patients have survived on treatment with a high calorie low-fat diet, with carnitine and medium-chain fat supplements,[9,10] but the early presentation in severe cases often precludes effective therapy being instituted in time in unsuspected cases.

Cases of CACT can readily be detected by tandem mass spectrometry newborn screening, as there is a significant elevation of palmitoylcarnitine (C16). It is likely that the sensitivity of newborn screening is very high. CACT cannot be distinguished by screening from carnitine palmitoyltransferase deficiency type II (see below), as C16 is also elevated in that condition. Elevated C16 levels can be confirmed by acylcarnitine profiling in leukocytes or cultured skin fibroblasts. To distinguish between CACT and CPTII, enzyme assay or mutation analysis is needed. In our own experience of newborn screening in New South Wales, Australia, we detected only 2 cases in over 900,000 babies; both had very high C16 levels, but died, at 22 and 72 hours after birth, before the newborn screening result was available.

Carnitine Palmitoyltransferase Deficiency Type II (CPT II)

The late-onset form of this disorder was the first disorder affecting fatty acid metabolism to be described, in 1973.[11] This late-onset form presents with exercise-induced muscle

cramps or frank rhabdomyolysis with myoglobinuria and very high creatine kinase levels in plasma. Typically, a young man undertakes a cross country run or similar activity, and becomes symptomatic. CPT II has been mapped to chromosome 1p32. There is a common mutation frequently seen in late onset CPT II, c.388C>T, (Ser113Leu) which is present in around 60% of disease-causing mutations. More severe mutations lead to neonatal-onset disease, which is usually fatal in the first 1 to 4 days of life, and may be associated with structural abnormalities of the brain and kidney[12] or an infantile form that presents predominantly with hypoketotic hypoglycaemia and its usual secondary manifestations.[12] There is no successful treatment for the neonatal presentation, but otherwise treatment is on the same principles as for other defects affecting long-chain fatty acids: avoidance of fasting, limiting the intake of long-chain fat and using supplementation with medium-chain triglycerides and carnitine.[12] Medium-chain triglyceride loading before exercise has been found successful in late-onset disease.

The more severe forms of CPT II can be detected by newborn screening, but it is not clear that this strategy will detect most late onset disease. Patients with late-onset CPT II, in our experience, do not have an abnormal plasma acylcarnitine profile except at times of acute stress. Elevated dried blood spot levels of C16 on newborn screening point to either CPT II or CACT, and as discussed above, enzymology or mutation analysis is required to differentiate these 2 disorders.

In summary, the 4 carnitine cycle disorders described in man can all be detected by newborn screening. However, late-onset CPT II seems unlikely to be detected, and the sensitivity is not known for CPT I (this may be quite high) or for the transporter defect, where probably not all cases can be detected without a large false positive rate. Mothers with mild transporter deficiency may also be detected, and specific uptake assays in cultured skin fibroblasts will be needed. Treatment of the carnitine transporter defect and of CPTI and late-onset CPT II is very effective. Treatment may also be effective in some cases of the translocase defect and later infantile onset CPT II, but neonatal presentations of both these disorders generally precludes successful treatment.

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Fatty Acid Oxidation Defects

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Abstract

Fatty acid oxidation defects (FAODs) are a group of inborn errors of metabolism that carry a risk for morbidity and mortality. Successful management of these conditions involves early diagnosis, proper management of clinical situations that predisposes to metabolic crisis as well as adequate treatment of a metabolic crisis. The advent of tandem mass spectrometry based newborn screening has reduced the morbidity and mortality in some FAODs. This article discusses how to recognise FAODs and how to manage them.

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Key words: Newborn screening, Tandem mass spectrometry

Introduction

Fatty acids are aliphatic monocarboxylic acids and commonly have carbon chains that range from 4 to 28 carbons. They can be saturated or unsaturated. Fatty acids serve many important biological functions. These include being a source of energy especially during prolonged fasting as well as being a vital building block in cell membrane and the production of essential molecules such as lipoproteins and cell signalers.

The main sources of fatty acids are dietary intake, release from fat stores (lipolysis) and synthesis by the human body. The human body can synthesise all fatty acids except for linolenic acid and linoleic acid. Hence the latter are also known as essential fatty acids.

There are 2 pathways for breaking down fatty acids. The main pathway is beta-oxidation and this occurs in the mitochondria. The minor pathway is omega-oxidation and this occurs in the endoplasmic reticulum. Hence, defects in fatty acid oxidation can occur either in the beta or the omega oxidation pathway. In this paper, the focus will be on defects in beta-oxidation.

Types of Fatty Acid Oxidation Defects (FAODs)

The beta oxidation defects can involve the acyl-CoA dehydrogenases or the 3-hydroxylacyl-CoA dehydrogenases. e.g. very-long-chain acyl-CoA dehydrogenase deficiency (VLCADD), medium-chain acyl-CoA dehydrogenase deficiency (MCADD), short-chain acyl-CoA dehydrogenase deficiency (SCADD), multiple acyl-CoA dehydrogenase deficiency (MADD or GAII) and long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD).

MCADD is the most common with an incidence of 1 in 10,000 to 20,000 births. LCHADD and VLCADD are rare disorders occurring at approximately 1 in 100,000 births. There are 2 forms of SCAD deficiency: a severe form occurs approximately 1 in 100,000 and a mild form which is more common and is thought to be benign. There are also rarer FAODs but these will not be discussed here.

Signs and Symptoms

Beta-oxidation defects can present with acute symptoms or chronic symptoms. Examples of acute signs and symptoms include

- **Encephalopathy** (e.g. lethargy which can progress to coma)
- **Urine ketones that are absent or present in small amounts (hypoketotic or non-ketotic) when large amounts are expected**. The human body is expected to produce large amounts of ketone bodies when a person is not feeding well or when a person is hypoglycemic. If urine ketones are absent or present in small amounts (e.g. + or ++ on a urine dipstick), this would generally be considered to be hypoketotic and inappropriate for the above mentioned scenarios.
- **Blood glucose level may be normal or low.** In fatty

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acid oxidation defects, the encephalopathy is usually caused by accumulation of fatty acids. Hence the blood glucose level may be normal during such episodes and the presence of a normal blood glucose level does not exclude the diagnosis of a fatty acid oxidation defect.

- **Hepatomegaly may be present**
- **Plasma ammonia may be elevated**
- **Liver transaminases may be elevated**

These clinical features are usually triggered by physiological stresses such as prolonged fasting, febrile illness, vaccinations etc. Each episode carries with it a high risk for morbidity (neurological sequelae that is usually permanent) and mortality.

There are also some symptoms which are particular to some of the conditions. For example, chronic skeletal muscle weakness can be seen in VLCADD, SCADD, LCHADD and chronic cardiomyopathy is seen in VLCADD and LCHADD.

Diagnosis

Several tests can be used to confirm the diagnosis of an FAOD. Plasma or urine acylcarnitine analysis usually shows the characteristic abnormalities especially if the specimen was taken during an episode of a metabolic crisis. Urine organic acid analysis also frequently show the characteristic abnormalities if the specimen was taken during an episode of a metabolic crisis. It is important to note that in some individuals, these abnormalites disappear during periods of good health hence there is a risk for a false negative result. In such cases, skin fibroblasts can be sent for enzyme analysis and/or in-vitro fatty acid oxidation analysis.

As the genes for these conditions have been identified, DNA testing is also available. In general, it is relatively easy to send specimens for the above tests. However, the interpretation of their results requires domain knowledge of the disease as well as the sensitivity and specificity of each test. Hence, consultation with a metabolic physician may be wise so as to avoid misinterpreting false negatives and false positives.

Newborn Screening for FAODs

The strategy of waiting for these affected individuals to present symptomatically and then diagnosing and treating them is unsatisfactory as it carries with it a risk of morbidity and mortality. Thus, the advent of screening to diagnose these conditions pre-symptomatically is an important step preserving good health in these affected individuals.

The leading way to screen for these disorders is during the newborn period through tandem mass spectrometry acylcarnitine analysis. Each of the above disorder has a relatively distinct pattern of abnormalities and hence a baby with an abnormal profile would be flagged for further evaluation.

For most paediatricians, their participation in the screening process will involve sending the sample and following up a positive screen. In the event of a positive screen, the parents should be contacted and informed of the screening result. An assessment of the baby's status should be done as a matter of urgency e.g. is the baby feeding poorly or vomiting or lethargic? The family should be educated on the need for regular feeds and to avoid fasting, as fatty acid oxidation only occurs during fasting. If the baby is ill (even if it is mildly ill), treatment must be started immediately (IV 10% glucose) and a paediatric metabolic specialist contacted. If the infant is well, referral to metabolic specialist for diagnostic/confirmatory testing should occur.

The main advantage of screening is the reduction in morbidity and mortality. For example, the MCAD screening programme has been shown to be effective in reducing deaths and severe adverse events in children up to the age of 4 years[1] and suggests that the neurological outcome is better in those who were diagnosed through screening.[2] Screening is also more cost effective.[3-5] There are some limitations to screening.[6] There is a risk for false negatives ("normal" result in an affected person) and this can occur if the screening test was done when the baby was not metabolically stressed (e.g. beyond the testing window) or if the baby was carnitine deficient. There is also a risk for false positives ("abnormal" result in an unaffected person) and these can result in parental anxiety, unnecessary testing, detection of carriers and the detection of benign variants e.g. mild SCAD. High quality laboratories have minimum false positive and false positive results. Finally, screening programmes are costly to set up and require much expertise if it is to be well run.

Management of FAOD

After a patient has been diagnosed to have an FAOD, each family should have a written action plan for management of illnesses and this should be available to all services involved in the child's care.

In managing an individual with an FAOD, the main principles are (i) avoiding situations/conditions that predispose to a metabolic crises and (ii) starting treatment before they get into a crisis. Hence, avoid fasting and consider restricting the fat intake to <25% of total caloric intake. Medium chain triglyceride (the predominant fat in coconut milk) should be restricted in MCADD. The patient may need carnitine supplementation (except in VLCAD where this is controversial). During periods that predispose to metabolic crises, increased calories and frequency of feeding and close observation are required.

If a child is not feeding well or is showing early signs of

crisis (e.g. lethargy), the child must be admitted for further management. The cardinal principle revolves around stopping catabolism, in particular, lipolysis. This means giving glucose (which will stimulate insulin production and insulin will inhibit lipolysis) and high calories (but avoiding IV lipids!). As specific therapies may be indicated for a particular FAOD, such as riboflavin for MADDD, involvement of a specialist metabolic physician is recommended.

RESOURCES

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2. Acute Metabolic Illness Protocols. Available at: http://www.childrenshospital.org/newenglandconsortium/NBS/Emergency_Protocols.html. Accessed 11 November 2008.

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Diagnosis of Tetrahydrobiopterin (BH_4) Responsive Mild Phenylketonuria in Japan over the Past 10 Years

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Abstract

Background: A novel therapeutic strategy for phenylketonuria (PKU) has been initiated in Japan. Hyperphenylalaninemia (HPA) results from a phenylalanine hydroxylase (PAH) enzyme deficiency or a deficiency of its cofactor, tetrahydrobiopterin (BH_4). BH_4 can normalize blood phenylalanine levels in BH_4 deficiency, but typically not in PKU. However, since 1999 it has been reported that many HPA patients (serum phenylalanine <20 mg/dL) showed a gradual decrease of serum phenylalanine levels after 24 hours from BH_4 loading. The BH_4 responsiveness seems to be regulated in mild PKU by PAH mutations, and affected by the BH_4 dose and administration period. Methods and Results: In 2002 we formulated a provisional diagnostic criteria for patients with BH_4-responsive PAH deficiency, and newly diagnosed 19 patients in 100 HPA cases between 2002 and 2006. The incidence in the recent 5 years for BH_4-responsive mild PKU among patients with PAH deficiency was 25 %. Conclusion: A total of 31 patients was detected in the past 10 years, and the incidence detected using the provisional diagnostic criteria had increased to 25% among PAH deficient patients. BH_4 treatment for BH_4-responsive mild PKU is a new and effective pharmacotherapy, which replaces or liberalises the phenylalanine-restricted diets for a considerable number of mild PKU patients.

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Key words: BH_4-responsive PAH deficiency, Biopterin, Tetrahydrobiopterin

Introduction

Tetrahydrobiopterin (BH_4) can normalise blood phenylalanine levels in BH_4 deficiency, but typically not in phenylalanine hydroxylase (PAH) deficiency. However, in 1999 Kure et al reported 4 patients with PAH deficiency showed a decrease in blood phenylalanine elevations after BH_4 loading.[1] In 2000, Shintaku et al found that 5 out of 15 patients with mild PKU (serum phenylalanine <20 mg/dL) showed a gradual decrease of serum phenylalanine at 24 hour with BH_4 loading, although no patients with classical phenylketonuria (PKU: serum phenylalanine >20 mg/dL) responded to BH_4.[2] Shintaku et al examined 12 patients with BH_4-responsive PAH deficiency discovered by PKU screening and evaluated the responses in the BH_4 loading tests and formulated a provisional diagnostic criteria, which was the percent decline in serum phenylalanine from initial values after single-dose (>20%), four-dose (>30%), and 1-week BH_4 (>50%) loading tests.[3] We administered this provisional diagnostic criteria to neonatal PKU screening between 2002 and 2006 in Japan and detected 19 patients with BH_4-responsive mild PKU.

Materials and Methods

In all of the 100 patients with HPA detected by neonatal PKU screening, we examined biopterin metabolism by pteridine analysis, dihydropteridine reductase assay and single-dose BH_4 loading test. Four-dose and 1-week BH4 loading tests were conducted in patients who had normal BH_4 metabolism and had decreases in plasma phenylalanine concentrations by over 20% in the single-dose test.

An oral BH_4 (Asubio Pharma, Tokyo, Japan) loading test was performed after demonstrating serum phenylalanine concentrations of greater than 6 mg/dL upon instituting a normal diet, which was maintained during loading tests. In the single-dose BH_4 loading test, BH_4 (10 mg/kg) was administered before breakfast; blood samples were collected

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Table 1. Incidence of BH4-responsive mild PKU in Neonatal Mass-screening in Japan

Before and after implementing a provisional diagnostic criteria in 2002		BH_4-responsive HPA	HPA	Incidence
Total	1995-2006	31	234	13%
Before	1995-2001	12	134	9%
After	2002-2006	19	100	19%

at 0, 4, 8, and 24 hours after loading. In the four-dose BH_4 loading test, BH_4 was administered at doses of 10, 10, 5, and 5 mg/kg at 0, 24, 36, and 48 hours, respectively. Blood samples were obtained at 0, 4, 8, 24, and 52 hours after loading. In the 1-week BH_4 loading test, BH_4 was administered for 1 week at 20 mg/kg/day divided into 3 doses daily. Blood samples were obtained before loading and after 4 and 7 days respectively. Serum phenylalanine concentrations were determined by using an automated amino acid analyser (L-8800; Hitachi, Tokyo, Japan). Serum pteridine was measured by high performance liquid chromatography (LC-10; Shimazu, Kyoto, Japan) after iodine oxidation. Dihydropteridine reductase (DHPR) activity was measured in Guthrie card specimens as described previously.[4]

Results and Discussion

Among these 100 patients, 19 patients had normal biopterin metabolism, and their mean values of percentage decline in serum phenylalanine from initial values were 40, 43, and 52 after single-dose, four-dose, and 1-week BH_4 loading tests respectively. The incidence of BH_4-responsive mild PKU in neonatal PKU screening in Japan was 19% between 2002 and 2006 (Table 1). Before 2002, 12 patients with BH_4-responsive mild PKU were detected among 134 patients with hyperphenylalaninemia (HPA). The diagnosis of BH_4-responsive mild PKU was made using the provisional diagnostic criteria and the incidence of BH_4-responsive mild PKU in neonatal PKU screening increased from 9% to 19% after 2002. A total of 31 patients with BH_4-responsive mild PKU were detected in 234 HPA cases and the incidence was 13% in neonatal PKU screening in Japan over the past 10 years. However, among the 100 patients found by neonatal PKU screening between 2002 and 2006, 4 were affected by BH_4 deficiency and 21 were affected other diseases (for example, neonatal hepatitis), and the remaining 75 patients were affected by PAH deficiency. Therefore the incidence of BH_4-responsive mild PKU among PAH deficiency in neonatal screening was 25% (19 out of 75) between 2002 and 2006. The incidence has increased to 25% after implementing the provisional diagnostic criteria, so that BH_4 treatment is thought to be available for a considerable number of mild PKU cases.

Conclusion

Between 2002 and 2006, 19 patients with BH_4-responsive mild PKU were newly detected by using the provisional diagnostic criteria described above, and during this period, the incidence among patients with PAH deficiency was 25%. A total of 31 patients were detected in the past 10 years in Japan, and the incidence of BH_4-responsive mild PKU detected using the provisional diagnostic criteria has increased to 25%. BH_4 treatment for mild PKU is a new and effective pharmacotherapy, which replaces or liberalises the phenylalanine-restricted diets for a considerable number of mild PKU patients.

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Disorders of Vitamin B12 Metabolism Presenting Through Newborn Screening

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Abstract

Elevated propionyl C3 carnitine is the most common abnormality seen in tandem mass spectrometry newborn screening profiles, with an incidence of 0.15% seen in our South Australian newborn screening programme. The most common cause for this result in our population is vitamin B12 deficiency but differential diagnoses include the inherited disorders of propionic and methylmalonic acid metabolism and cobalamin deficiencies. An approach to confirmatory testing and subsequent management of infants with elevated propionic carnitine is presented.

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Key words: C3 carnitine, Methylmalonic aciduria, Propionyl carnitine

Detection of disorders of B12 metabolism in the newborn period by tandem mass spectrometry relies on finding elevated propionyl (C3) carnitine or methylmalonyl (C4DC) carnitine. Although sensitive, C3 carnitine is not specific for a single condition. It is a marker of vitamin B12 deficiency and propionic academia as well as the methylmalonic acidurias, caused by deficiencies of the methylmalonyl coenzyme A mutase apoenzyme (*mut*o, *mut*-), or its cofactor adenosylcobalamin, as well as the cobalamin abnormalities, *cblC, cblD*, and *cblF* deficiencies, all of which have associated homocystinuria.

