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Received 01 June 2021.
Revised 17 June 2021.
Accepted 17 July 2021.
Published Online 24 Sept 2021

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Diagnostic Advances for Inborn Error of Metabolism (IEM) and Screening Interventions in Selected Asian Countries

***Abstract*—**The establishment of a viable program for new born screening in the Asian region has suffered unimaginable set-back partly due to several factors such as economic, cultural, and geographical differences. This has mostly become exacerbated partly in developing and developed economies due to lack of proper awareness. In many parts of the world, the screening of infants for the congenital disorder is an important part of neonatal activities with an inherent advantage of minimizing the morbidity and mortality rates in newborn. The recent development of state-of-the-art procedure has therefore made it possible for the new born screening of a wide range of strange disorders. This review succinctly presented the overview and importance of new born screening, awareness, spectrometry diagnostic tools and the potential future collaboration among member countries in this region.

***Keywords*—Inborn error of metabolism (IEM), New born screening (NBS), Plasma acylcarnitine profiling, Plasma amino ACD profiling, Urine organic acid profiling**

1 INTRODUCTION

The new born screening (NBS) is an vital developments in infant health treatment for the early detection of metabolic, hormonal-related and infrequent genetic disorders which can lead to serious health issues [1]. It has the ability to prevent catastrophic health outcomes and severe health problems including death, provided the screening is done properly and timely [2]. NBS is focused on having recognizing an managing the newborn affect as early as possible so as to reduce the rate of morbidity, mortality, and disability which are associated with inherited disorders [3] . This is made possible since the screening helps the timely identification of a number of congenital, hereditary and metabolic abnormalities [4] . However, it is noteworthy that the screening involved more than just the test, but it portrays a comprehensive system that has been designed for early detection of patients with targeted disorders and the initiation of the appropriate therapeutic intervention targeted to address it to prevent permanent disability and death[5]. Other components of this system, beside testing include: educating the involved individual, following them up, managing their condition, and time intervention[6] .

In the 1970s, the immunoassays were developed for thyroxin and thyroid-stimulating hormone. This resulted into the inclusion of congenital hypothyroidism (CH) to the panel of the newborn screening. Over the years, the scope of the screening programs has expanded from a single test to currently covering more than 30 primary conditions and more than 20 secondary disorders [7]. The inclusion of conditions or disorders in newborn screening is determined using a set of standard criteria as proposed by Wilson-Jungner, in 1960 [8,9]. These standard criteria encompass the understanding of the inherent disorder, valid testing procedure, treatment, and cost considerations for the screening program. The disease to be screened must be a vital health condition, of which the natural history is adequately comprehended and easily identified at the early stages [9]. Using these criteria, a suitable acceptable test by the population and with the intervals for repeating the test must be determined. Moreover, acceptable treatment procedures and the availability of the appropriate diagnostic facilities are required elements required of the standard criteria proposed by Wilson- Jungner. Other important criteria include: introduction of a case-by-case treatment policy of patients to be included in the programs; an economic balance between the benefit and the cost of finding the case [10] . However, with the

introduction of the recent technologies in NBS, there is a rising in the number of disorders that do not meet all of the Wilson and Jungner indicators.

Statistics have shown an increasing trend of over 1 billion children born with different forms of congenital disorder out of which about 20 % leads to irreconcilable irreversible dysfunction [11]. There has been rightly predicted to increase in years to come, hence the need for an urgent intervention to curtail the prevalence of inborn errors most especially in developing and under-developed countries [12]. This is because any delay in early diagnosis or engagement could significantly result in an irreversible developmental impairment and growth discrepancies [13]. It is crucial to notice that these disorders are one of the major reason why we have morbidity and mortality among the pediatrics [14–16]. Most of these disorders are treatable and if there is any delay in the diagnosis and treatment may lead to various adverse outcomes which ranges from a Moderate to dire neuropsychological disorder, cognitive impairment, physical deformity and, for most cases, neonatal mortality [2,17].

Although each of the disorders is individually rare, their collective incidence is relatively substantial with ratio 1:1500 to 1:5000 of live births. However, most infants, shortly after birth do not have the symptoms with some of these disorders but the symptoms appear later in life with subsequent metabolic decompensating or gradual chronic progression. These symptoms include developmental delay, seizure, hypoglycemia, lethargy, jaundice, and abnormal odor of breath, sweat or urine among others [16] .

2 CLASSIFICATION OF INBORN ERROR OF METABOLISM

According to Ferreira et. al [18], the international classification of inherited metabolic disorders (ICIMD) comprises 1450 disorders classified into 24 subcategories with a total of 124 groups. The abnormalities of intermediate metabolism are represented by the very first 13 groups. Moreover, disabilities of intermediary metabolism entail mechanisms that either facilitate the breakdown of lighter weight nutritional molecules actually belonged to one of the 3 main energy molecules or translate them into substrates for diverse molecule biosynthesis [18].