Mass newborn urine screening for methylmalonic aciduria has been performed on 1,745,753 newborn samples collected at age 21 days in Quebec since 1975.[1] This programme has provided valuable information on the spectrum of methylmalonic aciduria: from severe, neonatal acidosis to benign asymptomatic organic aciduria. In this population, the incidence of symptomatic methylmalonic aciduria is 1 out of 83,131 births with persistent benign methylmalonic aciduria seen in a further 1 out of 49,900 births. Importantly for this latter group, low to moderate methylmalonic acid excreters without determined biochemical phenotypes had normal outcomes and, when reviewed at 1 year, the MMA excretion had resolved in half of these individuals.[1]

In the South Australian newborn screening programme, elevated C3 carnitine is the most common abnormality seen in the acyl carnitine profile. Repeat blood spot sampling has been performed in 280 infants out of 191,464 screened (0.15%), with a higher recall rate (0.6%) seen in the central Australian (Northern Territory) population. Nine babies with higher metabolite levels or ratios have been recalled for clinical review and further testing (rather than re-sampling) and 8 of these have been shown to be deficient in vitamin B12. All 8 babies tested have had elevated plasma methylmalonic acid but only mildly elevated urine methylmalonic acid was seen in only 2 infants, and in the others, it was normal. We have seen no cases of methylmalonic aciduria or propionic aciduria but 1 case of cobalamin D deficiency presented clinically in the early days of the programme.

The foundation of confirmatory testing is urine organic acid analysis, looking for methylmalonic acid, propionic acidemia metabolites and ketones. Urine and/or plasma amino acid analysis should be performed with particular attention paid to levels of methionine, glycine, homocystine. Plasma total homocysteine and Vitamin B12 estimation should be performed but B12 levels may not reliably predict deficiency. If available, stable isotope quantitation of plasma and urine MMA should be performed. Of great

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Table 1. Acute Management after Recall will Depend on Whether the Baby is Sick or Well.

If Baby is sick	**If Baby is well**
Contact metabolic specialist	Counsel parents
Admit to hospital	Take diagnostic samples
Check blood pH	Encourage breast feeding (low protein content)
Take diagnostic samples	Give vitamin B12 1 mg intramuscular injection if deficient or significant level of MMA
Stop all protein (short term)	Warn parents of possible decompensation with viral illness
Calorie supplement parents are concerned	Write action plans for if/when
(dialysis or haemofiltration may be required in propionic acidemia)	Provide copy of action plan to:· • Parents • Local hospital • Local medical service
Give B12 1 mg IMI	Arrange follow up
Counsel parents	
Cardiac and renal function testing	

assistance is the clinical response to treatment with vitamin B12. Specific enzyme assays on cultured skin fibroblasts are available in specialist laboratories in Basle, Switzerland (Dr Brian Fowler) and Montreal, Canada (Dr David Rosenblatt).

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Glucose-6-Phosphate Dehydrogenase Deficiency: Correlation between the Genotype, Biochemistry and Phenotype

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Abstract

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common genetic enzyme defect present in many people from African, Middle Eastern, Mediterranean and Asian countries. Individuals with the enzyme deficiency may remain asymptomatic, develop an acute haemolytic crises to infections or Fava beans, neonatal jaundice or chronic non-spherocytic haemolytic anaemia. Electrophoretic mobility may be fast, slow or normal. Over 160 mutations have been described, mostly due to single amino acid substitution. Although correlation of the genotype and biochemistry with the clinical phenotype of G6PD deficient individuals remains somewhat variable, there is better correlation among individuals presenting with chronic non-spherocytic haemolytic anaemia, which is related to the NADP structure of the enzyme.

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Key words: Allelic frequency, Favism, Haemolytic anaemia, Molecular mutation, Neonatal jaundice

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is the first and rate-limiting enzyme of the pentose phosphate pathway. By maintaining erythrocytic glutathione in a reduced state (GSH), the enzyme protects cells and haemoglobin against oxidative damage. Deficiency of the G6PD enzyme has been estimated to affect about 400 million people worldwide, including those living in sub-Saharan Africa, Middle-Eastern and Mediterranean countries and many parts of Asia.[1] The G6PD gene is located on the long arm of the X chromosome (Xq28), resulting in an X-linked recessive mode of inheritance.[2] While females with the disorder may be heterozygous or more rarely homozygous, males will always be hemizygous.

Classification

G6PD deficiency may be characterised in different ways, according to the clinical presentation of affected individuals, biochemical properties of the enzyme or molecular genotype at the DNA level.

The clinical manifestations of individuals with G6PD deficiency are highly variable.[3] Deficient individuals may remain asymptomatic throughout all or most of their lives, since the deficiency may have been detected during routine screening at birth. Some individuals with G6PD deficiency present with acute haemolysis induced by the ingestion of Fava beans (favism), medications, infections or other as yet unknown agents or oxidant stressors. In a few cases, the presentation is that of chronic haemolytic anaemia or neonatal jaundice. There are over 400 allelic variants of the G6PD enzyme. Variants have been classified by the World Health Organization (WHO) into 5 classes based on the residual enzyme activity (REA) and clinical manifestations.[4] Class 1 (where there is complete enzyme deficiency) is associated with chronic non-spherocytic haemolytic anaemia. Class 2 is a severe enzyme deficiency (REA <10%) that is associated with acute haemolytic anaemia, while class 3 is a moderate deficiency (REA 10% to 60%). Class 4 has very mild or no enzyme deficiency (REA >60%) and is usually asymptomatic. Class 5 has increased enzyme activity.

The biochemical properties of the G6PD enzyme include its electrophoretic mobility in pH 8.6 buffer, Michaelis constant (Km) for substrate and thermo-stability. An altered enzymatic state will have normal, fast or slow electrophoretic mobility as compared to the normal enzyme.[5]

The active G6PD enzyme is made up of 2 (dimer) or 4 (tetramer) identical sub-units. The primary structure of

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each sub-unit has a molecular weight of 59,265 daltons and consists of 514 aminoacids.[6] The protein-encoding segment of the gene consists of 13 exons spanning 18 kb long.[2] Molecular sequencing has identified over 160 mutations, most of which are the result of single base changes from missense mutations, resulting in single amino acid substitution. Very few variants are the results of in-frame deletions that consist of 1 to 8 codons.[6]

Various researchers attempted more recently to correlate the clinical phenotype and biochemical characteristics of the G6PD enzyme with molecular genotype.

Correlation

Alfinito et al[7] studied 31 G6PD deficient unrelated males in Naples, Italy, including 17 who had been referred following haemolytic crises and 14 who were asymptomatic military personnel detected through screening. The most common variant was G6PD Mediterranean (45%), followed by G6PD Seattle (26%), G6PD A^- (13%), G6PD Cassano (6%) and single cases of G6PD Maewo and G6PD Cosenza. Molecular lesions in the group with haemolytic crises showed a predominance of G6PD Mediterranean (70%). In the group of asymptomatic G6PD-deficient patients, the molecular variant most frequently found was G6PD Seattle (56%), followed by G6PD Mediterranean, Cassano, Cosenza and Neapolis.[7]

Pietrapertosa et al examined fifty-four G6PD deficient unrelated males from Apulia, Italy, for 4 mutations that were known to be more prevalent in the region.[8] The allelic frequency of G6PD Mediterranean (563CT), Seattle (844GC), A^- (202GA) and Montalbano (854GA) in their study population was 48%, 33%, 7% and 3.7% respectively. Four other subjects (7.4%) had an unknown variant. The results suggested that the Apulia population had a polymorphic G6PD molecular deficiency. Most patients (88%) with the G6PD Mediterranean variant had severe (class 2) enzymatic deficiency and normal electrophoretic mobility. Over half of those with G6PD Mediterranean also presented in haemolytic crises. The G6PD Seattle variant was equally seen in subjects with severe and moderate enzymatic deficiency. All patients with G6PD Seattle had slow electrophoretic mobility and were mostly asymptomatic. Most patients (75%) with the G6PD A^- variant had severe enzymatic deficiency together with fast electrophoretic mobility and presented in acute haemolytic crises caused by unknown agents. The G6PD Montalbano variant was associated with severe enzymatic deficiency and normal electrophoretic mobility but did not present with haemolytic crises. Pietrapertosa et al[8] concluded from their data that G6PD enzymatic activity was a poor predictive parameter of acute haemolytic crises and not correlated with clinical features.

Although enzymatic levels remained poorly correlated with acute haemolysis, there appeared to be better correlation of the genotype with phenotype in individuals who presented with CNSHA (WHO class 1). Mason and colleagues beautifully illustrated how a majority of CNSHA mutations were clustered near to the dimer interface and structural NADP molecule or deletion mutations. These mutations in the dimer surface disrupted dimer-dimer contacts between the 2 sub-units or disrupted the structure in the interface by introducing a differently charged or different sized residue.[9] Hirono et al[10] found that class 1 variants that were mutated in a small region of the molecule between residues 385Cys, 386Lys, 387Arg and 410Gly had a raised Km NADP and were extremely thermolabile in low (10 μM) NADP. The enzymes that were rendered inactive in low NADP were reactivated on exposure to high NADP, suggesting that the mutations affected NADP binding and stability.[10]

Babies with G6PD deficiency frequently develop significant hyperbilirubinemia in the first week of life, requiring phototherapy. Several authors had shown that G6PD-deficient babies developed higher serum bilirubin levels than those with normal G6PD values, even when no evidence of other factors known to cause hyperbilirubinemia was present.[11,12] Furthermore, babies with G6PD deficiency were more likely to require exchange transfusion than those without.[11]

Ainoon et al[13] studied 86 G6PD deficient Malaysian Malay male babies through DNA analysis of umbilical cord blood samples. G6PD Viangchan (871GA), Mediterranean (563CT) and Mahidol (487GA) accounted for 37%, 27% and 15% respectively of the cases studied. Of the 71 neonates who developed jaundice, 57 (80%) required phototherapy, with only 1 progressing to severe jaundice requiring an exchange transfusion. They found no significant difference in the incidence of neonatal jaundice, mean serum bilirubin level, mean age for peak serum bilirubin, percentage of babies requiring phototherapy or mean duration of phototherapy between the 3 common variants that they studied.[13]

While several studies showed variable correlation between genotype, biochemistry and clinical characteristics of G6PD deficiency, there appeared to be much better correlation between molecular structure and chronic non-spherocytic haemolytic anaemia. Residual enzyme activity was generally poorly predictive of the clinical presentation.

Reasons

Several reasons were suggested for the variable clinical manifestations of G6PD deficiency.[14] In females diagnosed with G6PD deficiency, clinical expression was related to the random inactivation of the chromatin of the G6PD-normal or G6PD-deficient chromosome, a phenomenon

first proposed by Lyon.[15] Among males with G6PD deficiency, the response of different individuals with the same mutation to a single drug dose may vary widely, depending on the acetylator status of an affected individual.[16]

The age group and dietary pattern of the G6PD deficient individual influences the clinical presentation. While a G6PD deficient adult may consume Fava beans in the diet, a newborn baby would have minimal exposure to Fava beans so that favism would not be reported in the neonatal period. Even among affected individuals with similar G6PD mutations, not all would have the same reaction towards Fava bean. It had been proposed that superimposed genetic deficiencies may account for the highly variable haemolytic response to Fava beans.[14]

Among babies with G6PD deficiency, neonatal jaundice has often been ascribed to insufficient conjugation of serum bilirubin with liver glucuronide, rarely to increased haemolysis alone. In 1996, the cause of Gilbert's syndrome was identified as a polymorphism in the promoter region of the uridine diphosphate glucuronosyl-transferase-1 (UDPGT-1) gene important for bilirubin conjugation.[17] Subsequently, it was shown that a major factor influencing bilirubin levels in babies with G6PD deficiency lay in the expression of this UDPGT-1 gene.[18, 19]

Conclusion

Correlation between the genotype, biochemical characteristics and clinical phenotype of G6PD deficient individuals presenting with acute haemolysis remains somewhat variable. Nevertheless, there is better correlation between the molecular variants and the clinical presentation of WHO class 1 individuals with chronic non-spherocytic haemolytic anaemia. This correlation has been associated with the NADP structure of the enzyme. Further study will aid in unravelling the complex interaction of molecular variants, environmental factors (such as infection and medications), oxidative stress and residual enzymatic activity on the clinical expression of G6PD deficiency in affected individuals.

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External Quality Assurance Programme for Newborn Screening of Glucose-6-Phosphate Dehydrogenase Deficiency

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Abstract

Introduction: The nationwide neonatal screening for glucose-6-phosphate dehydrogenase (G6PD) deficiency in Taiwan was started on 1 July 1987. A network of G6PD referral hospitals distributed all around Taiwan was organised for follow-up, confirmatory testing, medical care and genetic counselling. To assess the reliability of confirmatory and screening tests, an external quality assurance (QA) programme for G6PD assay was developed. Materials and Methods: Lyophilised quality control (QC) materials and dried blood spots were prepared from erythrocytes and whole blood for confirmatory and screening tests, respectively. The external QA surveys were carried out every 1 to 2 months. The QA results were evaluated and compared to the consensus result and reference value. The test results were submitted through internet by participating laboratories and the summary reports were published on a webpage (http://www.g6pd.tw) within 2 weeks. Results: Twenty-one referral laboratories in Taiwan and 16 screening laboratories in Germany, Lebanon, Mainland China, Philippines, Thailand, Taiwan, Turkey, and Vietnam have been participating in the QA programme. From 1988 to 2007, 144 QA surveys for confirmatory testing were sent to referral laboratories. Among the 2,622 reports received, 292 (11.1%) were found to be abnormal. Interlaboratory coefficient of variation (CV) for the confirmatory test has reached below 10% in recent years. The significant improvement in interlaboratory CV was found to be correlated with the preventive site visits to the referral laboratories since November 2004. From 1999 to 2007, 52 external QA surveys for the screening test were performed. Among 504 reports received, 97 (19.2%) were found to be abnormal. From the 5040 blood spots tested by the screening laboratories, 95 false negative (1.9%) and 187 false positive (3.7%) results were reported. Conclusions: The external QA programme has been useful for monitoring the performance of the referral hospitals and screening laboratories and helpful for the participating laboratories to improve their test quality.

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Key words: External Quality Assurance, Glucose-6-Phosphate Dehydrogenase, Newborn Screening

Introduction

Glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) deficiency is the most common enzymopathic disease in Southeast Asia and other tropical areas worldwide.[1-3] This X-linked genetic disorder (MIM 305900) has been found to be an important cause of neonatal jaundice and acute haemolytic anaemia in the southern Chinese population in Taiwan.[4,5] In order to reduce the complications of G6PD deficiency, such as kernicterus, permanent neurological damage and death, nationwide neonatal screening for G6PD deficiency was started on 1 July 1987 after a pilot project conducted between November 1984 and June 1987 had demonstrated the practicality and the efficiency of neonatal screening of G6PD deficiency in Taiwan.[6,7] The effective collection rate has reached more than 99% of all newborns in Taiwan since 1996 and the overall incidence rate of G6PD deficiency was found to be about 2%.[8-10] The screening programme in Taiwan consists of 3 screening centres and 22 referral hospitals. The referral hospitals, which were located all around Taiwan including

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the outlying islands, were organised to provide confirmatory testing, medical care and genetic counselling for the screen positive cases. In order to assess the reliability of the confirmatory test performed by the referral laboratories, an external quality assurance (QA) programme for the determination of G6PD activity in erythrocytes has been carried out since January 1988.[11,12] In 1999, an external QA survey for the G6PD screening test was incorporated into this QA programme to assess the reliability of the G6PD screening test for the neonatal screening centres.[12] This report presents the results of the external QA programme for the G6PD confirmatory and screening test for the past 20 and 9 years, respectively.

Materials and Methods

External QA Programme for Quantitative Assay of G6PD Activity in Erythrocyte

Standardised procedures for quantitative analysis of G6PD activity in erythrocyte and methods for the calibration of spectrophotometer and micropipette were distributed to all participating laboratories. Erythrocyte G6PD activity was recommended to be determined kinetically at 37°C using the reagent kit (Cat. No. 345) produced by Sigma Chemical Co. (St. Louis, MO, USA) and Trinity Biotech (Co. Wicklow, Ireland) with maleimide as the inhibitor.[13] The quality control (QC) materials with different G6PD activity used for quantitative assay were prepared as described previously.[11] Briefly, the G6PD activity of the red blood cells (RBC) was assayed.[13] The RBC with normal and deficient G6PD activity were lysed and then mixed with each other in different proportions to prepare QC samples with different G6PD activities. These haemolysates were then dispensed into glass bottles and lyophilised.

Periodically, within 1 to 2 months, 3 (July 1992 to December 2007) or 5 (January 1988 to June 1992) QC materials were sent to each participating laboratory on dry ice by speed post delivery. The results of G6PD activity analysis were requested to be returned by facsimile (and internet submission since 2005) within 8 days. The external QA results were evaluated and compared to the median of all reports received and the reference value determined by our laboratory. The reported result was considered to be erroneous when: (i) more than two-thirds of the G6PD values were outside 80% to 120% of the median; and/or (ii) G6PD values were inconsistent with median values. Besides written summary reports, the summary has also been published on the website http://www.g6pd.tw as soon as the report was released since 1995. For participants with system errors detected by this external QA programme, troubleshooting was proceeded either by telephone contact or personal visit from the reference laboratory. In order to further reduce the error rate and improve the interlaboratory CV, a routine preventive site visiting programme to the participating laboratories has been installed since November 2004.

External QA Programme for Neonatal G6PD Screening Test

The QC materials with different G6PD activity were prepared from whole blood and spotted onto newborn screening blood collection filter paper. In brief, the G6PD activity of whole blood obtained from normal and G6PD deficient donors were measured by the quantitative confirmatory test method[13] as mentioned above and followed by centrifugation to separate the RBC and plasma. After washing with normal saline, the RBC with different G6PD activities were mixed in different proportions for preparing QC materials with different G6PD activity. These combined RBC were then mixed with plasma (45%) and spotted onto the blood collection filter paper used for neonatal screening.

Periodically, within 1 to 2 months, 10 QC specimens were randomly picked for each survey and distributed to each neonatal screening laboratory by speed post delivery. Reports were requested to be returned by facsimile (internet submission since 2005) within 4 days for screening centres in Taiwan and 8 days for overseas screening centres. For each QA survey, the G6PD activity of the QC dried blood spots was determined by quantitative assay[13] to set the reference values before the QC specimens were send out. The results reported by the screening centres were evaluated against the consensus result and compared with the quantitative reference values determined by our laboratory. The summary report for each survey was published on website http:/www.g6pd.tw within 2 weeks after the survey samples had been sent.

Results

External QA Programme for Quantitative Assay of G6PD Activity in Erythrocyte

Twenty-one referral laboratories have been participating in this external QA programme for quantitative assay of G6PD activity in erythrocyte. These include 9 medical centres, 7 regional hospitals, 3 local hospitals and 2 independent clinical laboratories, which have been providing confirmatory G6PD quantitative assays to the G6PD screen positive cases reported by neonatal screening centres in Taiwan. From January 1988 to December 2007, 144 QA surveys of G6PD quantitative test were carried out for these referral laboratories. In total, 2662 reports were received for these surveys. The reporting rate increased gradually from 81% in 1988 to 100% since 1996 (data not shown). Two hundred and ninety-two reports (11.1%) were found to have abnormal QA results, which were attributed

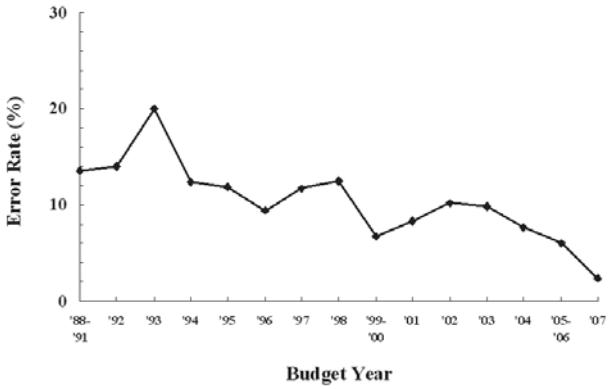

Fig. 1. Error rate found in the external QA survey for quantitative assay of G6PD activity in erythrocyte.

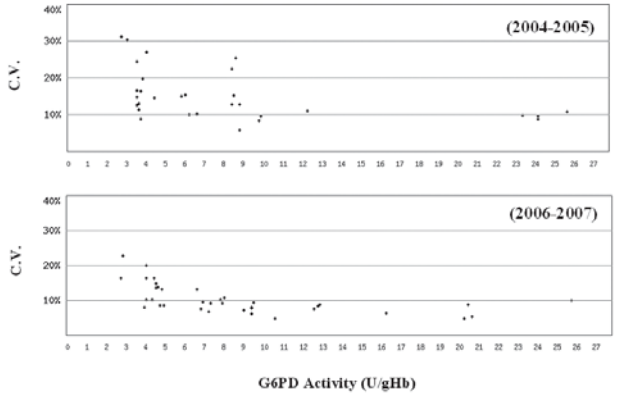

Fig. 2. Interlaboratory CV of the external QA survey for quantitative assay of G6PD activity in erythrocyte at different G6PD activity.
CV: coefficient of variation.

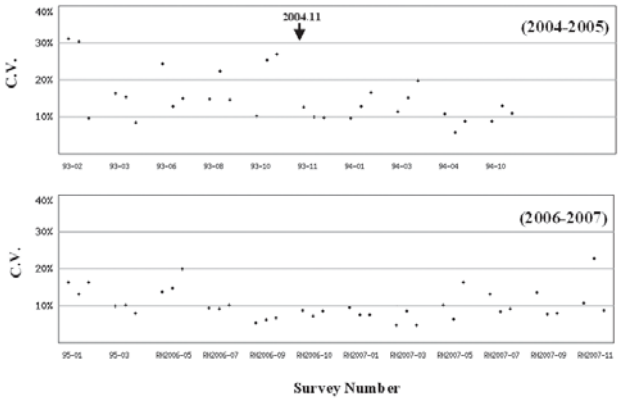

Fig. 3. Interlaboratory CV of the external QA survey for quantitative assay of G6PD activity in erythrocyte. The 2004.11 mark indicates the start of the routine preventive site visiting programme.

mainly to clerical (12.0%), experimental (18.5%), and instrumental errors (44.2%). Since 2006 the error rates have been decreased to less than 6% (Fig. 1). Most of the experimental and instrumental errors were found in those laboratories that did not execute internal QA properly. The average interlaboratory CV has been reduced from 15.1% between 2004 and 2005 to 10.3% between 2006 and 2007 (Fig. 2). Recently (2006-2007), almost all the interlaboratory CVs in the critical G6PD activity range between 6 and 13 U/gHb for the diagnosis of G6PD deficiency had been controlled within 10% (Fig. 2). The significant improvement of interlaboratory CV was found to be correlated with the preventive site visits to the participating laboratories since November 2004 (Fig. 3).

External QA Programme for Neonatal G6PD Screening Test

In addition to the 3 neonatal screening centres in Taiwan, 13 overseas neonatal screening centres/laboratories (6 in Mainland China, 2 in Philippines, and 1 each in Germany, Lebanon, Thailand, Turkey, and Vietnam) have also joined in this external QA programme (Table 1). From March 1999 to December 2007, 52 QA surveys for G6PD screening test were performed. Five hundred and four reports were received for these surveys and the reporting rate was 100%. Ninety-seven reports (19.2%) were found to have abnormal QA results, which contained 95 false negative (1.9%) and 187 false positive results (3.7%) (Table 1). The significant increase in number of errors in year 2003 and 2004 were contributed by those screening centres which changed their testing method during that period of time and as well as by screening centres that had newly joined the QA programme. Most of the false negative and false positive results were found in QC samples with borderline G6PD activities of 3.0-4.3 U/gHb and 4.4-6.0 U/gHb (normal newborn cutoff of the reference method: 4.4 U/gHb), respectively (Table 2). The false negative and false positive decisions in these borderline cases are most likely caused by different cutoff levels used by those screening laboratories. For example, all 6 screening centres using the same PerkinElmer Neonatal G6PD Kit (ND-1000) have reported different cutoffs (1.6, 1.8, 2.0, 2.1, 2.6, and 2.9).

Discussion

The external QA programme with preventive site visits to the referral laboratories has successfully controlled the interlaboratory CV under 10% in the critical G6PD activity range for diagnosis of G6PD deficiency. The well-controlled interlaboratory CV provided a basis for uniform interpretation using the same reference range of confirmatory test data generated from all G6PD referral hospitals nationwide in Taiwan. The screening centres may use the data generated by the external QA programme to compare the decisions made by other screening centres and to adjust the cutoff used in their own screening test accordingly.

The results of this G6PD QA programme revealed the

Table 1. Results of External QA Survey for Neonatal G6PD Screening Test

Period	No. of Laboratory	Report		Specimen		False positive	False negative
		n	error (%)	n	error (%)		
1999	8	39	0 (0.0%)	390	2 (0.5%)	0	2
2000	8	48	0 (0.0%)	480	0 (0.0%)	0	0
2001	8	48	7 (14.6%)	480	23 (4.8%)	23	0
2002	8	47	6 (12.8%)	470	15 (3.2%)	15	0
2003	10	48	17 (35.4%)	480	51 (10.6%)	33	18
2004	12	66	28 (42.4%)	660	92 (13.9%)	72	20
2005	12	48	9 (18.8%)	480	16 (3.3%)	16	0
2006	12	71	19 (26.8%)	710	54 (7.6%)	15	39
2007	16	89	11 (12.4%)	890	29 (3.3%)	13	16
Total		**504**	**97 (19.2%)**	**5040**	**282 (5.6%)**	**187**	**95**

Table 2. External QA Results of Neonatal G6PD Screening Test at Different Ranges of G6PD

G6PD activity (U/gHb)	No. of specimen	Reported decision		False positive	False negative
		Positive	Negative		
0.1 ~ 1.9	791	791	0	0	0
2.0 ~ 2.9	373	362	11	0	11 (2.9%)
3.0 ~ 4.3	390	306	84	0	84 (21.5%)
4.4 ~ 6.0	954	131	823	131 (13.7%)	0
6.1 ~ 26.8	2532	56	2479	56 (2.2%)	0
Total	**5040**	**1646**	**3394**	**187 (3.7%)**	**95 (1.9%)**

G6PD activity in the QC specimens were determined as described in the text
Cutoff: 4.4 U/gHb

importance of external QA. These external QA programmes for quantitative and qualitative analysis of G6PD activity provide a good system to monitor the performance of the screening and diagnostic services for G6PD deficiency. The external QA programme might also serve as a guide for participating laboratories to improve the quality of their service. Although an external QA programme can help laboratories reduce analytical errors, it is indispensable for every laboratory to establish and to carry out strictly their own internal quality control to achieve better quality of clinical laboratory service.

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Neuroblastoma Screening in Japan: Population-based Cohort Study and Future Aspects of Screening

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Abstract

Introduction: It is unknown whether screening for neuroblastoma has the benefit of reducing the incidence of advanced disease or mortality due to neuroblastoma. Japanese nationwide mass-screening for 6 month old infants was launched in 1985 and was performed using quantitative high-performance liquid chromatography (HPLC) between years 1990 to 2003. Materials and Methods: We compared the incidence rates (IR) and the mortality rates (MR) per 100,000 births of neuroblastomas diagnosed before 6 years of age between 2 cohorts: children born during the years 1980 to1984 (Pre-screen cohort, n = 7,620,203) and 1990 to1998 (Screen cohort, n = 10,878,918). We then proposed the optimal timing and procedures for future screening. Results: Cumulative IR in the Screen cohort was significantly higher than the Pre-screen cohort (29.80 vs. 11.96, *P* <0.0001). On the other hand, IR of neuroblastoma diagnosed after 24 months old in the Screen cohort was significantly lower than in the Pre-screen cohort (*P* <0.0001). The cumulative MR of the Pre-screen cohort was 5.35, whereas that of the Screen cohort was 2.82 (*P* <0.0001). Conclusions: HPLC mass-screening for neuroblastoma at 6 months of age found a marked increase in incidence in younger children (less than 12 month old) and a significant decrease in mortality rates overall. To reduce overdiagnosis of regressing cases and to identify preclinical stages of unfavourable cases, we propose using HPLC-screening at 18 months of age.

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Key words: Effectiveness, High-performance liquid chromatography (HPLC), Incidence, Mortality, Screening

Introduction

Neuroblastoma, one of the most malignant childhood solid tumours, accounted for about 15% of cancer mortality in children.[1,2] Since neuroblastoma usually produces catecholamine, urinary levels of their metabolites vanillylmandelic acid (VMA) and homovanillic acid (HVA) are useful markers for diagnosis. To improve outcomes in neuroblastoma by the early detection of preclinical tumours in infancy,[3,4] nationwide mass-screening test for neuroblastoma at 6 months of age was launched in Japan between 1984 and1985.[5] Initially, this test used a qualitative spot test for VMA and was replaced by the quantitative HPLC test in 1990. In 2003, acknowledging that there was overdiagnosis of occult tumours that would have spontaneously regressed or matured, the Japanese government decided to halt neuroblastoma screening. In response to this, a research group had been established to clarify the effects of screening at 6 months of age for neuroblastoma and recently reported the effectiveness of this, using a retrospective population-based cohort study.[6]

In this study, we reconsidered the effectiveness of nationwide Japanese neuroblastoma mass screening, especially quantitative HPLC screening, and proposed an adequate screening system for 18 month old children.

Materials and Methods

Study Design

Quantitative HPLC analysis of urinary VMA and HVA levels for screening was introduced around 1990 in most areas of Japan.[6] Using HPLC, the positive criterion was that the value of VMA or HVA was higher than the mean + 3 SD μg/mg creatinine. We compared the incidence (IR) and mortality rates (MR) in the patients diagnosed until 72 month of age in the cohort born between 1980 and 84 (Pre-screen) and that born between 1990 and1998 (Screen). Data on patients with neuroblastoma were obtained via the

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cancer registry of 2 major Japanese societies caring for neuroblastoma patients (Japanese Society of Paediatric Surgeons and Japanese Society of Paediatric Oncology). Both registries had data of all screening cases registered and follow-ups for at least 5 years. These databases were approved for use by our consortium by the ethics committee of each of these societies, as well as that of Hiroshima University. Patients were staged according to the INSS or Evans staging system.[7,8] Patients of any age with stage 1 or 2 disease, and those less than 12 months with stage 3 or 4S disease treated with either surgery or surgery plus chemotherapy, and patients aged 12 months or older with stage 3, and those with stage 4 disease were typically treated according to the protocol by the Japanese Neuroblastoma Study Group.[9]

Matching the death cases in our database to the cases in the death certificate files from the Ministry of Health, Labor and Welfare in Japan revealed that our database included 62.5% of all neuroblastoma cases diagnosed in Japan.[6]

Statistical Analysis

IR and MR per 100,000 births of neuroblastoma diagnosed at less than 72 months of age were estimated using the registry data. Multiple comparison for proportions were performed after applying variance stabilisation using arcsin transformation to evaluate the significance level for a large number of comparisons.

Results

Compliance rates for screening between 1990 and1998 was 85.9%. In the Pre-screen and Screen cohorts, 570 and 2025 cases were diagnosed respectively. The Screen cohort included 1537 cases detected by screening.

The IR in the Screen cohort was significantly higher than in the Pre-screen cohort. (29.80 vs. 11.56, P <0.0001, Table 1). In cases diagnosed before 5 months of age, IR in both cohorts remained within similar ranges. The IR of neuroblastomas diagnosed between 6 and 72 months of age was 10.27 in the Pre-screen cohort and 27.80 in the Screen cohort (P <0.0001). IR of clinically diagnosed neuroblastomas between 6 and 72 months in the unscreened group of the Screen cohort (11.35) was similar to that of the Pre-screen cohort (10.27). In the Screen cohort, IR of both localised and stage 4S neuroblastomas were markedly elevated, while IR of disseminated stage 4 was not. The IR of neuroblastomas diagnosed between 24 and 72 months of age significantly declined in the Screen cohort (3.24 *vs.* 1.87, P <0.0001). In all neuroblastomas diagnosed before 72 months of age, stage 4 disease was also significantly lower in the screened subgroup of the Screen cohort (P <0.05).

Out of the 570 and 2025 cases in the Prescreen and Screen cohorts, 255 and 192 patients died respectively; cumulative MR were 5.35 and 2.82 respectively. (P <0.0001). In comparison with the MR of cases diagnosed between 6 and 72 months of age in the Pre-screen cohort, that of the Screen cohort were significantly lower (P < 0.0001). MR in patients with neuroblastomas diagnosed between 6 and 72 months of age in the Pre-screen cohort was similar to that in unscreened subgroup in the Screen cohort (4.89 vs. 4.79).

Discussion

In 2 well-known prospective studies of neuroblastoma screening,[10,11] screening did not seem to reduce mortality, although a high incidence of early-stage disease was noted in these studies. Previous reports from Japan have commonly noted an increased incidence in these infants, but some of them had also observed a reduction in mortality rates.[12,13] Our recent population-based study, using a nationwide Japanese cohort, revealed a significant reduction in mortality by screening.[6] The controversy surrounding this issue may be derived from several factors, including age at screening, screening methodology, compliance rates, study design, diagnostic activity/ability, and socio-economic factors. The most critical problems for this controversy were the low mortality rates for neuroblastoma and the difference in incidence. In a previous effort initiated in Quebec using a cohort consisting of about 500,000 children, the number of observed neuroblastoma death cases was only 22, which may be too small to adequately evaluate for a reduction in mortality rate. In a Germany study which was larger than the Quebec programme, IR of neuroblastoma in the control cohort was 7.3, which was clearly low compared to the incidence typically reported.[1,2] In the present study, IR in the control cohort was 11.96 and more than 100 deaths occurred in each cohort, allowing us to detect significant differences in MR. Only then can the effectiveness of screening for neuroblastoma become apparent for HPLC-screening. In fact, the IR of advanced (INSS 4) neuroblastoma detected more than 2 years of age was significantly decreased. The difference in cumulative MR due to neuroblastoma diagnosed before age 72 months between screened and unscreened groups in the Screen cohort was 22.3 per million.

The MR of the unscreened subgroup in older patients did not show any decrease during these 2 decades. These data indicate that no major advances have been made in neuroblastoma treatment in cases of disseminated disease in older patients. The recent advent of intensive myeloablative therapy with stem cell transplantation may significantly improve survival in advanced disease but would not increase the total number of cases cured. Thus

Table 1. Numbers of Patients, IRs, and MRs of Neuroblastoma Diagnosed Younger than 72 Months of Age

	Pre-screen	**Quant-screen**
Year of birth	**(1980-1984)**	**(1990-1998)**
No. of birth	**7,620,203**	**10,878,918**
No. of screened children (%)	**–**	**9,342,132 (85.9%)**
Total cases of neuroblastoma	570	2026**
Total IR	11.96	29.80
RR (95% CI)	–	2.49 (2.27-2.73)
Cases diagnosed at 1-5 months (IR)	81 (1.70)	135 (1.99)
Cases diagnosed at 6-72 months(IR)	489 (10.27)	1891(27.81)**
Cases in screened children (IR)		1781(30.52) [1536(26.31)]
Cases in unscreened children(IR)		109(11.35)
Localized stage (1-3) (IR)	199 (4.18)	1478(21.74) [1434(24.56)]
Disseminated stage 4 (IR)	251 (5.27)	291(4.28) [231(3.96)]
Stage 4S (IR)	10 (0.21)	101(1.49) [97(1.66)]
Total deceased cases among children with	255	192**
neuroblastoma diagnosed at 0-72 months		
Total MR	5.354	2.824
RR (95% CI)		0.53 (0.44-0.64)
Deceased cases diagnosed at 0-5 months (MR)	22(0.462)	18(0.264)*
Deceased cases diagnosed at 6-72 months (MR)	233(4.892)	174(2.558)**
Screened children (MR)		128(2.091) [20(0.343)]
Unscreened children (MR)		46(4.789)

CI: confidence interval; HPLC: high-performance liquid chromatography; HVA: homovanillic acid; IR: cumulative incidence rate until 72 months of age (per 100,000 births); RR: relative risk; VMA: vanillylmandelic acid

[ ]: Number of screening-detected cases, ** $P < 0.01$, * $P < 0.05$.

early diagnosis and prevention of dissemination in unfavourable disease, in particular screening, may be the most effective means of improving outcomes in neuroblastoma. Thus, the most appropriate age for screening to detect unfavourable disease at early stages with minimum detection of favourable tumours should be elucidated. Most neuroblastomas detected through screening at 6 months of age had favourable characteristics[14] and would either spontaneously regress or mature into ganglio-neuromas.[15] In one study it was observed that for restricted local neuroblastomas detected by screening approximately 40% to 60% regressed spontaneously.[16] However, a whole genome expression profiling study of neuroblastomas detected by screening failed to molecularly differentiate tumours at high risk for progression from those in which the natural history was instead likely to be that of spontaneous regression or differentiation.[17] This phenomenon suggested that some favourable infant tumours might turn into unfavourable types and early detection of these unfavourable tumours is most desired (Fig. 1).[18] According to several studies which adopted "wait and see" strategies,[16,19] urinary VMA and HVA levels would have declined to normal ranges by 18 months of age in almost all regressing tumours (Fig. 1). To decrease overdiagnosis without sacrificing efficacy, a pilot programme of neuroblastoma screening for 18-months-old children has been proposed and launched in several prefectural committees. In the recent report evaluating Japanese screening,[6] neuroblastoma cases that were not picked up during screening at 6 months of age (false negatives) were estimated as 15.2% within the whole cohort of neuroblastoma cases diagnosed between 6 and 72 months of age. In this report, sensitivity, specificity and positive predictive values of HPLC screening were calculated as 84.7%, 99.9%, and 21.1% respectively. Neuroblastoma screening for 18 month old children is also expected to reduce false negative cases.