There are various categories of disorders in the newborn which include genetic, metabolic, hematologic, endocrine, immunologic and cardiac disorders [17]. If these disorders are not detected earlier, they have the potential of placing a heavy financial burden on the individuals and the society at large as several of these disorders are correctable and manageable. Metabolic disorders are a complex and heterogeneous group of monogenic disorders exhibiting clinical symptoms from attendant errors in the genetic code resulting in a lowered or deficient activity of enzymes at the single pathway of intermediary metabolism [19]. The specificity of enzymes to a larger extent determines the ability of the body to inhibits the generation of toxic side products of metabolism which could result in metabolic diseases [20]. The chains of reactions at the metabolic pathways, therefore, enable the introduction of different intermediate metabolism which helps to identify the specific enzymatic defects responsible for any inherent error of metabolism [21] .

The enzymatic defects could lead to an inborn error of metabolism could be traced to sides reactions. Previous research revealed the possibility of the formation of undesirable side reactions during the enzyme metabolism. This metabolic compromise is generally referred to as enzyme metabolism promiscuity. An example of enzymes promiscuity is the one found in protein structure in which the integration of side-chain amino acid which is dissimilar to the main polypeptides could result in a compromised in the integrity of the metabolic processes [20] . During this process cell structure must have a means of combating the damaged small molecules by the compromised enzymes and side reactions [19] .

More than 500 known inherited metabolic disorders have been reported by many researchers [22] . This number increases as the knowledge of human metabolism increases and the availability of new technologies required to detect metabolic disorders [23]. The various examples of IEMs include: phenylketonuria, congenital hypothyroidism, phenylketonuria (PKU), methylmalonic academia, maple syrup urine disease (MSUD), isovaleric acidaemia (IVA), glutaric acidaemia, tyrosinemia, citrullinemia, long-chain 3 hydroxy acyl CoA dehydrogenases deficiency (LCHADD), medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, disorders of glycogen storage,

lysosomal storage; and mitochondrial diseases amidst others [24]. The most commonly screened metabolic disorders are phenylketonuria and congenital hypothyroidism which may be due to many factors [25]. However, with the advent of technological advancement in screening, a lot of newborn errors and conditions can be adequately and promptly screened once [2]. All of these IEMs are classified into various classifications which include disorders of: amino acid, urea cycle, organic acid, and fatty acid oxidation.

2.1 Amino acid inborn disorder (AAD)

The amino acid disorder is the metabolic inborn errors which are traceable to enzyme deficiencies resulting from the high accumulation of metabolites to such a level that can lead to organ damage or permanent failure over a while [26]. The most frequently affected organs include the brain, liver, and kidneys, with symptoms becoming apparent weeks or months after birth as a result of the cumulative effect of the damage to these organs [27]. The most common example of the amino acid disorder is phenylketonuria which is usually resulted from the deficiency of phenylalanine hydroxylase which converts phenylalanine to tyrosine in the body [28]. The damaging effects of elevated plasma phenylalanine could lead to neurological complications which included mental retardation, delayed or missed developmental milestone, which is noticeable only when the levels become elevated for months after birth [28]. Interestingly, the most commonly administered treatment is usually the dietary restriction of phenylalanine and supplementation of a tyrosine as needed [29]. Other disorders in this group include maple syrup urine diseases, which is caused by the deficiency of branched-chain keto-acid dehydrogenase enzymes which leads to elevation of plasma leucine, isoleucine, and valine [30], classical homocystinuria, which is as a result of methionine accumulation [31]; and tyrosinemia type 1 which is as a result of the accumulation of phenylalanine and tyrosine [32].

2.2 Organic acid inborn disorder (OAD)

Organic acidemias are groups of inborn errors of metabolism which result in the accumulation of organic acids that causes disturbance in the acid-base balance and alterations in pathways of intermediary metabolism. They are also regarded as organic aciduria, are mostly a group of inherent metabolism impairment characterized by absorption of genetically altered organic acid metabolites which results in an accumulated toxic matter in new born urine. Many organic acidemias are usually asymptomatic during the neonate or early childhood phase, but there are mild or moderate manifestations which might not be visible before adolescence, or may not receive medical treatment in anyway [33]. During an early stage of the disorder, the infant involve develop an acute metabolic acidosis accompanied by high anion discrepancies [33]. This often presents with severe, recurrent episodes of clinical and biochemical decomposition. Treatment includes the restriction of specific amino acid, the therapeutic use of vitamins that are involved in the metabolic pathway. This group of disorders includes propionic academia, isovaleric academia, 3-methylcrotonyl-CoA-carboxylase deficiency, Cobalam in defects, glutaric aciduria type 1, HMG-Co-A Lyase deficiency. Ma et al., [33] reported from the result of their investigation that the amino acid disorder accounted for half of the patients (3132 patients), while the organic acid inborn error accounted for just 26 % of the total patients analyzed (5568 patients) in Xinxiang city of China.