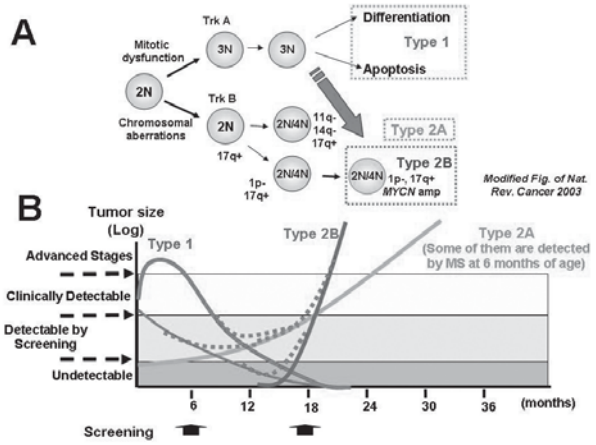

Fig. 1. Neuroblastoma development hypothesis.
A. This was modified figure described previously.[20] Usually, neuroblastoma was divided into 3 types: Types 1 (low risk), 2 (intermediate) and 2B (high risk). Type 1 tumours were infant low risk tumours which show triploid (3N) and regress/mature spontaneously. Type 2 tumours which were diagnosed at older children, have several genetic aberrations and progress into advanced stages. Type 2B tumours are the most aggressive ones with *MYCN* gene amplification. In the present study, early stages of Type 2 tumours are not found out in the tumours detected by screening but Type 2 tumours in elder children significantly decreased, suggesting that some Type 1 tumours might turn to become Type 2 tumours.

B. Time course of each type of tumour in neuroblastoma. According to several studies adopting "wait and see" strategies,[16,19] most regressing or maturing tumours were diminished until 18 months of age and majority of unfavourable type 2A or 2B tumours progress after 18 months of age. Thus, we propose a pilot programme of neuroblastoma screening for 18 months old children.

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Legal Issues in Neonatal Screening

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Abstract

Legal issues arise if some persons or institutions feel wrongfully treated whether or not this feeling is justified. In neonatal screening, the following topics may be causing legal issues: no screening programme where such a programme should be (UN Convention for the right of the child); neonate(s) not screened for conditions within the established programme; no consent when it should have been given; error(s) in sampling, analysis, reporting; no follow-up available, error(s) in confirmatory diagnostics and treatment; irregular storage of dried blood spot specimen. Legal issues can be solved easily when responsibilities of parties concerned have been established and documented. Unfortunately, legal systems vary from country to country and what has become "normal" practice in one jurisdiction may still be battled about in another. The management of a neonatal screening programme should try to define as best as possible the performance criteria and to have the programme assessed and accredited to certain internationally accepted standards. It diminishes the chances for errors and it helps to avoid legal issues.

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Key words: Legal, Neonatal, Performance, Rights, Screening

The title of this presentation comprises 2 concepts. Neonatal screening does not need extensive introduction since it has become part of routine public health in many countries around the globe. It consists of a series of events, starting with taking a blood sample from the newborn infant and analysing this sample for one or more components that are indicative of the presence or possible development of a congenital metabolic disorder. If the results indeed point to such disorder, then the child is referred to a paediatrician for confirmatory diagnostics, usually by analysing a venous blood sample, treatment by medication or diet, and eventually, long-term follow up for one to several years to ensure that the child develops as normally as possible with as few deteriorating health effects as possible.

Legal issues is a more difficult concept which can be understood as matters related to the legal system. A legal system is defined in several ways such as an explicit, institutionalised, and complex mode of regulating human conduct, or as a set of rules which influence behaviour. In addition, a legal system is always connected to social and moral rules and finally, there is a relationship between the concepts of law and ethics. Legal issues arise when there is a discrepancy between an individual's presumed rights and the perceived situation in practice. In other words, identical practical situations may be viewed by one individual as normal whereas another may conclude that his rights are violated and consequently will take legal steps to protect his rights.

To understand when and where legal issues exist or may arise, it is necessary to answer 4 questions:

1. Which rights are valid in a certain situation?
2. Are these rights real or presumed?
3. What is the practical situation in which someone feels unhappy?
4. Is that unhappiness realistic or perceived?

To make this more concrete, let's consider the following examples.

1. A car collision. The person whose car has been hit normally has a right for damage reimbursement, i.e. a real right; if the reimbursement does not take place, he can start a legal procedure.
2. House on fire. Most communities have stipulated the period in which the fire brigade should be present to fight the fire. If the fire brigade is too late, the house owner normally has a right to claim damage reimbursement from the local municipality; again, if the

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reimbursement does not take place, he can start a legal procedure to enforce his claim.

3. In healthcare the situations may be more difficult. If the surgeon in spite of correct administrative data amputates the wrong leg, the patient can claim damage reimbursement and otherwise he can sue the surgeon. On the other hand, if a smoker gets lung cancer, can he really claim anything from the tobacco company; if he exposes his lungs to cigarette smoke, does he have a real or a perceived right for healthy lungs?

Back to neonatal screening. Can a parent claim the availability of a neonatal screening programme for a certain condition, e.g. phenylketonuria, in his jurisdiction? Can he claim reimbursement if it is not available? Can he argue that his jurisdiction should have the same screening policy as another jurisdiction where screening for phenylketonuria is available? What are his real rights?

In this respect, it is interesting to read the United Nations Convention on the Rights of the Child[1] which was ratified by virtually all UN member states. In Article 24, it reads that "States Parties recognise the right of the child to the enjoyment of the highest attainable standard of health and to facilities for the treatment of illness and rehabilitation of health" and that "States Parties shall strive to ensure that no child is deprived of his or her right of access to such healthcare services". On the one hand, the text emphasises the right of the child, e.g. to be screened neonatally, and on the other hand, it does not go further than that the States shall strive to achieve this. The parent mentioned above therefore cannot claim anything in this respect.[2-4] He can only try to persuade the politicians in his jurisdiction to make neonatal screening available.

In case there is a neonatal screening programme running, it should stipulate performance criteria such as coverage of the newborn population, timeliness of sampling, analysis and reporting results, availability of treatment, follow-up etc, and last but not least the conditions of storage of dried blood spot specimens and its potential use.[5] In such a situation, there may be a legal issue if a child is not sampled at all, not sampled within the stipulated time window, results are lost, the child is not referred in time, or the use of the stored specimens for other purposes.[6] Depending on the local organisation the parent can claim damages with the programme management, the person not having taken the sample, the laboratory not having performed the analysis in the prescribed way etc.

In general, the more precise a situation has been defined, the easier it is to determine whether rights are valid and have been violated and the better the chance on success when claiming for damage reimbursement. The management of a neonatal screening programme should try to define as best as possible the performance criteria and to have the programme assessed and accredited to certain internationally accepted standards. It diminishes the chances for errors and it helps to avoid legal issues.

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Inborn Errors of Metabolism Presenting as Neonatal Encephalopathy: Practical Tips for Clinicians

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Abstract

Inborn errors of metabolism constitute an important cause of neurological disease in the neonatal period and can present clinically as encephalopathy. Although it is relatively rare, it is important to have a high index of suspicion. Appropriate investigations and a step-wise approach to diagnosis allow for early institution of treatment and can prevent significant morbidity and mortality. The aim of this article is to give a brief outline of the various inborn errors of metabolism to consider in neonatal encephalopathy and to provide a framework for investigation and diagnosis.

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Key words: Hyperammonemia, Metabolic acidosis, Refractory seizures

Neonatal encephalopathy is a clinically defined syndrome of disturbed neurological function in the infant during the first week after birth, manifested by difficulty initiating or maintaining respiration, depression of tone and reflexes, altered level of consciousness and often seizures. Encephalopathy can be subtle in the neonatal period and can be missed unless signs are specifically looked out for.[1] This can range from poor suck and jitteriness to seizures and apnoea. Seizures in a neonate may not be the typical tonic clonic seizures, but a series of brief jerks or spasms of muscles of the face, limb, and tongue. They can also present with brief fixation or eyelid flutter, sucking movements or apnoea.

There are many causes of neonatal encephalopathy and inborn errors of metabolism are rare causes. However, it is important to have a high index of suspicion as early diagnosis and intervention can significantly alter the prognosis. Acute encephalopathy due to metabolic disorders usually result from accumulation of a toxic substance e.g. ammonia or deficiency of an essential product e.g. ATP.[2] Most of these metabolites are able to cross the placenta and therefore the baby is usually well at birth. A typical history would be a baby who was well initially, but only to present with poor feeding, lethargy or seizures after a few days.

Inborn errors of metabolism presenting with encephalopathy can broadly be divided into 2 main groups, one with significant biochemical abnormalities and another group with none. It is essential to do baseline investigations to further classify these patients. Baseline investigations would include a plasma electrolytes, ammonium, amino acids, lactate, acid-base status and glucose level. Urine sample for ketones and organic acids are also useful (Table 1).

Encephalopathy with Significant Biochemical Abnormalities

(i) Hyperammonemia

When there is significant hyperammonemia, the main metabolic disorders to exclude are urea cycle defects and organic acidurias.[3] As these disorders mainly affect the protein metabolism, they often present after the baby has established full feeds. The presence of significant metabolic acidosis in organic acidurias distinguishes the 2 groups of disorders as acidosis is usually severe in organic acidurias. Other rare causes of hyperammonemia to consider are fatty acid oxidation disorders and transient hyperammonemia of the newborn.

Ammonium is neurotoxic. Neurological damage and the eventual prognosis is dependent on the duration and severity of hyperammonemia. Therefore, it is essential to institute urgent treatment. All protein intake should be stopped and adequate caloric intake ensured. Treatment includes intravenous sodium benzoate or sodium phenylbutyrate. Severe or refractory cases may require hemodialysis or

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Table 1. Investigations for Neonatal Encephalopathy

Baseline investigations
Electrolytes, calculate anion gap
pH and blood gases
Glucose
Plasma ammonium
Plasma lactate
Urine ketones
Plasma amino acids
Urine organic acids
Second-line investigations
Plasma carnitine and acylcarnitine profile
Urine amino acids
Urine reducing sugars
CSF amino acids
CSF glucose
CSF neurotransmitters

hemofiltration. Other medications may be required depending on the metabolic disorder suspected.

(ii) Metabolic Acidosis

A common laboratory feature of many inborn errors of metabolism during an acute illness is metabolic acidosis with an increased anion gap. A few of these conditions can present acutely in the neonatal period with mainly neurological symptoms. These can be divided into the main anions that cause the acidosis.

Lactic acidosis

Lactic acidosis can be a common acute feature in critically ill neonates, often as a result of poor circulatory perfusion or seizure activity. However, persistent lactic acidosis or increased lactate in the cerebrospinal fluid is suggestive of metabolic disease. In a neonate with encephalopathy and persistent lactic acidosis, one needs to exclude mitochondrial defects or pyruvate metabolism defects.[4]

Organic acidurias

Organic acidurias such as methylmalonic acidaemia (MMA) and propionic acidaemia (PA) are disorders of branched-chain amino acid metabolism.[5] Metabolic decompensation often occurs in the first week of life after establishing feeds and is heralded by signs of encephalopathy accompanied by marked metabolic acidosis. This is also usually accompanied by hyperammonemia as a result of a secondary inhibition of the urea cycle. Urine organic acid analysis would show excretion of the abnormal organic acid and is often diagnostic.

Acute management of organic acidurias includes removal of offending substrate and ensuring adequate caloric intake. Administration of carnitine facilitates the excretion of the organic acids. In addition, cofactors such as vitamin B12 or biotin are often given during the acute illness as the patient might have a vitamin B12 responsive MMA or some patients with multiple carboxylase deficiency are responsive to biotin.[3] Treatment for secondary hyperammonemia may be necessary.

(iii) Hypoglycaemia

Hypoglycaemia can be a presenting feature in many metabolic diseases. The timing of hypoglycaemia in relation to feeds can be very useful in directing the next line of investigations.[6] If hypoglycaemia occurs shortly after a feed, one should always suspect hyperinsulinism. Disorders of gluconeogensis can present after fasting of a few hours e.g. Glycogen storage disease type I. Hypoglycaemia can also be a presenting symptom of galactossaemia.

Fatty acid oxidation defects are also an important group of conditions to consider in hypoglycaemia.[7] These neonates have an impaired capacity to use stored fat as a source of energy during periods of fasting. Other clinical features include hepatomegaly, myopathy and Reye syndrome. Urine organic acid analysis, measurement of plasma carnitine and acylcarnitine profile are the main line of investigations. Confirmatory studies can be performed on skin fibroblast or mutation analysis.

Encephalopathy with no obvious biochemical abnormalities

However, there are several other causes of neonatal encephalopathy that may not have any biochemical clues. Non-ketotic hyperglycinemia (NKH) typically presents in the first few hours or days of life with refractory seizures and progressive obtundation.[8] The presence of hiccups may be a clue. Diagnosis is based on an increased CSF: plasma glycine ratio.[9] Treatment in classical NKH is often disappointing and mortality is high. Survivors will have profound neurological sequelae.

Maple syrup urine disease (MSUD) can also present with deepening encephalopathy in the first week of life. There is accumulation of branched chain amino acids (BCAA), due to decreased activity of branched-chain ketoacid dehydrogenase complex. Leucine in particular is neurotoxic. These patients may not have any significant biochemical abnormality apart from ketosis and occasional hypoglycaemia. Plasma amino acid analysis reveals increased leucine and allo-isoleucine levels. Other BCAAs e.g. valine and isoleucine can be elevated too.

Molybdenum cofactor deficiency or isolated sulfite oxidase deficiency can also present with encephalopathy

and intractable seizures in the neonatal period. As the disease progresses, they have pyramidal signs, choreoathetosis and severe mental retardation. A characteristic lens dislocation may be presents in infancy. A low plasma uric acid is found in molybdenum cofactor deficiency. A positive urine sulfite dipstick can point towards this diagnosis, but is low in sensitivity and specificity. Urine amino acid analysis typically shows excretion of large amounts of sulfite, thiosulfate, S-sulfocysteine and cystine. The enzyme deficiency can be diagnosed via assays of cultured fibroblast or liver.

Glut-1 deficiency syndrome can also present with intractable seizures in the first week of life. This is due to a defect in the protein that transports glucose across the blood brain barrier. Diagnosis is suggested by a CSF: blood glucose ratio of <0.6. Confirmatory testing includes red blood cell glucose uptake studies and mutation analysis.[10] Treatment involves a ketogenic diet to provide alternative fuel to the brain.

Vitamin-responsive seizures are another rare cause. Pyridoxine-dependent seizure can begin in the first week of life or even as early as in utero. These seizures are non-responsive to traditional anticonvulsants. These patients have a dramatic response to pyridoxine which is evident both clinically, as well as, on the electroencephalogram. Other similar vitamin responsive seizures include pyridoxal phosphate and folinic acid.[11]

Conclusion

It is important to consider inborn errors of metabolism when faced with a neonate who is encephalopathic. Baseline investigations are important in streamlining the diagnostic process and the need for more extensive and sometimes, expensive diagnostic tests. Early diagnosis and intervention is critical in determining the morbidity and mortality from such cases.

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Financing Newborn Screening Systems: US Experience

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Abstract

Newborn screening (NBS) in the United States (US) has existed since the early 1960s and is required in all 51 state jurisdictions. It is generally recognised that NBS provides a significant public health benefit by preventing or markedly decreasing the adverse medical consequences of conditions included in the screening panel. There is currently no US national NBS policy, so instead there are 51 independent state programmes that vary widely in their policies, infrastructures, procedures and services. Not surprisingly, US NBS programme costs and methods of financing also vary. Surveys have increasingly found a reliance on fees to pay for screening tests, short-term follow-up and other parts of state NBS systems. This article reviews some of the current US NBS financing issues and methodologies.

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Key words: Fees, Financing, Newborn screening, Paediatrics, Screening

Introduction

Newborn screening (NBS) for phenylketonuria (PKU) began in the US in the early 1960s.[1] The intended impact of screening was to reduce or eliminate the mental retardation known to result from PKU, and thus, to keep patients from being institutionalised at the government's expense. As NBS began in each state, a legislative requirement to screen all newborns was usually enacted in order to ensure full population coverage and to define sustainable financing from government funding. At the time, unfunded mandates from state governments were rare and it was relatively easy to develop an overall government cost savings justification for NBS. Savings arose since PKU patients were routinely housed in government mental institutions. Annual institutionalisation expenses for a single patient are significant and the magnitude of the expenses multiplies over a lifetime. Therefore, detection of relatively few cases of PKU results in overall government cost savings when testing costs are compared to the costs of institutionalisation over a lifetime.[2]

As state NBS programmes expanded to include other conditions in the 1960s and 1970s, the financing issues became more complex. The primary funding issues centred on the fact that all screened conditions did not impact government spending to the same degree. Some screened conditions did not necessarily result in institutionalisation, so government costs savings were not as obvious. The result was a gradual move towards NBS programmes that could be sustained without government financial support. Thus, state public health departments began to consider NBS fees in the late 1960s, and now almost all programmes have some level of fee.[2] Figure 1 provides a graphical look at the fees that exist in state NBS programmes today (discussed later).