2.3 Urea Cycle Inborn Disorder (UCD)

This is a neonatal error which makes it impossible for an inborn baby to absorb waste particles during the process of proteins digestion into amino acids. The accumulation of waste products could portend a fatal consequence which include damage to the brain and in most cases the new born relapse into coma. They are hereditary disorder which in many case treated with dietary modification and medications [34]. Urea cycle disorders are inherited metabolic disorders from the nitrogen metabolism which involves any of the six enzymes in the nitrogen pathway [34]. For diagnosis of these disorders, quantitative plasma and urine amino acids, measurement of orotic acid in the urine, enzymes assays and mutation or DNA analysis [35]. Treatment is aimed at protecting the neurologic integrity. However, liver transplant maybe indicated in few instances where there is acute liver failure, Examples of this disorder include argininosuccinic aciduria, argininaemia, ornithine transcarbamylase deficiency, citrullinemia [36].

2.4 Fatty Acid Oxidation Disorder (FAOD)

Moreover, the fatty acid oxidation disorder is inborn errors that involve enzymes along the fatty acid oxidation pathway. The symptoms of this disorder are generally referred to as the Reye syndrome-like which include lethargy, nausea, and vomiting. These conditions include medium-chain acyl CoA dehydrogenase deficiency, long-chain hydroxylacyl CoA dehydrogenase deficiency, very-long-chain acyl co-A dehydrogenase deficiency, carnitine uptake defects, tri-functional protein deficiency. Ma et al., [32] reported that the fatty acid beta-oxidation disorders in Xinxiang city of China accounted for 19.4% of all cases with an incidence ratio of 1:1617. The fatty acid oxidation is generally referred to as an inborn error due to its inherent disorientation or impairment of either the fatty acids or mitochondrial β-oxidation. The early symptoms of this disorder at the neonatal stage include acute cardiomyopathy with an attendant long-term effect of liver dysfunction and hypoglycemia in infants. Episodic rhabdomyolysis is often the immediate symptom during or after teenage years, while most children show these symptoms at any developmental stage. Usually, potentially

Table I: Selected inborn error of metabolism, symptoms, and treatment.

Disorders	Main clinical manifestations if untreated
Congenital(primary) hypothyroidism	Often asymptomatic in neonates; may manifest itself with prolonged jaundice, muscle hypotonia, weak drinking, and later severe developmental delay, growth arrest, macroglossia, and constipation. Note: TSH screening does not detect secondary or tertiary hypothyroidism!
Carnitine acylcarnitine translocase(CAT) deficiency	Hypoketotic hypoglycemia, myopathy, cardiomyopathy
Glutaraciduria type I (GA I)	Macrocephaly, frontotemporal brain atrophy, hyperpyretic encephalopathic crises, development of a dystonic-dyskinetic movement disorder
Isovaleric acidemia (IVA)	Neonatal onset: weak drinking, vomiting, seizures, encephalopathy, coma, marked ketoacidosis, sweaty odour; mild, late-onset, and asymptomatic variants of the disease are common
Phenylketonuria	Developmental delay, permanent brain damage, severe mental retardation, epileptic seizures
Tyrosinaemia hereditary type I	Liver and renal dysfunctions, rickets, chronic Fanconi
Tyrosinaemia-tyrosine aminotransferase deficiency	Skin and eye disorders with or without mental retardation
Propionic acidaemia Methylmalonic acidaemia	May present: 1. Neonate- Overwhelming illness neurological abnormalities, acidosis to thrive, vomiting acidosis 2. Infancy- failure to thrive, Vomiting and mental retardation 3.Older- Intermittent 4. Mental retardation
Urea cycle disorders	May present: -Neonate: Often lethal due to ammonia intoxication - Infancy: Vomiting, anorexia, with particularly protein intolerance -Older: intermittent drowsiness
Maple syrup urine disease (MSUD	Weak drinking, somnolence, hypo- or areflexia, altered muscle tone, epileptic seizures, respiratory insufficiency, coma, urine smells like maple syrup (neonatal onset)
Galactosemia	When milk (lactose) is ingested, severe hepatic dysfunction, jaundice, coagulopathy, hepatomegaly, cataracts (neonatal onset)

Table I cont'd:

Disorders	Main clinical manifestations if untreated
Medium-chain acyl-CoA dehydrogenase deficiency (MCAD	Fever, vomiting, or fasting leads to a catabolic state with hypoglycemia, epileptic seizures, coma, manifestations resembling Reye syndrome (hypoketotic hypoglycemia, hepatopathy, hyperammonemia, encephalopathy) (neonatal onset is possible)
Long-chain 3-OH-acyl-CoA dehydrogenase deficiency (LCHAD)	Neonatal onset: hypoketotic hypoglycemia, lactic acidosis, cardiomyopathy, elevated creatinine kinase; Later: manifestations resembling Reye syndrome, polyneuropathy, retinitis pigmentosa
Carnitine palmitoyltransferase I (CPT-I) deficiency	Fasting intolerance, Reye-like manifestations (hepatoencephalopathy)
Carnitine palmitoyltransferase II (CPT-II) deficiency	Neonatal type with myopathy, cardiomyopathy, hepatopathy, Reye-like manifestations; adult type with exercise-induced rhabdomyolysis
Very-long-chain acyl-CoA dehydrogenase deficiency (VLCAD)	Neonatal onset possible, as in LCHAD; otherwise, cardiomyopathy and Reye-like manifestations (hepatoencephalopathy) develop in the first months of life

fatal symptoms may occur quickly in children within a few days of fasting, but adults can need up to four days of fasting. Table I shows the summary of some selected inborn error of metabolism, their clinical manifestation, and the treatment procedures.