NBS screening in the US originated at the state level and there has never been a national NBS policy. Despite the lack of national NBS policy, there has been sporadic federal financial support for state NBS programmes, often as part of genetics funding activities. In the mid-1970s, there was specific federal funding legislation that applied directly to genetic disease screening. However, in 1981 genetic diseases became a part of block grant funding to the states, and newborn screening and genetic service activities became a part of the Health Resources and Services, Maternal and Child Health Branch (HRSA/MCHB) Special Projects of Regional and National Significance (SPRANS). In the late 1980s, special supplemental SPRANS funds were used to encourage universal NBS for sickle cell diseases to help enact a 1987 consensus recommendation from the National Institutes of Health.[2]

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Other SPRANS projects have indirectly impacted NBS over the years including genetics planning and implementation grants and grants for various state data projects. Since 1999, HRSA/MCHB has funded the National Newborn Screening and Genetics Resource Center (NNSGRC) through a cooperative agreement with the University of Texas Health Science Center at San Antonio.[3] Regional cooperation/collaboration for NBS follow-up activities has also been encouraged by HRSA/MCHB. During the 1980s, HRSA/MCHB funding supported a national collaboration that resulted in the 'Council of Regional Networks for Genetic Services.' Since 2004, HRSA/MCHB cooperative agreements have supported 7 redefined regional collaboratives for genetics and newborn screening and a national coordinating centre in an effort to continue to enhance clinical genetic services and other family support needs in geographic areas lacking such resources.[4]

The specifics of NBS financing over time can be tracked through various survey reports. A 1983 survey reviewing the impact of reduced federal support of genetic services showed that block grant funding had resulted in prioritisation of NBS by public health departments to in order to preserve their funding.[5] Those states that were not charging a fee for NBS then, were under pressure to begin one, and by 1985, about half of all programmes were collecting a NBS fee.[6] By 2001, 13 NBS programmes reported that fees were their sole funding source and 19 other NBS programmes reported fee revenue comprised at least 60% of their newborn screening revenue.[7] In 2006, fees existed in 45 NBS programmes[8] and today, the number stands at 47.[9]

Methods

The usual method for fee collection is by charging for a screening 'kit' that includes the filter paper collection device and accompanying data submission form(s). A mailing envelope into which multiple forms may be placed often accompanies kit orders, although mailing costs may or may not be included in the kit cost. Lancets and other specimen collection supplies are usually not included in the kits and must be supplied by the specimen collection facility. Kit costs usually include screening laboratory costs and may include certain other system costs including programme administration, public relations, education, and/or screening follow-up among others. In order to speed up the process of specimen transport, there is a trend towards courier services, and this cost is sometimes included in the kit pricing. The components included in NBS fee calculations can be extensive and complex, and fee amounts vary accordingly.[9]

Kit charges are usually payable at the time the kit order is filled. Since kits are ordered in advance of birthing, payments may be extended over a certain time window (varies from 30 to 120 days) to facilitate cash flow at the specimen collection facility (where costs are usually recovered from third party insurers). A limited number of NBS programmes bill specimen submitters periodically (usually monthly) based on specimens received at the screening laboratory. In cases where 2 or more specimens may be required by NBS policy, the initial kit charge often includes the cost of the second or subsequent kits.

Private health insurance usually includes payment for NBS as part of maternity benefits and public welfare insurance (Medicaid) also includes NBS. While it is usually the submitter who must recover insurance costs, at least one NBS programme bills insurers directly. Since Medicaid payments are state regulated, reimbursement methods and amounts vary. In a limited number of states, Medicaid funds are transferred directly to the NBS programme, but in most cases, these payments are part of negotiated hospital diagnosis related grouping (DRG) agreements. For this reason, it is sometimes difficult to increase NBS kit or service fees since the DRG agreements cover specified time periods and a NBS cost increase during the contract period may not translate to a corresponding DRG increase. In no case does inability to pay for state-required NBS services at the patient level prevent screening. Various mechanisms exist to accommodate non-payment including averaging such occurrences into the overall fee basis.

Results

As of August 1 2008, 47 state NBS programmes report collecting a fee for NBS (see fig. 1).[9] Nine programmes require 2 screens on each newborn, as indicated by an asterisk in the table, and for each, the fee given is for an initial screen. In cases where an initial fee is not easily separated from the two-screen fee, the fee has been halved to approximate the cost of a single initial screen. In Arizona, there is a higher fee of $10 for the second screen and in Colorado the second screen costs $10 less. In at least 2 states, a small surcharge is included in the fee to accommodate additional programme costs and in at least 1 state, newborn hearing screening is included in the screening fee. The average initial screening fee for those charging a fee is $51.89 (with the adjustments noted for those with multi-specimen fees), although not all programmes provide identical services nor do they have identical screening panels. Current fees for US programmes can be found at http://www2.uthscsa.edu/nnsis/ (accessed September 23, 2008).

Fee income is usually processed in one of two ways – either as a deposit to the state's general revenues or as income for dedicated programme use. NBS programmes

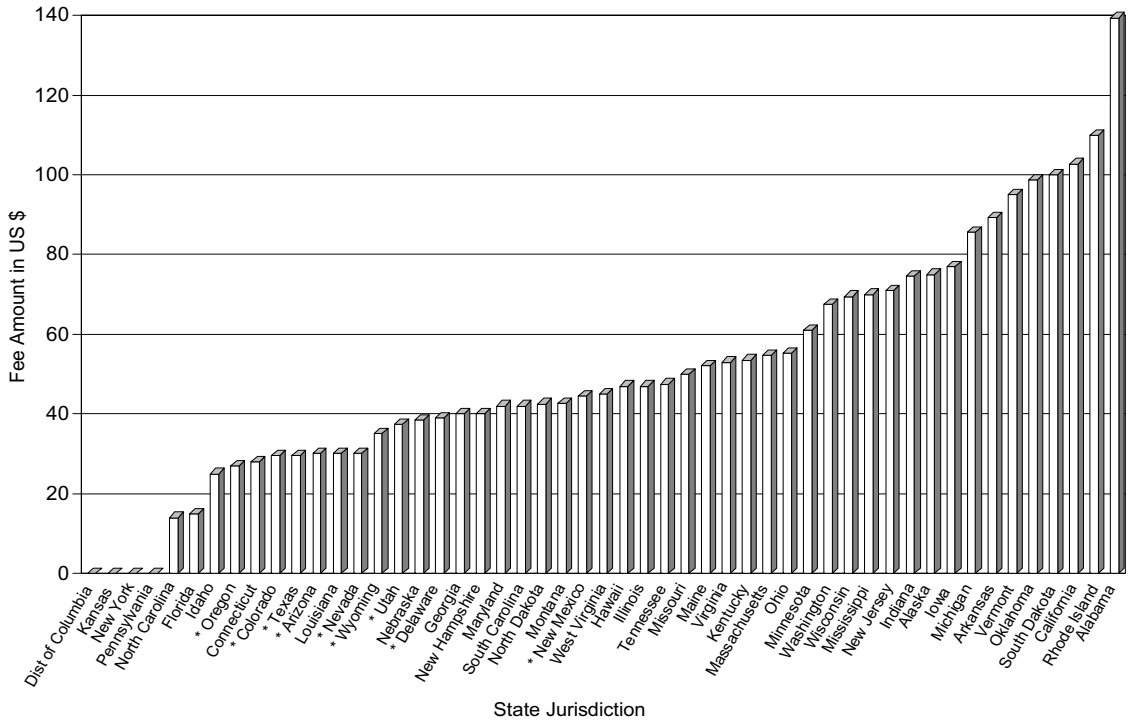

Fig. 1. Fee amounts currently[9] charged in US newborn screening programmes in ascending order.

may have to compete for the use of general revenue funds along with other government programmes, while competition for dedicated funds is usually not necessary. In either case, political considerations have been known to affect fund usage.[10] In cases where a contracted laboratory provides screening services, the fee may be collected by that laboratory. The contract may require payment of part of the fee to the NBS programme for follow-up/education and related administrative costs. Most fees do not include costs for start-up of new screening procedures. Lack of start-up funding usually means that a separate request to the state legislature is needed to add expensive technologies for screening expansion. Thus, NBS expansion in a government setting is often slower than in the private sector (other factors may also contribute such as lower salaries received in the government sector and lack of trained personnel).

Discussion

At the national level, most NBS funding comes from fees with limited augmentation from federal block grant funds and legislative appropriations. The US General Accounting Office (GAO, now Government Accountability Office) reported that in 2001, 64% of 2001 NBS programme funds came from fees, 5% from the Maternal and Child Health Services block grant, and 19% from other state funds.[8] A 2007 survey confirmed that 90% of US NBS programmes had a fee, 61% obtained some funding from the federal maternal and child health block grant programme, and 33% obtained some support from state general revenues, although the relative amounts were not reported.[7] The GAO Report also noted that laboratory costs outweighed non-laboratory costs approximately 2 to 1 in 1999.[8] NBS programme expansions have likely caused this ratio to shift towards increased non-laboratory costs since larger numbers of conditions are being simultaneously detected that require additional follow-up and education.[2]

Many US NBS systems continue to lack extensive long-term tracking and comprehensive education programmes,[11] and most provide little or no funding for medical interventions or counselling.[12] Some programmes include payment (or partial payment using a sliding fee schedule based on family income) for PKU formula, but little else. As programmes have expanded, payment for other medical interventions (including specialty care, metabolic formulas and foods, drugs, counselling, and surgical interventions) have been identified as major policy considerations, but financing them as part of NBS remains controversial. There are special public assistance programmes for children and families with special health care needs, including those related to NBS conditions, but services and accessibility vary.[8]

While a national coding system for Medicaid reimbursement for medical laboratory services exists, there are currently no codes for NBS tests, and this has been cited as a hindrance to receiving reimbursements from insurance by some programmes. A national NBS fee coding model has been suggested for discussion,[2] but no actions have yet resulted. Recent Congressional action has resulted in the passage of authorisation legislation that directly impacts newborn screening,[13] but until appropriations are approved, forward movement on the activities included in the Act will be limited. It is likely that appropriations for federally supported NBS activities will help in the national harmonisation process, but it is unlikely that services and fees will become uniform nationally without a stricter national policy in this regard. Regardless of the funding stream, NBS personnel remain dedicated to maintaining quality screening systems.

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Developmental Dysplasia of the Hip: Universal or Selective Ultrasound Screening?

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Abstract

Developmental dysplasia of the hip (DDH) is an intriguing condition that evolves during infancy. It would be thus foolhardy to expect a screening tool at birth to be both highly sensitive and specific. Uncertainty regarding an optimal screening method is compounded by a general lack of sound epidemiological data. Clinical screening remains widely used. Some reports estimated that it did not pick up 60% of children who eventually needed surgery. Ultrasonography, it was hoped, would improve detection rates. There are 2 approaches to ultrasound; universal screening, which is adopted by some European countries, or selective screening of high-risk infants. The problems with universal ultrasound screening are high false positive rates and high costs. The benefit was a possible 6- to 10-fold reduction in surgery for late DDH. Similar reductions though had also been reported if ultrasound was used selectively for infants with clinical and historical risk factors. A literature review on this topic is presented. There are pros and cons for both screening strategies. This is reflected in the different protocols that exist among various countries. For healthcare systems that are considering their options, universal ultrasound screening is generally not cost-effective and should not be the preferred screening strategy.

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Keywords: Dysplasia, Hip, Screening, Ultrasound

Introduction

Physical examination using the Ortolani and Barlow tests is the mainstay of screening for developmental dysplasia of the hips (DDH). Abnormalities detected through clinical screening ranged from 0.4 to 168 per 1000 newborns,[1] and the reported incidence for Singapore (4.7 per 1000)[2] and Malaysia (0.7 per 1000),[3] which share similar demographics, also differed significantly. This heterogeneity could be due to true difference in prevalence among populations, experience of the screener[4] as well as the exact criteria for diagnosis. Compounding this uncertainty is the intriguing nature of hip dysplasia. It most likely represents a spectrum of developmental disorders that may manifest only after the neonatal period.[5]

A practical one-point-in-time diagnostic test for DDH, which is critical to evaluating screening strategies, does not exist. This contributes to the pervasive practice of treating newborns with a positive screening result. More recently, a report based on the Medical Birth Registry of Norway which looked at total hip replacement (THR) rates for a birth cohort of more than 2 million newborns over a 38 year period found that only 8% of those who underwent THR due to dysplasia had unstable hips at birth.[6] The authors cast doubt on the effectiveness of clinical testing for neonatal hip instability. A similar conclusion was arrived at from an earlier United Kingdom study.[7]

To compare the effectiveness of different screening strategies, the "true" incidence of DDH also needs to be looked at. This is because the majority of newborn hip abnormalities become normal without treatment.[8] Thus, to reliably conclude which is the best screening strategy, adequately powered randomised controlled trials are needed, with a precisely determined endpoint, true DDH.

In the astonishingly large number of studies found in the literature, true DDH was defined differently, due to the many methods of case ascertainment. Most referred to hips that were still abnormal after 4 to 6 weeks, when it was thought that the initial neonatal immaturity of the hips would have largely resolved by then. The other significant problem causing poor quality of evidence was the inadequate follow-up of newborns with a negative screening result, falsely assuming that none in this group would have developed DDH. Extended follow-up of a birth cohort, which is necessary for study validity, is not commonly incorporated in most protocols because obviously it is extremely resource-intensive.

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Universal Ultrasound Screening

The availability of ultrasound since the 1980s has helped greatly in the diagnosis and management of DDH. The methods based on Graf[9] and Harcke[10] are commonly used. Because it is non-invasive and can pick up problems in the cartilaginous hip much earlier than radiology, efforts to use it on clinically normal hips began, with the aim of reducing missed cases of DDH. Ultrasound has been used to screen whole population of newborn babies, especially in Europe. However the main problem with ultrasound screening mirrors that of physical examination, in that the vast majority of sonographically abnormal hips in the neonatal period turn out to be normal eventually.[11,12]

Synder et al[13] reported their experience since 1985 with universal ultrasound screening. To overcome the problem with high false positive rates if performed too early, infants were screened at 6 weeks old. They reported a gradual decrease in hip surgeries, and hence recommended universal ultrasound in populations with a very high prevalence of DDH like in Poland (68 per 1000).[14] In Synder's report 1500 sonograms or more might have been needed to prevent 1 case of hip surgery.

Bialik et al[15] used an algorithm to reduce unnecessary treatment for sonographically abnormal hips. As a result, only 10% out of 995 sonographically abnormal hips required treatment. Non-treated hips all developed normally at the age of 1 year. Unnecessary treatment, either surgical and non-surgical, carries a risk for avascular necrosis of the hip. Using historical controls Von Kries et al[16] calculated that there was an 80% reduction in the incidence of first operative procedure for DDH since 1996 when universal ultrasound screening was made routine in Germany.

For universal ultrasound screening to work optimally, the following conditions should preferably be met:

i) The population prevalence of DDH is high.
ii) Infants should be screened after 6 weeks old, which means a detailed follow-up programme for a birth cohort is needed.
iii) A protocol is used to reduce unnecessary treatment of mildly dysplastic but stable hips. A dedicated paediatric orthopedic and physiotherapy team is essential.
iv) The cost of universal hip ultrasound, including trained radiologists, hardware and follow-up, is acceptable to the local healthcare system.

Selective Ultrasound Screening

Selective ultrasound screening strategies are generally risk factor-based, aiming to lessen the burden of screening every newborn with ultrasound. In a meta-analysis Lehmann et al[17] estimated that the odds for DDH by various risk factors were 5.5 for breech delivery, 4.1 for female gender and 1.7 for a positive family history. Other commonly cited variables are musculoskeletal abnormalities (torticollis, foot deformity, sacral dimple), abnormal hip examination (clicks, limited hip abduction) and intrauterine growth retardation. Approximately 7% to10% of a birth cohort would have a risk factor for DDH.[18-20]

Descriptive studies based on this selective, risk-based ultrasound screening strategy yielded contradictory results, with only some reporting reduced cases of late DDH.[18-21] This would not be surprising because 60% of DDH infants had no associated risk factors.[22] As such the only probable rationale for using the selective strategy would be for it to complement clinical screening, especially if the latter was not performed by experienced personnel.[4]

Which Strategy?

Considerations for choosing a particular screening strategy usually revolve around 2 factors, effectiveness and cost. Despite the scores of published studies on DDH, there remained a paucity of randomised controlled trials (RCTs) dealing with effectiveness of screening methods. In fact there was only 1 RCT[23] and 1 quasi-randomised study[24] comparing universal versus risk-based screening by ultrasound.

Rosendahl et al[24] allocated 11,925 newborn infants to receive either general, selective or no ultrasound screening in addition to the clinical examination. Follow-up was till 27 months old. They found that general ultrasound screening resulted in a higher treatment rate than in either the selective or in the no ultrasound screening groups (3.4%, 2.0% and 1.8%, $P < 0.0001$). For infants not subjected to treatment, ultrasound screening resulted in a higher follow-up rate because of non-conclusive early findings. Prevalence of late DDH was lower for general ultrasound screening, but the differences were not statistically significant (0.3, 0.7, 1.3 per 1000, $P = 0.11$). They concluded that "the effect of ultrasound screening in reducing … late DDH was at best marginal despite a considerable increase in diagnostic and therapeutic efforts". About 1000 sonograms were needed to pick up 1 late DDH compared to clinical examination alone.

Holen et al[23] conducted the only true RCT comparing general versus selective ultrasound screening. After 6 to 11 years of follow-up, out of a total of 15,529 infants, there was 1 case of late DDH in the general group compared to 5 in the selective group (0.13 versus 0.65 per 1,000, $P = 0.22$). Again a large number of infants (nearly 2000) needed to be screened to detect an additional case of late DDH if general ultrasound screening was used.

Given the unexpectedly low prevalence of late DDH in the 2 studies, a much larger sample size than initially assumed would have been needed to achieve statistical significance, if at all. However, taking both studies together,

given the lower prevalence of late DDH in the general ultrasound screening groups, it would appear that this was the better strategy in terms of preventing late DDH. In health economics terms though, this advantage would come at much higher screening, follow-up and treatment costs per quality adjusted life year (QALY). Emotional anxiety of parents of false positive infants should also not be under-estimated or discounted.

In the face of considerable debate over the effectiveness of ultrasound screening, it is not surprising that very few cost studies had been done. Clegg et al[25] studied surgical and screening costs before and after routine universal ultrasound and found that costs were comparable. After routine ultrasound was implemented, the higher screening cost was offset by fewer number of surgeries performed, and at a lower surgical cost per surgery because they were done earlier (and as such surgically less complicated). Hernandez et al[26] used a decision analysis tool incorporating assumed probabilities of a particular outcome and resource expenditure to determine the utility (value) of clinical versus ultrasound screening strategies. They concluded that ultrasound screening (either selective or universal) had a lower value when compared to clinical screening alone. Brown et al estimated that costs for screening 100,000 newborns using the different methods to be £4 million for universal ultrasound, £3 million for selective ultrasound and £1 million for clinical screening alone.[27]

Conclusion

Although limited in number, the available high quality evidence does not support using ultrasound either universally or selectively to screen for DDH, both from the effectiveness and cost perspectives. This conclusion is reflected in the various published national guidelines.[28-30] As such universal or selective ultrasound screening should not be done outside a well-designed research setting. Training doctors to be proficient in clinical screening for DDH remains the priority.[4]

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Minimising Harm from Newborn Screening Programmes

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Abstract

The challenge of newborn screening programmes is to maximise benefits and minimise harms. These harms include pain inflicted as a result of taking the test, reduced by pain relief and training of specimen takers; from false positive and negative test results (impacting both affected families and healthcare professionals), minimised effectively by taking the sample at the correct time, precise and specific tests, appropriate disorder definition, well chosen cut-offs (which may be informed by a large series of diagnosed cases of the screened disorders) second-tier tests, age adjusted normal ranges and anxiety which may be appropriate but limited by the availability of information. Programme audit is important in early detection of problems.

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Key words: Newborn metabolic screening; Quality

Introduction

Newborn baby metabolic screening programmes are public health programmes which minimise the morbidity and mortality from inborn errors of metabolism. However, these inborn errors of metabolism are rare conditions and relatively few babies get significant benefits from the screening programme while there is the potential for many more to be harmed, albeit in a minor way. The screening programme has components of sampling, laboratory testing, repeat and diagnostic testing, treatment, policy/planning; funding, and audit. Each of these components can impact adversely on both affected and unaffected infants.

Method

Components of a typical newborn baby metabolic screening programme were examined for negative impacts on affected and unaffected infants, and for strategies for minimising the negative impacts.

Results

Figure 1 shows the interrelation of the different screening programme components. The likely harms resulted from the components include the following.

Policy and Planning – Test Cut-offs

Setting test cut-offs must take into account also the true biological value and the imprecision of the test at that level to ensure all infants with values at the cut-off are recalled. Assays using blood dried on paper are inherently less precise than the equivalent serum tests which must be taken into account. Improvement of assay performance so the test has minimum variation around the cut-off means less both false positive and false negative results. There must be understanding of assay bias if comparing with literature and other programmes and use of the International Society for Neonatal Screening (ISNS) minimum dataset and kits calibrated against ISNS reference materials[1] can assist harmonisation. Each local programme needs to make value judgements of the cost of additional recalls against the benefits of finding additional, probably mild cases of screened disorders. Additional specificity can be gained by using cut-offs varied with infant maturity and age at specimen collection.[2]

It has been suggested[3] that disorder cut-offs are defined by the use of large studies combining all the positive tests from many screening programmes rather than by using statistically determined cut-offs. This is a commendable approach (historically well used by screening programmes but without the benefit of large case numbers obtainable by world-wide collaboration) but may need modification to take into account disease severity and timing of sample.

All positive tests rightly generate anxiety in families and it is proper that programmes seek to minimise this. The anxiety however may be appropriate and short-lived[4] but over the screened community early detection may minimise anxiety and the stress of caring for affected, untreated children. False alarms cause desensitisation of laboratory and follow up personnel which may result in failure to take

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appropriate speedy action when a true case presents. False positive tests can result in the expense and inconvenience of unnecessary treatment unless they are followed by appropriate diagnostic tests.

False negative tests impact on those with the disorder and with screening programme credibility. There are those which are unavoidable (screening marker not raised, or not reliably raised at the time of testing e.g. blood glycine in non-ketotic hyperglycinemia, immunoreactive trypsin in cystic fibrosis screening); and those which are avoidable due to programme failures. False negative tests can be minimised by good laboratory practices.[5] Greater clarity of disorder definition and of community expectations can minimise the impact of false negative test results.

Sample Collection

It is now recognised that neonates feel pain, including that from medically induced procedures. All affected and unaffected infants will be inflicted. Recent recommendations from the Royal Australasian College of Physicians Paediatrics and Child Health Division for minimisation[6] include:

- Do not warm the heels of the neonates as this does not reduce pain or aid blood collection
- Consider use of oral sucrose (0.5-1 mL 24%, 2 min before)
- Encourage breast or bottle feeding
- Ensure parent or carer holds baby
- Use automated retractable lancet

Samples sometimes cannot give reliable test results when they are badly collected perhaps due to insufficient blood collected, layered blood, spills etc, or because they are not taken at a time for which the programme has validated cut-offs. Or because the analyte of interest is not abnormal at that time e.g. samples taken are too early for detection of amino acid disorders. A recent study testing pairs of satisfactory and unsatisfactory portions of the same sample showed both low and high bias depending on the cause of the inadequacy giving increased false positive or negative tests depending on the disorder. Samples collected too early may give false positive screens for congenital hypothyroidism, cystic fibrosis and congenital adrenal hyperplasia, and false negative for amino acid and fatty acid oxidation disorders. Unless age-adjusted normal ranges are used, samples collected too late can also give false negative results for cystic fibrosis and fatty acid oxidation disorders. Strategies for minimisation are training and education of specimen collectors and laboratory assessment of the effects of the particular problem with the sample and accepting a result which may be quantitatively inaccurate but in screening terms an accurate result. Delays in sending samples can also produce harm from delayed diagnoses, minimised by collector education and provision of courier or stamped envelopes.

Policy and Planning – Disorder definition

The decision limit for a positive test impacts both false positive and false negative test numbers. First, the disorder must be defined, taking into account that while these conditions are monogenic they present in a spectrum of disease ("metabolome") and the mild end of the spectrum may not have significant clinical benefit from early diagnosis. The Australasian programmes have begun to define conditions e.g. cystic fibrosis, "Cystic fibrosis (CF): Patient with one or more characteristic phenotypic features (including meconium ileus); *or* a history of CF in a sibling; *or* a positive newborn screening result AND 2 CFTR disease-causing mutations or a sweat chloride concentration greater than 35 mmol/L". The aim of definitions like these is to enable screening programmes to determine programme metrics in a timely manner, to reduce false positive tests and to exclude as a screening objective detection of e.g. CF so mild that it presents as cough or infertility in older adults.

Laboratory Testing

Newborn screening laboratories typically test large numbers of samples of which a low proportion have positive tests and a lower proportion are affected by the screened condition. It is too easy for lack of attention to lead to muddled samples and screening laboratories lack the benefit of past history clinical laboratories have to aid in detection of this type of error. The typical screening programme has a large number of repetitive action involved in punching and testing samples which put staff at risk from overuse and pain syndromes. Development of more automated testing systems minimises both human errors and risk of occupational health harms.

An important strategy for improving specificity one is the use of more specific first line testing for example use of immunoassays with more specific antibodies, analyte ratios such as phe/tyr to improve PKU screening. Second tier tests such as succinylacetone in tyrosinemia screening, ph/tyr ratio in PKU screening, specific 17-hydroxyprogesterone or steroid profiles in CAH screening have proven benefits on test specificity.[7]

Treatment

Potential harm resulting from unnecessary or inappropriate treatment can be minimised by the use of confirmation before treatment (e.g. screening test plus appropriate clinical symptoms, screening test plus diagnostic test). Complete diagnostic test algorithms are available (the American College of Medical Genetics - ACMG ACT sheets).[8] Ongoing involvement of local specialist paediatricians is important to ensure locally appropriate,

Fig. 1. Interaction of screening programme components.

up-to-date protocols for repeat testing following screen-positive results are available.

Programme Audit

Programme audit is critically important in recognising and solving problems which can arise from changed circumstances e.g. kit antibodies, laboratory information systems programming, locally changed specimen collection practices. Such things as recall rates for the different screening tests, coverage, sensitivity, specificity and positive predictive value of the different tests could be monitored. Programme audit must inform programme policy.

Discussion

All newborn metabolic screening programmes do a lot of good, and all do some harm. The programme challenge is to maximise the former while minimising the latter. Screening programme components other than those above can also affect outcomes, e.g. insufficient funding and uninterested government departments. Most programmes have laboratory funding but not specimen collection, diagnosis and treatment funding from the same source, which creates difficulties in programme management. The strategies for minimisation of harm discussed above are primarily those from the part of the programme most easily managed, the laboratory.

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Newborn Screening in China: Phenylketonuria, Congenital Hypothyroidism and Expanded Screening

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Abstract

This study was to investigate the current status of neonatal screening in China, to further clarify the incidences of hyperphenylalaninemia (HPA) and congenital hypothyroidism (CH). From 2000 to 2007, a total of 17,961,826 newborns had been screened for HPA and 1527 cases were detected, giving a HPA prevalence of 1:11,763. At the same time, 18,284,745 newborns had also been tested for CH, with 8918 cases being detected (1:2050). It is remarkable that the mean number of newborns screened per year had increased 5 times between 2000 and 2007. In Shanghai, 116,000 newborns were screened using tandem mass spectrometry and 6 different were detected. The overall prevalence of an inborn errors of metabolism identified was 1 in 5800 healthy newborns, with hyperphenylalaninemia being the most common. Neonatal screening had developed rapidly in China in recent years, and a pilot study using tandem mass spectrometry has been started. The biggest challenge is still to increase coverage to the entire country, especially in the mid-western area.

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Keywords Congenital hypothyroidism, Hyperphenylalaninemia, Neonatal screening, Phenylketonuria, Tandem mass spectrometry

Introduction

The diseases on the neonatal screening panel are difficult to diagnose by normal medical examination. Neonatal screening can make diagnosis and treatment possible even before the occurrences of signs or symptoms. Prompt recognition, diagnosis and treatment are important as the patient could benefit from the early, presymptomatic diagnosis. Abnormal amino acids, organic acids, and fatty acids metabolism are the major causes of many inborn errors of metabolism and many other diseases.[1,2] The diseases commonly screened for in the newborn period are principally inborn errors of metabolism which have a diverse spectrum in different countries, with different rates of incidence.

As an important measure for preventive medicine, neonatal screening programme in China was started in 1981, and it has now become one of the most popular measures for control and treatment of congenital problems. The aims of this study were (i) to describe the current status of neonatal screening for hyperphenylalaninemia (HPA)congenital hypothyroidism (CH), and their incidence; (ii) to report the recent development of neonatal screening using tandem mass spectrometry in Shanghai, and to further clarify the incidence of these diseases and their spectrum.

Methods

Dried blood spot (DBS) specimens were collected from 3-day old babies using a heelstick and spotted onto filter paper (S&S 903), and sent to the local neonatal screening laboratory. All screening laboratories participated in the external quality assessment activity organised by National Neonatal Screening Quality Control, National Center for Clinical laboratories (NCCL). The parents were provided information about the screening and they can indicate their choice in written form.

Neonatal Screening for Hyperphenylalaninemia and Congenital Hypothyroidism

The data on neonatal screening for HPA and CH were collected from the neonatal screening centres from the whole country by National Neonatal Screening Quality Control Laboratory, National Center for Clinical Laboratories. Phenylketonuria (PKU) screening was

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achieved by fluorometric method, quantitative enzymatic method, and bacterial inhibition assay. The cut-off value was 120 μmol/L for hyperphenylalaninemia and differentiation between PKU and tetrahydrobiopterin (BH_4) deficiency was performed in majority of patients. Thyroid stimulating hormone (TSH) was tested by time-resolved fluorescence immunoassay (TRFIA), fluorescence enzyme immunoassay (FEIA) and enzyme-linked immunosorbent assay. The cut-off value was 10 IU/L for CH screening.

Neonatal Screening Using Tandem Mass Spectrometry

Neonatal screening using tandem mass spectrometry was actually performed only in Shanghai. It was started in early 2003. The blood samples were drawn from newborns delivered at 50 local maternity and children's health hospitals or general hospitals in Shanghai. The dried blood samples on 3.2 mm filter paper were punched and derived using n-butanol hydrochloride with isotope internal standard, which was purchased from the American Cambridge Isotope Laboratory. The analysis was carry out on the API 2000 tandem mass spectrometer as previously reported.[3] The quality control samples were provided by the Department for Screening Neonates, Centre for Disease Control and Prevention, USA.

The diagnosis of disease was performed according to the tandem mass spectrum profiles, with appropriate confirmatory diagnostic testing, and gas-chromatographic mass spectrometry for organic acids analysis. A few diseases were confirmed by DNA analysis and therapeutic outcome.

Results

Neonatal Screening for Hyperphenylalaninemia and Congenital Hypothyroidism

According to the data collected by the National Center for Clinical Laboratory, China had 143 neonatal screening laboratories in 2007 and 117 centres had reported their screening data. For screening of phenylalanine, the fluorometric method was used in 69.7% of the laboratories, bacterial inhibition assay was used in 21.2%, and quantitative enzymatic method in 9.1%. For screening of TSH, time-resolved fluorescence immunoassay was used in 56.8% of the laboratories, enzyme-linked immunosorbent assay 28.8% and fluorescence enzyme immunoassay method in 14.4%.

From 2000 to 2007, a total of 17,961,826 newborns had been screened for phenylalanine and 1527 cases were detected, giving a hyperphenylalaninemia prevalence of 1:11,763. At the same time, 18,284,745 newborns had also been tested for TSH and 8918 cases were detected. Hence, the prevalence of CH was 1:2050. The annual number of newborns screened and cases identified are shown in Table 1. It is remarkable that the mean number of screened per year had increased 5 times from 2000 to 2007. The national coverage of neonatal screening was almost 33.2% in 2007.

Table 1. Annual Number Screened and Cases Identified in China (2000-2007)

Year	Number screened for HPA	Cases of HPA identified	Number screened for CH	Cases of CH identified
2000	1,112,980	94	1,113,682	469
2001	1,520,750	142	1,520,000	582
2002	1,139,273	117	1,139,054	547
2003	1,140,663	119	1,153,004	614
2004	1,993,782	141	1,992,571	945
2005	2,802,270	224	2,813,883	1 361
2006	2,929,236	219	2,944,022	1 701
2007	5,322,872	471	5,608,529	2 699
Total	17,961,826	1527	18,284,745	8 918

Neonatal Screening Using Tandem Mass Spectrometry

From January 2003 to 2007, 116,000 neonatal samples in Shanghai were analysed using tandem mass spectrometry. Twenty newborns were screened positive and were also confirmed to have inborn errors of metabolism, and these included 6 different kinds of diseases (Table 2). Thus the resulting overall prevalence of an inborn error of metabolism identified in newborn screening using tandem mass spectrometry in Shanghai was 1 in 5800 healthy newborns. Classification of these disorders into different groups revealed 11 patients with amino acidemias (1:10,545), 7 with organic acidemias (1:16,571) and 2 with fatty acid oxidation disorders (1:58,000). The result shows that hyperphenylalaninemia was the most common disease of all inborn of errors of metabolism in this group.

Discussion

Neonatal screening began with the screening of PKU in

Table 2. Results of Expanded Newborn Screening by Tandem Mass Spectrometry for 116,000 Newborns in Shanghai

Disease	n
PKU/hyperphenylalaninemia	10
Maple syrup urine disease	1
Methylmalonic acidemia	3
Propionic acidemia	1
3-methylcrotonyl-CoA carboxylase defection	3
Short chain acyl-CoA dehydrogenase deficiency	2
Total	20

the early 1960s. It was soon followed by multiple screening for many other diseases and DBS on filter paper continue to be the preferred method used for population-based newborn screening. So far, mass screening of newborns for inborn errors metabolism is a tremendous achievement in the field of preventive medicine. In China, neonatal screening first started in 1981. The first paper on the incidence of IEM in Shanghai in 1984 reported a figure of 1 in 15,930 for PKU, and 1in 6309 for CH.[4] However, based on the results of 5.8 million neonatal screening samples collected from the main screening centres, the incidence of hyperphenylalaninemia and CH were 1 in 11,144 and 1 in 3009 respectively.[5] The results of this paper with almost 18 million newborns screened again showed that the prevalence of hyperphenylalaninemia was 1 in 11,763, similar to that reported previously. However, the prevalence of CH was 1 in 2050 which was higher than that previously reported. In fact, the most common screening diseases in most neonatal screening centres in China were HPA and CH.

PKU was the first treatable inherited metabolic disease. HPA might be caused by a deficiency of phenylalanine hydroxylase, the classical type, or by tetrahydrobiopterin (BH_4) deficiency. BH_4 is an essential cofactor for the aromatic amino acid hydroxylases. Deficiency of BH_4 may lead to phenylketonuria phenotype, with mental retardation as well as other severe neurological disorders.[6,7] With neonatal screening for HPA gradually spreading across the entire China, many patients who were detected and treated early had a good physical and nearly normal mental development. However, some patients with HPA caused by either a deficiency of BH_4, a cofactor of phenylalanine, tyrosine and tryptophan hydroxylase, or a deficiency of 6-pyruvoyl-tetrahydropterin synthase (PTPS) or dihydropteridine reductase (DHPR) have clinical symptoms that are confusingly similar to that of classic PKU and are often misdiagnosed as the classical PKU. However, their prognosis are much worse than classical PKU and cannot be solely treated with the PKU regime.[8,9] Thus, to distinguish these cases from those suffering from classical PKU is not only important for appropriate, prompt and specific treatment but also it is also beneficial for genetic counseling.