3 OVERVIEW OF DIAGNOSTIC ADVANCES IN NEW BORN SCREENING

Newborn screening (NBS) was first developed in the 1960s by Robert Guthrie as a method of rapid pre-symptomatic detection and later adoption of prevention steps for infants born with metabolic disorders [37]. The successful implementation of Guthrie test had positively influenced the introduction and development of newborn screening for other metabolic conditions. Dried blood spots (DBS) is another methods being utilized in early-1970s, for newborn screening due to the ease of specimen collection and transportation with minimal used of sample. However, these conventional methods was gradually replaced by more efficient and specific methods of screening which include chromatography, fluorimeters and the most recent of them all, tandem mass spectrometry. This has helped the expansion of the newborn screening programs in many countries especially the developed ones [38]. The mass spectrometry method is a common analytical tool used to classify and measure by ions fragmentation depending on their molecular mass-to - charge ratio and calculating their pressure [39].

Although mass spectrometer (MS) is not a new technology but the use of one of its variants (electrospray tandem mass spectrometry) for new born screening is a classical technique that replaced the conventional Guthrie test. This is usually applied in new born screening with two different modes and it is done either by directly injecting the sample into the ionization source of the MS or by the separating the mixtures of compounds by liquid chromatography (LC) or gas chromatography (GC) before detection by MS is performed (16,38,40). The tandem mass spectrometry can be used to rapidly identified and quantify a large variety of different analytes from a single sample (such as blood or urine). These are conducted by separating the ions on the basis of their molecular mass-to-charge ratio and quantifying their intensity [23]. Many of inherited metabolism defects can be detected very quickly and with automated systems, including those which cannot be readily detected by other methods [27]. One can easily choose which analytes to screen, thus making it possible to choose which disorder to test or screen for.

Two approaches are often considered in the diagnosis of these disorders based on the biochemical mechanism that results in abnormal metabolic profile IEM. Firstly, the possible abnormal levels of the metabolic biomarkers are screened for inside urine, blood or plasma samples before the disease manifests [41]. This abnormality usually occurs when there is accumulation of the normal metabolites of the pathway that are upstream of the blockage or there is the accumulation of normal metabolites of other upstream pathways which cannot be fed into the blocked pathway. The second approach is based on the test for detecting specific pathognomonic biomarkers. This abnormality can occur when the surplus intermediates are channeled through pathway they do not normally use [42]. The biochemical procedure depends on the ability to identify abnormally high levels of the main substrates or of by-products that arise from alternative pathways upstream of the enzymatic

blockage [43]. These can be detected along with lower levels of the product of that enzyme or any of its downstream metabolites [44]. The new advances in new-born screening (i.e. plasma amino acid profiling-HPLC, plasma acylcarnitine profiling-LCT-MS, and urine organic acid profiling-GCMS) have enabled a better understanding and a more rapid diagnosis of inherited error of metabolism as carefully discussed in the succeeding sub-sections.

Basic Metabolic tests are also conducted in a routine laboratory using the biological samples of urine, blood and plasma in assessing the complete blood count, serum electrolytes, bicarbonate blood gases, blood glucose, urine pH electrolytes, ammonia, creatine kinase among others. They are not diagnostics all by themselves yet they are important in screening IEMs before advanced biochemical testing like MS/MS are performed. Table II gives examples of the metabolic tests according to Society for Study of Inborn Error of Metabolism (SSIEM) classification.

Table II: Metabolic tests for diagnosis of IEMs (According to SSIEM)

Disorder Group	Basic Metabolic tests for Diagnosis	Diagnostic modalities of choice
Disorders of amino acid, organic acid metabolism	GELAK (glucose, electrolytes, lactate, acid-base ammonia, ketones), homocysteine	Tandem mass spectrometry (MS/MS), U/HPLC quantitative amino acids, urine GC-MS, Succinylacetone (tyrosinemia type 1)
Disorders of fatty acid metabolism	Glucose,, other sugars, insulin, acid base, lactate, ammonia, ketones, uric acid, creatine kinase	Fasting studies, genetic testing, MS/MS, Urine GC-MS

3.1 Plasma Amino Acid Profiling using HPLC

Amino acids (AAs) are basic structural protein units and precursors of neurotransmitters, porphyrins and nitric oxide [45]. The amino acids and their derivatives, play very important function in the biological processes in human body and these include the synthesis of protein and metabolic pathways [45]. When amino acid is catabolized, they form organic acids that can replenish the kerb cycle and ammonia which is eliminated through the urea cycle [46,47]. Any defects in the amino acid metabolic pathways could result in abnormal levels or concentration of single or multiple amino acids and their downstream plasma or urine metabolites [45][46]. This usually follows an autosomal recessive mode of inheritance in which the mutation from the metabolic genetic block of both parents culminate into the lack or partial activity of enzyme needed in amino acid metabolism [36,45]. As a result of this, it is expedient to check the level of these free amino acids in physiological samples like plasma, as one or several compounds may play the role of a biomarker for one specific or a group of metabolic disorders [48]. Examples that are normally screened for are phenylalanine (for PKU); allo-isoleucine and valine for maple syrup urine disease (MSUD) [49].

Giuseppe et al., [49] emphasized the need to derivatives AA pre and post-column so as to have a good chromatographic separation which will aid in detecting the levels of AA in the plasma and other physiological samples. However, this procedure is time-consuming and may lead to interference. To, therefore, mitigate these inherent interferences quantitative and qualitative methods have been developed which is done without derivatization. Initially, only a few underivative amino acids were successfully conducted using HPLC or CE coupled to electrochemical detectors but now, even those in trace amounts can be detected using MS/MS with high sensitivity and specificity. In recent studies, the underivatized amino acid is analyzed for complex biological matrices like plasma using fast, straightforward and sensitive HPLC-ESI-MS/MS method [50,51]. In order to cover many important intermediates of metabolic pathways which are necessary in making diagnosis of aminoacidemia, this method is not only fast, of high sensitivity and specificity, but also provide the avenue to monitor 40 underivatized AAs with the inclusion of the key isomers and to quantify some of them [49]. This is unlike other methods like FIA-MS/MS which does not allow for the distinguishing of isomer and isobaric compound because they appear as a single peak. This occurs in cases like that of leucine which has 3 isomers (leucine, isoleucine, and allo-isoleucine) and is also isobaric with 3-hydroxyproline and propionyl-glycine. This often results in false positive results if further analyses are not

performed [52][53]. The whole set of AAs or a part of it can be thus analyzed from the plasma sample and this can be done in two different gradients (10 or 5 mins). For instance, where the whole set of 'AA' is to be analyzed, the 'AAs' can be classified into three divisions, within the same acquisition method based on their retention time. For those that play a significant role in the detection of metabolic disorders such as Val, Leu/Ile/a-Ile, Met, Phe, Cit, and Tyr, a shorter chromatographic run of 5 min could be used and this allowed the simultaneous monitoring of a reduced number of amino acids, and thus maximized the detection sensitivity and productivity as supported by [49].

3.2 Plasma Acylcarnitine Profiling using LC-T-MS

The breakdown of fatty acid and amino acid results in the production of acylcarnitines as intermediates and this is usually generated from the conversion of acyl co-A species through the action of carnitine acyltransferase [54]. Acylcarnitine is usually found in tissues and body fluids. The transportation of activated fatty acid (acyl-CoAs) from the cytosol into the matrix (where fatty acid oxidation takes place) depends on the production of carnitine conjugates [55]. It is worthy to note that a net efflux of acylcarnitine species from the mitochondria of the cell into the cytosol and ultimately into the plasma is very essential in cases of impaired fatty acid oxidation to prevent the accumulation of acyl coA intermediates which are potentially toxic in the mitochondrion [55,56]. However, there is paucity or no information on how the acylcarnitines are discharged into the extracellular space, changes in the plasma and urinary acylcarnitine profiles are used for the detection of fatty acid and amino acid oxidation defects [57]. Newborn child having these disorders accumulates disease-specific acylcarnitines that correlates with the acyl coenzyme-A compounds in the affected mitochondrion pathway [57]. An example of this is the accumulation of propionyl carnitine in individuals with propionic and methylmalonic acid disorders [58].

The acylcarnitine profile can thus be regarded as the assessment for detecting inborn errors of fatty acid and branched-chain amino acid catabolism [59]. It is primarily used for the clinical diagnosis of inherited impairments and complex metabolic syndrome [55]. This profile identifies and quantifies the acylcarnitines in the blood plasma [57]. Several metabolic routes such as amino acid and amino acid oxidation pathways are majorly responsible for the production of a broad spectrum of short, medium and long-chain acylcarnitine species [55]. The interpretation of the acylcarnitine profile, therefore, requires an individual to be able to recognize the abnormal concentration of specific analytes or patterns of analytes and their inherent metabolic origin [58]. The occurrence of acylcarnitine with different isomers at a very low concentration makes their quantification a daunting task. Hence, direct infusion of ESI-MS/MS is commonly used for the establishment of the acylcarnitine profile. However, this technique does not permit isomeric acylcarnitine species' discrimination. Even though the LC-T-MS was developed to allow the separation of isomeric compounds, the exact compound on chromatograph is difficult to detect and quantify since their concentration in biological samples is quite low [49,58]. To mitigate this limitation, a robust and highly sensitive Liquid Chromatography-Tandem Mass Spectrometer (LC-T-MS) can be utilized to comprehensively quantify the arrays of acylcarnitine species with a special focus on amino acid-derived intermediates at the isomeric form and lower concentration [58].