Patient with BH_4 deficiency have specific urinary pterin profiles that can be indicative of a specific enzyme defect. Therefore, urinary pterin analysis is utilised to rule out BH_4 deficiencies among HPA patients. The incidence of BH_4 deficiency in Caucasian newborns was approximately 1% to 3% of those with HPA.[6] According to a study of our 341 HPA patients from the neonatal screening centre in Shanghai, 44 confirmed cases of BH_4 deficient patients resulted in an incidence of BH_4 deficiency of 12.9% among those with HPA, which was much higher than the Caucasian counterparts. Hence, it is crucial to set up screening for BH_4 deficiency among all HPA patients diagnosed with HPA.

CH is another congenital disorder with a prevalence of 1:2050 in China. This high prevalence warrants our special attention. We believe that the high prevalence is related to 3 factors. First is the evolution of methodology for TSH measurement. At the beginning of the neonatal screening period, the radioimmunoassay (RIA) method used to be popular and the prevalence was low because of the lack of sensitivity of this test. In recent years, time-resolved fluorescence immunoassay, enzyme-linked immunosorbent assay and fluorescence enzyme immunoassay method were used in all screening laboratories and the prevalence has been increased. A second factor is the revised cut-off value. Ten years ago, the cut-off value of TSH for neonatal screening was 15 IU/L to 20 IU/L in most neonatal screening laboratories. Now the cut-off value is set up at 10 IU/L so that milder cases of CH could also be detected. A third factor is the deficiency of iodine. Although nationwide iodinification of salt was implemented in the early 1990s, mild cases of iodine deficiency still exist in some mountainous or remote areas. Hyperthyrotropinemia or mild hypothyroidism is still on the rise.

Neonatal screening has evolved conceptually from a laboratory test for a disorder into a public health system. Previously, many children were not screened for inborn errors of metabolism as these were considered rare diseases and thus were not treated in the early stages. This led to delayed mental and motor development, and sometimes even death. The introduction of the analysis of amino acids and acylcarnitines by tandem mass spectrometry to population-based neonatal screening has tremendously increased the number of treated cases of detectable forms of inborn errors of metabolism. Advancement in newborn screening technology, coupled with recent advancement in the diagnosis and treatment of rare but serious neonatal congenital conditions provide increased opportunities for effective management of patients and their families.[10-12] According to the American College of Medical Genetics (ACMG), a uniform neonatal screening panel of 29 disorders including 6 amino acid disorders, 9 organic acidemias, 5 fatty acid oxidative disorders, 3 hemoglobinopathies and 6 others was advocated in recent years.[13]

China is a fast developing country. Development in managing the morbidity and mortality of inborn errors of metabolism should take precedence over socio-economic and technology development. Among all the neonatal diseases, more than 500 inborn errors of metabolism are especially important firstly, because of their relative frequency, and secondly, because rational therapy has been or will be available in the near future. Our pilot study of neonatal screening using tandem mass spectrometry in

Shanghai had revealed that the prevalence of detectable disorders was 1 in 5800, and those affected patients can have substantial improvements in mortality and morbidity. We expect that newborn screening using tandem mass spectrometry could be further expanded, and more experience in neonatal screening, diagnosis and treatment of IEMs can be accumulated and shared among Chinese scientists and physicians.

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Newborn Screening in Bangladesh

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Abstract

Newborn screening started in Bangladesh in 1999. The programme started as part of a regional project of the International Atomic Energy Agency (IAEA) to screen for congenital hypothyroidism (CH). In the beginning the IAEA helped the country with equipment, filter papers, reagents, training and expert services. Since 1999, 2 pilot projects to screen newborns for CH were completed. Under these projects some 30,000 newborns were screened and 16 were identified with hypothyroidism. The government of Bangladesh approved a national project in July 2006 to screen newborns in some selected areas of the country for CH. Under the project some 200,000 newborns will be screened and laboratory facilities for newborn screening will be increased. Bangladesh has a large population of about 140 million. With the current birth rate some 2 million new births take place every year. The socio-economic situation of the country is also different. Per capita income of the country is one of the lowest in the world. About 85% of babies are still delivered at home. As such newborn screening is a big challenge for Bangladesh. However, the country is trying to overcome these challenges.

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Key words: Bangladesh, Congenital Hypothyroidism, Newborn Screening

Introduction

Bangladesh is a small country in South Asia, with an area of 147,570 sq km. However it has a large population of about 140 million. With the current population growth rate of about 1.48%, some 2 million births take place every year. Iodine deficiency is endemic in the country. Goitre and other iodine deficiency disorders (IDD) are very common and are known from ancient times.[1]

In South Asia, priorities for healthcare in children have centred around infectious disease, malnutrition and curative services.[2] Bangladesh's situation is similar, and the country's main challenges in healthcare are childhood problems like malnutrition, diarrhoea and other communicable diseases, with a high infant and child mortality rate. Newborn screening has emerged as a new challenge for the country.[3]

Bangladesh entered into an era of newborn screening in 1999. The programme started as part of an International Atomic Energy Agency (IAEA) Regional Project on Neonatal Screening for Congenital Hypothyroidism in East Asia (RAS/6/032). Initially, it was a great challenge. There was very little knowledge or experience to run such a programme. There was no awareness, no laboratory set-up and no trained manpower for newborn screening.

The Bangladesh Atomic Energy Commission with the support of IAEA started the newborn screening movement in Bangladesh. In the beginning IAEA helped the country with equipment, filter papers, reagents and expert services. IAEA also trained some of our physicians and laboratory people abroad in a few advanced newborn screening centres. The Government of Bangladesh had also a positive attitude towards the programme from the very beginning. Healthcare professionals especially showed a great interest towards the programme and supported it wholeheartedly.

Bangladesh presented their first data on newborn screening in the 4th Asia-Pacific Regional Meeting for Neonatal Screening held in Manila, Philippines in October 2001. The screening programme in the country thus entered into the international arena. At home, to promote newborn screening, a number of motivation and training programmes were attended by physicians, paramedics, policymakers and public representatives.

Due to efforts during the past few years the Government

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Table 1. Summary of Newborn Screening (Congenital Hypothyroidism)

Time period from January 2000 to December 2006	No. of newborn screened	No. of unsatisfactory samples	No. of positive samples	No. of babies recalled	No. of diagnosed cases
Total	31802	1263	438	362	16

of Bangladesh included a project on newborn screening in its Annual Development Programme (ADP) in July 2006. Under the programme about 200,000 newborns from different parts of the country will be screened for detection of congenital hypothyroidism (CH). The project is expected to cost 104 million taka (about US$1.5 million). The project will run up to June 2010.

Materials and Methods

Till now CH was the only condition screened. Screening was done mainly in large hospitals using filter paper. Cord blood was used for sample collection. After collection and proper drying, the samples were sent to laboratories by courier service or messenger. At present the samples are analysed in 6 different laboratories in different parts of the country. The Institute of Nuclear Medicine and Ultrasound situated in Bangabandhu Sheikh Mujib Medical University in Dhaka acts as a central laboratory and coordinates the activities of other laboratories. All the laboratories use the radioimmunoassay (RIA) method. TSH was measured and a value of 20 mIU/ L was taken as a cut-off point. Babies who screened positive were recalled and their serum were analysed for T4 and TSH.

Results

From January 2000 to December 2006 a total of 31,802 babies were screened. The samples were collected randomly from 10 selected hospitals mostly located in Dhaka city as well as some other parts of the country. Some 321,000 babies were born in the same period in these hospitals. It meant that more than 289,000 babies were not screened, thus screening coverage was only 9.9%. One thousand two hundred and sixty-three (4.0%) samples were found to be unsatisfactory due to various reasons and were rejected. In 438 samples (1.4%), TSH levels were more than 20 mIU/ L. Unfortunately only 362 babies (1.1%) could be recalled. Sixteen babies were eventually confirmed to have CH (Table 1). This gave an estimated prevalence of about 1 in 2000 newborns.

Discussion

Newborn screening began in the 1960s with the work of Robert Guthrie, a researcher in USA and widely recognised as "father of newborn screening". Guthrie and Susi[4] developed a bacterial inhibition assay (BIA) for phenylalanine in order to detect phenylketonuria (PKU), an inborn error of metabolism which causes severe mental retardation. Since then, newborn screening programmes have been adopted by most of the developed countries. However, the scenario in developing nations is different. In countries with depressed and developing economies, particularly in Africa and Asia, newborn screening is either not yet a priority or is just emerging as a priority.[5] Bangladesh is a developing country with many other problems. The universal newborn screening programme is a highly ambitious project for a country like Bangladesh.[6]

Increased global awareness has resulted in new national newborn screening programmes in South Asia. Bangladesh and some other countries in South Asia entered into a new era of newborn screening with the initiation of an International Atomic Energy Agency (IAEA)-sponsored regional project on neonatal screening for CH in 1999.

Congenital hypothyroidism is a treatable condition and a child can be saved from severe mental and physical retardation if detected and treated in first few weeks of life. Iodine deficiency is also endemic in Bangladesh and the prevalence of iodine deficiency disorders (IDD) is high.[7] For these reasons, Bangladesh is making it a priority to screen for CH.

The preliminary prevalence of CH in Bangladesh is about 1 in 2000 newborns which is higher than other published results.[8,9] However, for a country with a high incidence of iodine deficiency this was probably not unexpected. The actual prevalence rate may well be much higher.

A developing newborn screening programme in Bangladesh faces many problems. The socio-economic situation is a big barrier. More than 85% of deliveries occur at home and it is very difficult to bring them under the programme. Out of 438 newborns who were screened positive, only 362 could be contacted. Others did not have a complete contact address or any phone number. Some people gave their old addresses. However, now sample collectors are more particular in recording details of the contact address and the number of lost cases is getting fewer now. Similarly in the beginning, we had to reject a good number of samples as they were found to be unsatisfactory. Now healthcare providers are more trained in collection of samples and the number of unsatisfactory samples is now negligible.

The country still lacks an official policy for newborn

screening. That is one of the greatest weaknesses of our programme. Healthcare professionals are working to integrate newborn screening into the healthcare system of the country. Obtaining funds for screening 2 million babies per year is also a big hurdle. It is hoped that once the government formulates a policy that some donor agencies will make funds available. Bangladesh has a successful extended immunisation programme with a coverage rate of more than 90%. Perhaps neonatal screening can be modelled after this.

Conclusion

Newborn screening is a challenging programme for Bangladesh. The activities of the past few years have created a platform for the growth of future newborn screening programme in the country. It is hoped that at the end of the current national project in June 2010 the government will adopt a policy on newborn screening and it will become a sustainable programme in Bangladesh.

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Newborn Screening in Pakistan – Lessons from a Hospital-based Congenital Hypothyroidism Screening Programme

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Abstract

We are living in a time of unprecedented increase in knowledge and rapidly changing technology. Such biotechnology especially when it involves human subjects raises complex ethical, legal, social and religious issues. The establishment of newborn screening programmes in developing countries poses major challenges as it competes with other health priorities like control of infectious diseases, malnutrition and immunization programmes. Despite this, it is imperative that the importance of newborn screening programmes is recognised by developing countries as it has been proven through decades of experience that it saves thousands of babies from mental retardation, death and other serious complications. Pakistan has an estimated population of 167 million inhabitants, 38.3% of whom are under 15 years of age. Pakistan lacks a national programme for newborn screening. However, as individual practice at the local level, Aga Khan University Hospital (AKUH) and a few other hospitals are doing newborn screening for congenital hypothyroidism. The main hurdle in the implementation of newborn screening in Pakistan is the lack of good infrastructure for health. Eighty percent of deliveries take place at home. Moreover, little resources are available for children identified with a genetic condition due to the non-existence of genetic and metabolic services in Pakistan. In a 20-year audit of congenital hypothyroid screening at AKUH we found 10 babies with congenital hypothyroidism. However due to missing data links spanning several years, we were unable to calculate its true incidence during this period. In order to estimate the incidence of congenital hypothyroidism (CH) we reviewed in detail data over 10 months in 2008, a period where there was better compliance for repeat thyroid stimulating hormone (TSH) testing, and found 2 babies with CH. This gave an estimated incidence of 1 in 1600 live births.

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Key words: Developing countries, Neonatal TSH, Thyroid

Introduction

We are living in an era of unprecedented increase in knowledge and rapidly changing technology. This progress in biotechnology raises complex ethical, legal, social and religious issues. Newborn screening for detection of congenital metabolic and endocrine disorders exists as established programmes in most of the developed world but its establishment in developing countries poses major challenges as other health issues like control of infectious diseases, malnutrition and immunization programmes take precedence over it.

Pakistan, a developing country with an estimated population of 167 million inhabitants, 36.3% of whom are under 14 years of age, has as yet no newborn screening programme at the national level. The crude birth rate is 38 to 40 per 1000 and the infant mortality rate is 80 per 1000 livebirths.[1] The healthcare system is mainly hospital-based and primary health care facilities are practically non-existing. Here more than 80% of deliveries occur at home and 80% of these home deliveries are usually attended by unskilled birth attendants.[2]

Pakistan has a very high consanguinity rate of 46% to 61% due to strong cultural preferences.[3] As a result we face a huge burden of inherited diseases, which contribute significantly to the alarmingly high infant mortality rate in Pakistan. Due to the lack of diagnostic facilities and absence of national registries for diseases, prevalence of various inherited diseases are not known. β thalaessemia is probably the most common single gene disorder in our population, as Pakistan is situated in the β thalaessemia belt[4] and 5.4% of Pakistanis are carriers for β thalaessemia.[5] Other inherited diseases more prevalent in the Pakistani community are

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G6PD deficiency and congenital hypothyroidism (CH). Ali et al reported a G6PD deficiency frequency of 1.8% among healthy Pakistani adults.[6]

Although Pakistan lacks a national programme for newborn screening for any inherited disease, at the local level Aga Khan University Hospital (AKUH) and few others are doing newborn screening for CH.

We present our data on CH screening over a period of 20 years. We identified hurdles a newborn screening programme can face in a developing country like Pakistan.

Patient and Methods

CH screening started at AKUH in March 1987. In the first 2 years of screening all newborns delivered at AKUH and 4 other maternity homes working under the banner of AKUH were targeted, which included 5,000 livebirths. In the subsequent years due to logistic reasons CH screening was limited to babies born at AKUH only. We covered a period of over 20 years, from 1989 to October 2008, in which a total of 53,619 babies were screened.

Venous blood samples were collected on either the second or third day of life. Thyroid stimulating hormone (TSH) levels were measured by the chemiluminescence method till 1995; afterwards the method was switched to electro-chemiluminescence. Recall strategy (Fig. 1) was planned if TSH was more than 13 mIU/L.

Results

In the first 2 years of CH screening between 1987 and 1988, 5,000 babies were screened and of these 5 were found to have CH, which was confirmed on the basis of a low T4 and high TSH as has been presented by Lakhani et al.[7]

For our audit we requested for medical records to provide the number of livebirths at AKUH per year for the period mentioned. Although we got the number of births and their medical record numbers, it was not possible to get TSH values of these babies through their medical records, as these files were still not in electronic format. Also since the TSH results usually come back after the babies are discharged the coding was not done in the records even if the values are found to be high.

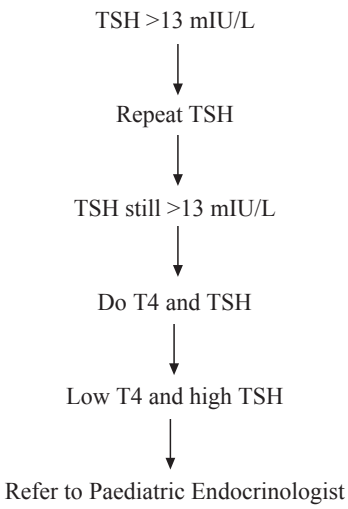

Fig. 1. Recall strategy for elevated TSH.

The next option was to request neonatal TSH data from the laboratory. We faced issues in this step as well**.** For the period between 1989 and 2008, we were unable to retrieve TSH screening results of 11,803 (22.0%) out of 53,619 babies born at AKUH. Thus screening data for only 41,816 (78.0%) babies was available for review. According to our lab reference values babies with TSH more than 13 mIU/L were to be recalled during the first 2 weeks of age for repeat TSH. There was another missing link here as repeat TSH values for the majority of babies were not available. Among the babies whose repeat TSH was more than 13 mIU/L, 10 babies were found to have low T4 and high TSH. None of these babies had radioisotope scanning of the thyroid gland done, thus the etiology of CH could not be ascertained. From January till mid-October 2008, we carefully analysed data for each and every patient. This period has lowest dropout rate with the highest compliance for repeat TSH. In this period we found 2 babies with TSH both in initial and repeat of more than 100 mIU/L. Both babies had low free and total T4 too. Based on just this year's data (10 months), a mathematical estimated incidence of CH would be about 1 in 1600 livebirths (Table 1).

Discussion

It is difficult to calculate the incidence of CH based on our data because in 22.0% of babies first TSH values were not available. Possible reasons for the missing records could be that AKUH medical records were shifted from paper to electronic and during this process some medical records were lost. A second possibility was that some of the babies were discharged before the second day of life when CH screening was supposed to be done.

Most of these discharges were on parental request. Parents were however given a lab request to get TSH screening done as out-patient, after 3 days of age. More than 80% in drop out rate was seen in repeat TSH, as the majority of parents did not come for follow-up TSH when they were recalled. Most of them probably thought that the baby had no problems because there were no symptoms. It is possible that among most of the babies who dropped out, some of them had repeated their TSH at some other laboratory for which data was not available to us. Lack of awareness among parents was another contributing factor for not repeating TSH levels. Also there are many private paediatricians in city and each one not only uses different

Table 1. CH Screening – A 20-year Audit

Year	Total birth	TSH done n (%)	CH cases detected
1989	1414	891 (63%)	1
1990	1669	776 (47%)	3
1991	1667	1400 (84%)	0
1992	1753	1554 (89%)	0
1993	1847	1700 (92%)	1
1994	1977	1701 (86%)	0
1995	2261	1680 (74%)	1
1996	2479	1976 (80%)	0
1997	2731	2114 (77%)	0
1998	2779	2403 (86%)	0
1999	2891	2296 (79%)	0
2000	2967	805 (27%)	1
2001	2909	2373 (82)	0
2002	3010	2434 (81%)	1
2003	3070	2516 (82%)	0
2004	3414	2824 (83%)	0
2005	3689	2753 (75%)	0
2006	3999	3164 (79%)	0
2007	3919	3282 (84%)	0
Jan-Oct 2008	3174	3145 (99%)	2
Total	53,619	41,816 (78.0%)	10

laboratories for testing, but also have different cut off values for raised TSH.