3.3 Urine Organic Acid Profiling using GC-MS

The organic acids (OAs) are water-soluble compounds emerging from the transitional metabolism of all main groups of organic cellular functions. It includes one or more carboxyl groups and other non-amino functional groups. vital metabolites from virtually all intermediary metabolism and exogenous compound [60]. Organic acid disorders are a heterogeneous group of metabolic disorders that result in the accumulation of organic acids which causes disturbance in the acid-base balance and alterations in pathways of intermediary metabolism [61]. A lot of the clinically important OA are otherwise normal components of urine profiles which accumulate under pathologic conditions. However, certain organic acids are completely undetectable under clinical circumstances, resulting from alternate mechanisms triggered in response to the failure of the enzyme role. An illustration of this is the 2-methylcitric acid in propionate metabolism

disorder [62]. The abnormality in the metabolic profile of organic acid is traceable to the alteration in the normal metabolic process which can be ably detectable in the urine metabolites but a lesser quantity in other body fluids [27]. This is feasible as urine contains hundreds of organic acids which could be from normal or abnormal metabolism [27]. Moreover, the diagnosis of organic acid disorders is firstly performed by analyzing the organic acids which could assist in recognizing the accumulation of single or several organic acids in the urine [61]. Their identification is key in diagnosing and treating IEMs. However, due to technological advancement, new born screening expansion for organic acid disorders and fatty acid oxidation disorders has been feasible using tandem mass spectrometer which has assisted in identifying increment many disorders in newborn [27].

Furthermore, gas chromatography-mass spectrometry (GC-MS) is often used to derivatize and detect the metabolites present in newborn urine which usually produce an optimal result for volatile metabolites. However, it can also be employed for analyzing primary metabolites when chemical derivatization schemes are utilized [63]. The traditional method which is often used firstly in the analysis of urine organic acid and it involves extracting acidic fraction from the urine sample using an organic solvent such as ethyl acetate which followed by the derivation of the extracted compound so as to make the organic acid more volatile, and the identification of metabolites is finally conducted using the gas chromatography-mass spectrometer [44]. However, the extraction step of from this traditional method could lead to loss of many neutral and positively charged compounds which may be of interest [44]. As a result, it is now being replaced by Shoemaker and Elliott urease method, wherein enzymatic removal of urea is done using urease instead of the extraction process. This method enables urine specimen used to have an abundance of organic molecules which can be separated and quantified using GC-MS [64]. In this method, the organic acids are separated using capillary gas chromatography which contains an immobilized, non-polar stationary phase. The metabolites identification and quantification are routinely conducted using the electron impact mass spectrometry with a mass range between 50 and 550 m/z [65]. **Table III** provides information on the interpretation of few metabolites.

4 STATISTICS OF IEM IN SELECTED ASIAN COUNTRIES

The emergence of new born screening has been proven to emanate out of the desire to mitigate the unprecedented increase in the number of children born with severe disabilities through early detection and treatment. Recent global and Asian statistics have shown an increasing trend of children born with different forms of congenital disorder and which often results in irreconcilable, irreversible dysfunctions. The need then arises for an urgent intervention to curtail the prevalence of inborn errors most especially in Asia-Pacific countries. The inborn error of metabolic (IEM) differs considerably among the selected Asia countries with specific screening policies. India and China account for the largest birth in the region [66]. Out of this region, countries like Japan, China, South Korea, and Singapore have been having successful newborn screening which has increased at an unprecedented rate in recent times, however, in India, government activities has recently been geared toward newborn screening [66]. It is worthy to note that the incidence of IEM in India is very high (1:1000) which could be attributed to the prevalence of consanguinity marriage [67]. The overall incidence of IEMs in Singapore was reported as 15 %; in China as 12 %(68)(68)(68)(68), in Hong Kong as 11% [68], in South Korea as 4 %, in Japan as 5 %, and in Taiwan as 7 % which account for an incidence ratio of 1:3165, 1:3795, 1:4100, 1:13205, 1:8557, and 1:7 030, respectively [68] **(Fig. 1).**

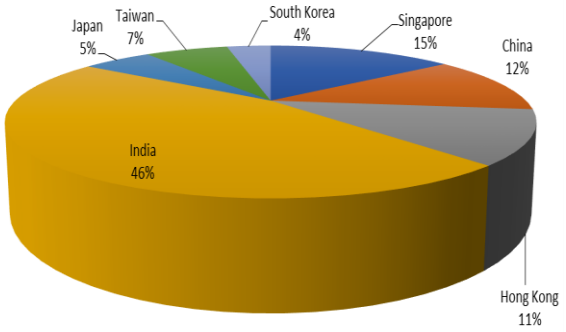

Figure 1: The Cumulative Incidence rate of Inborn Errors of Metabolism (IEMs) in selected Asia countries

Table III: Interpretation of tandem mass spectrometry for inborn errors of metabolism

Category	Analyte	Derangement	Interpretation
AMINO ACIDS	Citrulline	Elevated	Citrullinemia type1 or 2, arginine-succinic aciduria
	Methionine	Elevated	Homocystinuria
	Leucine+isoleucine, Valine	Elevated	Maple syrup urine disease
	Phenylalanine	Elevated	Phenylketonuria, Non-PKU pterin disorders
	Tyrosine	Elevated	Hereditary tyrosinemia type 1,2,3
	Glycine	Elevated	Ketotic/non-ketotic hyperglycemia
	Alanine	Elevated	Suggestive of mitochondropathy but not diagnostic
	Glutamine	Elevated	Hyperammonemia
ACYL-CARNITINES			
Carnitine	Free carnitine (CO)	Reduced	Primary/secondary carnitine deficiency
	Free carnitine (CO)	Elevated	Carnitine Palmitoyl transferase1 deficiency, iatrogenic
Organic acids	Propionyl carnitine (C3)	Elevated	Methylmalonic academia,propionic academia, vitamin B12 deficiency,
	Butyryl carnitine (C4)	Elevated	Ethylmalonic aciduria, ethylmalonic encephalopathy, short chain acyl CoA dehydrogenase deficiency, isobutyryl-CoA dehydrogenase deficiency
	Isovaleryl carnitine (C5)	Elevated	Isovaleric acidemia
	Triglyl carnitine (C5:1)	Elevated	Beta-ketothiolase deficiency
	3-hydroxyisovaleryl-carnitine OR 2-methyl-3-hydroxybutyryl carnitine (C5OH)	Elevated	Multiple carboxylase deficiency(holocarboxylase deficiency, biotinidase deficiency), HMG CoA lyase deficiency, 3-methyl crotonyl CoA carboxylase deficiency, Beta-ketothiolase deficiency, 2-methyl 3hydroxy butyric aciduria, 3-methylglutaconic aciduria
	Glutaryl carnitine (C5DC)	Elevated	Glutaric aciduria type 1 or 2 (multiple acyl coA dehydrogenase deficiency)
Fatty acids:Medium chain acyl carnitines	Hexanyl carnitine (C6) Octanoyl carnitine(C8) Decenonyl carnitine (C10:1) Decanoyl carnitine (C10)	Elevated	Medium-chain acyl-CoA dehydrogenase deficiency
Fatty acids: Very long chain acyl carnitines	Tetradecanoyl (C14), tetradecenoyl- (C14:1) and tetradecadienoyl-carnitine (C14:2)	Elevated	Very long-chain acyl-CoA dehydrogenase deficiency
Fatty acids: Long chain acyl carnitines	Hexa decanoyl (C16) Octadecanoyl (C18), Octadecadienoyl carnitine (C18:2)	Elevated	Carnitine palmitonyl transferase II deficiency
Fatty acids: Long chain hydroxyl acyl carnitines	3-hydroxy hexadecenoyl (C16:1-OH), 3-hydroxyhexadecanoyl (C16-OH), 3-hydroxyoctadecenoyl (C18:1-OH) and 3-hydroxyoctadecanoyl-carnitine (C18-OH)	Elevated	Long-chain 3-OH acyl-CoA dehydrogenase deficicency, trifunctional deficiency
Fatty acids: Multiple	C4-C18 saturated and unsaturated species	Elevated	Multiple acyl CoA dehydrogenase deficiency (Glutaric aciduria type 2)

The incidence of various IEMs screened out of the total births between 1997 and 2015 in Japan was 1:22000, 1:30000, and 1:26000 for organic acidaemia, fatty acid oxidation disorder and amino acid/ urea cycle disorders, respectively. Moreover, in Singapore out of the total number of new born screened between 2006 and 2014 for organic acidaemia, fatty acid oxidation disorder and amino acid/ urea cycle disorders, respectively. In Taiwan between 2001 and 2014 the ratio of organic acidaemia, fatty acid oxidation disorder and amino acid/ urea cycle disorders was respectively, 1:18000, 1:34000 and 1:17000.