It has been proved through decades of experience that newborn screening saves thousands of babies from death, mental retardation and other serious complications. It is imperative that the importance of newborn screening programmes is recognised by developing countries like Pakistan and serious steps are taken at the national level to ensure successful newborn screening for conditions like CH, because it is easily diagnosed and the treatment is also very cost effective when compared to the cost of care for a mentally retarded child. Public awareness programmes should be conducted to not only educate parents about the consequences of CH but also to stress the need to initiate therapy as early as possible after birth to ensure proper brain development.

In view of the issues/deficiencies that have been identified we propose the following steps to be addressed at both institutional and national levels.

- Designate 1 or 2 physicians, to whom laboratories will report to regarding raised TSH values. These physicians should then design a system where they not only inform the patients in a timely manner, but also track the follow-up visits.
- It is our estimate, that there are many false positives (raised TSH) when we used the cut-off of 13 micro IU/mL. We have reanalysed our data with a cut-off of 20 and 25 micro IU/mL, and found that our estimated recall would be 5% and 2.3% respectively which is significantly lower than the recall rate of 15% if the cut-off for repeat TSH is kept at 13 mIU/L. This is close to what other countries in the region have reported.[8]
- Another strategy/suggestion at the national level would be to apply for a grant, which would provide an incentive to get TSH repeated free of charge. In this way, we would probably have a big enough sample size to calculate the true incidence of CH.

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Organising Services for IMD in Thailand: Twenty Years Experience

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Abstract

The study of inherited metabolic disorders (IMD) in Thailand is in its infancy when compare with developed countries. Prior to 1987, majority of these disorders were clinically diagnosed since there were only a handful of clinicians and scientists with expertise in inborn errors of metabolism, lack of well-equipped laboratory facilities and government support. In developing countries, inherited metabolic disorders are not considered a priority due to the prevalence of infectious diseases such as HIV infection and congenital infections. A multicentre survey conducted in 1994 and 2001 revealed the existence of numerous cases of IMD from all over the country. Case reports and publications on IMD in Thai (and international) medical journals in past 20 years had undoubtedly raised its awareness among Thai paediatricians and scientists. In 2001, the Genetic Metabolic Centre was first established in Siriraj Hospital Faculty of Medicine, Thailand. Numerous new cases of IMD had been identified since then.

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Key words: Genetic metabolic centre, Inherited metabolic disorders

Introduction

Milestones of IMD in Thailand

The study of inherited metabolic disorders in Thailand started in 1987. Majority of IMD were clinically diagnosed since there were only a handful of clinicians and scientists with expertise in IMD. There was also a lack of governmental interest and support due to the high prevalence of infectious diseases and congenital infections. However a multicentre survey conducted between 1994 to 2001 revealed the existence of numerous cases of IMD from all over the country. Research collaborations with United States and Japanese experts and scientists were initiated since 1989.

The First Decade (1987-1997)

Majority of IMDs were clinically diagnosed, since there were no laboratory facilities available in Thailand. In May 1990, only 1 Thai delegate (the author) attended the 5th International Congress of IEM held in Asilomar, California, United States. In June 1993, the First Asia-Pacific Regional Meeting (APRM) of the International Society on Neonatal Screen (ISNS) was held in Sapporo, Japan. Experiences from these 2 international meetings had made a great impact on the development of IMD in Thailand. In July 1994, the First Asia-Pacific Conference on Medical Genetics was held in Bangkok, Thailand, supported by Mahidol University and International Center for Medical Research (ICMR), Kobe University, School of Medicine, Japan. Subsequently, there was a multicentre study for IMD initiated from 1994 to 1998 to collect data and explore the prevalence of IMD.[1,2]

From 1993 to 1997, a pilot project on Newborn screening was started at Siriraj Hospital, Mahidol University in Bangkok.[3] In November 1995, the 2nd APRM of ISNS was held in Hong Kong. The following year, 1996, the Department of Medical Science, Ministry of Public Health (MOPH), Thailand initiated a pilot project in newborn screening in Thailand.[4,5]

The Second Decade (1997-2007)

In 1998, the 3rd APRM of the ISNS was organised in Chiang Mai, Thailand and was attended by more than 200 people. The objective of the meeting was to raise awareness of IMD and to educate Thai physicians (paediatricians & obstetricians) and to help nurses understand the importance of newborn screening.

From 1998 to 2000, gas-liquid chromatography/ mass spectrometry (GC/MS) was first introduced in Thailand through collaborations with Japanese scientists which led

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to previously undiagnosed organic acid disorders.[7,8,21] Collaborations with US scientists (1993 to 2002) for tandem mass spectrometry also led to identification of newly diagnosed fatty acid oxidation disorders.[6,8] In 2003, the first Thai textbook on IMD, a collective data on IMD as well as 15 years' of experience of IMD referrals to Siriraj Hospital was completed.[9]

In 2003, the IX International Congress on IMD (ICIEM) held in Brisbane, Australia; 2 papers on urea cycle disorders and 1 oral presentation titled "IMD in Thailand – Siriraj Experience" were presented. In 2005 – the First Genetic Metabolic Symposium was held in Bangkok to raise awareness of IMD among Thai paediatricians.

The X ICIEM was held in Chiba, Japan, in September 2006. The author of this paper was invited to be one of the local organising committee and invited speakers in "IEM in Asia" Symposium. Twenty-five newly reported cases on IMD in Thailand were presented. The 6th ISNS meeting was held in Awaji Island, Tokushima, Japan and a poster "PKU infant from newborn screening at Siriraj Hospital" was presented. The invited lecture titled "Newborn Screening in Thailand: Challenges and Opportunities" was also presented. From 2005-2008, numerous publications on molecular characterisation of IMDs in Thai patients were accomplished.[13-18]

Establishment of Genetic Metabolic Centre in Thailand

Research collaboration with Chulabhorn Research Institute in Bangkok since 1998 has also helped us in the area of amino acid analysis, enzyme assays and mutation analysis from which almost 20 publications were published based on pioneering works on IMD in Thailand.[2,11,13-18]

In 2001, the Genetic Metabolic Centre was established at Siriraj Hospital Faculty of Medicine, the first of its kind in Thailand, with assistance from JICA (Japanese Intergovernmental Cooperation Agency) which provided Gas-liquid Chromatography-Mass Spectrometry (GC/MS) and technology transfer, together with funding from Chaofa-Maha Chakri Pediatric Building for High-Performance Liquid Chromatography (HPLC). Numerous new cases of IMD had been identified since then.

Multicentre Study of IEM (2001 to 2004)[1,2,6-8,10,11]

The following IMDs were identified: (i) *Carbohydrate disorders* – galactosemia, glycogen storage diseases (GSD type I, GSD type II, GSD type III), fructose[1,6] bisphosphatase deficiency); (ii) *Amino acid disorders* – PKU, hyperphenylalaninemia, tyrosinemia type I, MSUD, homocystinuria, albinism, NKH; (iii) *Urea cycle disorders* – ALD, OTC, ASD, unidentified UCD; (iv) *Organic acid disorders* –

Research Collaborations

(a) Research Collaborations with the United States (1987-1997)[1-3,6,8,12]	
Prof Edwin Kolodny (NYU)	Lysosomal enzyme assays
Dr Edwin Naylor (McGee Women's & Children, PA)	Tandem mass spectrometry (TMS)
Dr George Thomas (Johns Hopkins)	Amino acid analysis (AA)
Prof Hugo Moser (Kennedy Krieger Institute)	Peroxisomal disorders
Dr Robert Guthrie (New York)	Newborn screening (NBS)
Prof Saul Brusilow (Johns Hopkins)	Urea Cycle Disorders (UCD)
Dr Holmes Morton (Lancaster, PA)	Maple Syrup Urine Disease (MSUD)
Prof Vivian Shih (Boston, MA)	Amino acid analysis (AA)
(b) Research Collaborations with Japan (1997-2007)[2,3,7,8,21]	
Dr Toshiaki Oura (Osaka)	Newborn screening
Dr Hiroshi Naruse (Tokyo)	Newborn screening
Prof Isamu Matsumoto (Kanazawa)	Gas-Liquid Chromatography and Mass Spectrometry
Prof Seiji Yamaguchi (Shimane)	Tandem mass spectrometry
Prof Keiko Kobayashi (Kagoshima)	Mutation analysis (Citrullinemia)
Assoc Prof Toshihiro Shinka (Kanazawa)	Gas-Liquid Chromatography and Mass Spectrometry
Assoc Prof Kenji Hara (Fukuoka)	Gas-Liquid Chromatography and Mass Spectrometry

IVA, MMA, PA, alkaptonuria, multiple carboxylase deficiency (MCD); (v) *Mitochondrial disorders* – MCAD, translocase deficiency, carnitine deficiency; (vi) *Peroxisomal disorders* – RCDP, Zellweger, primary hyperoxaluria type I; (vii) *Lipidosis* – Niemann-Pick type I, Gaucher, Sandhoff, GM_1 gangliosidosis; (viii) *Mucopolysaccharidosis* – Hurler, Hurler - Scheie, Scheie, Hunter, Sanfilippo, Morquio, Maroteaux-Lamy, Sly, unidentified MPS; (ix) *Disorder of Purine Metabolism* – Lesch - Nyhan; (x) *Disorder of Copper Transport* – Menkes; (xi) *Leucodystrophies* – X-linked ALD, others; (xii) *Others* – lipoprotein lipase deficiency, hyper-lipoproteinemia, porphyria, cystinuria, methemoglobinuria, HMG CoA lyase deficiency.

Newborn Screening Programme in Thailand

A national neonatal screening programme has been implemented into public health infrastructure by the Ministry of Public Health (MOPH) since 1996. At present, approximately 80% to 90% of all newborns are being screened for congenital hypothyroidism (CH) and phenylketonuria (PKU).[19,20] However, many problems still exist due to a lack of systematic and holistic approach which include a lack of adequate follow-up, genetic counselling and problems with medical management. A national committee has recently been established in 2006 and problems are being solved.

Summary

Inherited metabolic disorders in Thailand is in its developing stage; however there was marked improvement in the past 20 years from clinical to biochemical diagnoses and more recently to the molecular level. There is also an increase in the number of well-trained clinical geneticists and biochemists who have taken a keen interest in the area of IMD. Though we have difficulties presenting the epidemiological data of each IMD and Thailand is not free from the prevalence of infectious diseases and congenital infections; the future of IMD in Thailand is certainly progressing well.

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Outcome of Organic Acidurias in China

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Abstract

From June 1998 to May 2007, 9566 urine samples were collected from patients with psychomotor deficits, seizures, vomiting and unconsciousness in Peking University First Hospital. Their urine organic acids profiles were analysed using gas chromatography - mass spectrometry (GCMS), GCMS solution and Inborn Errors of Metabolism Screening System software. In all patients, blood acylcarnitines were analysed using tandem mass spectrometry. One hundred and sixty-eight patients (1.76%) with organic acidurias were detected. Among them, 116 (116/168, 69.0%) had methylmalonic aciduria, 63 (54.3%) of these 116 patients had methylmalonic aciduria combined with homocysteinemia. Sixteen (9.5%) of those patients detected with organic acidurias had propionic aciduria, and 15 (8.9%) had multiple carboxylase deficiency. Seven (4.2%) had glutaric aciduria type 1. After dietary treatment, medicine and rehabilitation, clinical improvements were observed in more than half of the patients. Twenty-eight of the 168 patients (16.7%) recovered and led a normal life. The method of urine organic acid analysis by gas chromatography - mass spectrometry and blood acylcarnitines analysis by tandem mass spectrometry have been established and applied successfully in China, namely Beijing, Shanghai, Wuhan and Guangzhou. The prognoses of Chinese patients with organic acidurias have also improved significantly.

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Key words: Gas chromatography - mass spectrometry, Tandem mass spectrometry

Introduction

China has a huge population, of whom more than 0.3 billion are children under 14 years. According to the cumulative incidence of organic acidurias, more than 2000 out of 20 million newborn babies would be affected by organic acidurias annually. China is a developing country and is quite different from western countries, especially in terms of socio-economic systems. Chinese doctors face a great challenge in performing a nationwide study on organic acidurias.

Research on organic acidurias started in 1996 with great support by Japanese experts. In the past 11 years, the techniques of urine organic acids analysis by gas chromatography - mass spectrometry (GCMS) and blood acylcarnitines analysis by tandem mass spectrometry have been introduced in some university hospitals in Beijing, Shanghai, Wuhan and Guangzhou for high-risk screening.[1-3] Collaborative networks played a critical role in the development of the programme. Now, in China, 7 labs are providing services for high-risk screening, diagnosis, treatment and genetic counselling for organic acidurias. By implementing high-risk screening, more and more patients with various organic acidurias were detected.

Subjects and Methods

In Peking University First Hospital, from June 1998 to May 2007, 9566 urine samples were collected from patients with psychomotor deficits, seizures, vomiting, unconsciousness and multiple organs dysfunction of unknown causes. The urine organic acids profiles were analysed using Shimadzu GCMS (Shimadzu QP2010, Kyoto, Japan) and Screening System software.[4,5] Amino acids and acylcarnitines in dried blood samples were quantitatively analysed by tandem mass spectrometry.[2] Plasma or serum total homocysteine concentration were measured by a fluorescence polarisation immunoassay described by Abbott Laboratories.[6]

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Results

Prevalence

As shown in the Table 1, 182 patients (1.9%) with typical organic acidurias were detected. Among 182 patients with organic acidurias, methylmalonic aciduria was the most common disease[3] (116/168, 69.0%). Propionic aciduria was the second most common (9.5%). Fifteen patients (8.9%) had multiple carboxylase deficiency.[7]

Glutaric aciduria type 1, maple syrup urine disease, oxoprolinemia, ketothiolase deficiency, isovaleric aciduria, methylcrotonyl CoA carboxylase deficiency, alcaptonuria were detected in the rest of the patients.

Treatment and Outcome

After diagnosis, 132 (72.5%) patients were treated by diet, medicine and rehabilitation. Clinical improvement was observed in 96 (52.7%) patients. Unfortunately, 37 (20.3%) patients died. Thirteen (7.1%) patients did not have follow up.

(i) Methylmalonic aciduria

Methylmalonic aciduria is the most common detectable organic aciduria in China. Till May 2007, 116 patients with methylmalonic aciduria were detected. Fifty-three cases (45.7%) were isolated methylmalonic aciduria. They were treated by L-carnitine, cobalamin and special formula. Sixty-three cases (54.3%) were combined methylmalonic aciduria and homocysteinemia.[3] They were treated by cobalamin, folate, L-carnitine and betatine supplementations.

Out of 53 patients with isolated methylmalonic aciduria, 29 had onset during the neonatal period, and 14 died. Sixteen patients had the onset between 1 and 12 months, and 4 died. Eight of them had onset after the age of 1 year, and 1 died. Three of them had normal development later on in life.

Table 1. One Hundred Eighty-two Cases with Organic Acidurias Detected from 9566 High-risk Patients

Diseases	No.	%
Methylmalonic aciduria	116	63.7
Propionic aciduria	16	8.8
Multiple carboxylase deficiency	15	8.2
Glutaric aciduria type 2	11	6.0
Glutaric aciduria type 1	7	3.8
Maple syrup urine disease	5	2.7
Oxoprolinemia	3	1.6
Ketothiolase deficiency	3	1.6
Isovaleric aciduria	3	1.6
Methylcrotonyl CoA carboxylase deficiency	2	1.1
Alcaptonuria	1	0.5

The outcomes of 63 patients with combined methylmalonic aciduria and homocysteinemia were relatively better.[3] Nineteen patients had onset during the neonatal period, 5 of them died, 1 had normal development. Among the 26 patients who had onset after the age of 1 year, only 1 patient died. Twelve other patients had normal development.

Generally, the outcomes of the patients with combined methylmalonic aciduria and homocysteinemia with onset after the neonatal period are much better than those with isolated methylmalonic aciduria. The outcomes of patients who had onset at school age or later were more favourable. Among the 17 cases, 9 of them recovered completely, while 8 improved with a mild residual handicap.[3]

(ii) Multiple carboxylase deficiency

Among our patients with organic acidurias, the outcomes of patients with multiple carboxylase deficiency are the best. In my hospital, 15 patients aged between 1 month and 14 years were detected using urinary organic acids analysis. Biotinidase deficiency was detected in 9 of them. Thirteen patients were treated successfully by biotin supplement.[7] Two patients died before the treatment.

Propionic Aciduria

Generally, the outcomes of our patients with propionic aciduria were unfavourable. In our study, 13 out of 16 patients had onset in the neonatal period. Among the 9 patients who died, 4 were diagnosed by postmortem. One of the 7 patients who is still alive is 10 years old, with psychomotor retardation and epilepsy.

Glutaric Aciduria Type 2

Seven cases with lipid storage myopathy due to late-onset glutaric aciduria type 2 were studied.[8] These previously healthy patients began to develop progressive fatigue, muscular weakness and pain between the age of 8 years to 22 years. All patients had mild hepatomegaly, significant elevation of serum creatine kinase, creatine kinase-MB, lactate dehydrogenase, hydroxybutyrate dehydrogenase and carnitine deficiency. Muscle biopsy revealed lipid storage myopathy. All patients responded to high dose riboflavin (100-500 mg/d). Rapid clinical and biochemical improvement were observed. After 1 to 3 months of therapy, all of them could go back to school or work.

Conclusion

Organic acidurias are relatively common metabolic diseases in China,[1-3] but the precise incidence is not known yet. Affected patients manifest a wide variety of clinical,

biochemical and neurological symptoms involving multiple independent organ systems.[9-11] Diagnosis of organic acidurias especially in the patients with late onset symptoms were often not carried out until the manifestation of symptoms.[9-11]

Organic aciduria research is considered a novelty in China compared to many developed countries. Population distribution and diseases profile of organic acidurias are usually very complicated, thus making it difficult to effectively conduct screening of organic acidurias. Therefore, it is important to develop a nation-wide collaboration system.

Domestic and international cooperation have enabled many patients to be detected. Although the network system is in its infancy stage, it has contributed much to provide information, training of paediatricians and healthcare professionals.

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