The incidence of various IEMs screened between 2000 and 2015 in South Korea was recorded as 1:31000, 1:111000, and 1:29000 for organic acidaemia, fatty acid oxidation disorder and amino acid/ urea cycle disorders, respectively. Out of all the IEMs reported in Singapore, 3-methylcrotonyl-CoA carboxylase deficiency (MCCD) has the highest incidence (1:16000); followed by Short-chain acyl-CoA dehydrogenase (SCAD) (1:29500) and Carnitine uptake defect (CUD) with 1:35500. In the Philippines, the incidences of IEMs were reported to be 1:81576 for phenylkenoturia (PKU); 1:3926 for Congenital hyperthyroidism (CH); 1:108768 for galactosemia and 1:58 for Glucose-6-phosphate-dehydrogenase (G6PD) deficiency [69]. The different investigation has been conducted on the prevalence of IEMs among the Chinese population. An example of this is the investigation conducted by Wang (2019) using a wide range of twenty-two IEMs with a recorded incidence ratio of 1:3163 and the most prevalent of them are the PKU (1:19128) and mild hyperphenyalaninemia (M-HPA) (1:19128). Other IEMs and their incidence ratio include, carnitine uptake defect (1/26777), SCADD (1:28690), hypermethioninemia (1:30893), 3-MCCD (1:33412) and methylmalonic acidemia (1:40166) (36). To be specific, in Japan alone the propionic acidemia: PPA (1:41,000), phenylketonuria: PKU (1:46,000), very-long chain acyl-CoA dehydrogenase: VLCAD (1:93,000), carboxylase deficiency: CD (1:96000), methylmalonic academia: MMA (1:120,000), and medium-chain acyl-CoA dehydrogenase: MCAD (1:129,000) deficiencies recorded higher level of incidence [70]. Shibata et al., (2018) reported a high incidence ratio of propionic acidemia and methylcrotonyl-CoA carboxylase deficiency in Japan and South Korea with the former as a result of mutation from a weak phenotype. Moreover, in Taiwan the most common inherited metabolic error is the methylcrotonyl-CoA carboxylase deficiency (MCCD), phenylketonuria (PKU), carboxylase deficiency (CD), and primary carnitine deficiency (PCD), which accounts for about one out of forty-one thousand, fifty-eight thousand, sixty-one thousand and, seventy thousand live birth, respectively [70].

5 HISTORICAL PERSPECTIVES OF NBS POLICY IN SELECTED ASIAN COUNTRIES

There are mark-able four generations of newborn screening advancement in the Asian continent. The first era of this development happened in the early 1960s where the newborn screening was regarded as an advancement over the conventional public health institution, with pacific Asian countries like Japan, and China, at the forefront [71]. The second generation was in the 1980s in which the countries like China, Taiwan, Singapore, India, and Hong Kong showing a significant increase in newborn screening with congenital hypothyroidism as the prominent inborn error screened for [71]. Also, in the 1990s countries like Korea, Thailand, and the Philippines were at the developing stage and this period marked the third generation of newborn screening in the Asian continent. Moreover, the 4th generation was in the 2000s which identified countries such as Indonesia, Mongolia, Myanmar, Sri Lanka and Pakistan at the developmental but insignificant stage of post-natal screening only for congenital hypothyroidism even with no clear policy and limited funding [72]. However, there is limited or no formal newborn screening program with little information available on newborn screening activities in Bhutan, Cambodia, Laos, Brunei, Burma, Lebanon, Nepal, North Korea, East Timor, Indonesia, Pakistan & Papua New Guinea [72]. Presently, some of the nations in this region are just starting screening initiatives for targeted metabolic as well as other congenital anomalies, especially the countries with depressed economies [71]. Maks et al [66] evaluated the current status of expanded newborn screening in Hong Kong China with a special emphasis on the few numbers of incidences and their expanded spectrum which could be used to develop a broad-based program

for the local area. The result of their investigation revealed the cases of expanded newborn error of metabolism to be one out of 4122 births which accounts for an infinitesimal 0.00024% incidence from the number of children born. In Malaysia, NBS started in the 1980s with cord-blood testing for G6PD disorder [73]. The Malaysian Ministry of Health established a countrywide, step-by-step congenital hypothyroidism (CH) clinical guidelines for all newborns born in public hospitals in 2003 [73]. With the development of auditory and interventional treatments for hearing-impaired infants from the early nineties, some few clinics in the early 2000s adopted healthcare facility newborn hearing testing. Nevertheless, extended NBS (which includes abnormalities of amino acid metabolism, fatty acid oxidation, and organic acid metabolism) is not obligatory and has not yet been implemented into the Malaysian public health system.

6 LIMITING FACTORS AND FUTURE PROJECTION NBS POLICY IN SELECTED ASIAN COUNTRIES

Even though approximately half of the world's new birth is from the Asia regions, many of the countries the newborn screening (NBS) is mostly at the developmental stage. This is largely due to economic, cultural, and geographical differences which consequently led to little information about newborn screening in those countries [71]. Specifically, India and China account for most of the newborns in the Asian region but only China has recorded a significant number (80%) of PKU screening using mass spectrometry. However, Indian has shown limited response to newborn screening in recent times as reported by Casti et al., [74]. Moreover, in Pakistan and India; Bhinder et al., [75] reported a higher rate of consanguinity at 73 % and 60 % in Pakistan and Indian, respectively, which account for neonatal mortality in those countries. The developed and developing Asian countries are all confronted with one problem of the other in the development of a viable screening policy for the newborns. The developed countries are faced with the problem of searching for new conditions to improve and increase the coverage of their expanded screening policy. However, the developing Asian countries are bedeviled with inadequate infrastructure, the challenge of setting up screening panel, integration of expanded newborn screening to the existing public health, and management of the newborn after positive results [10,76]. Furthermore, among the six geographical regions identified by the International Society for Neonatal Screening (ISNS), the Asia-Pacific region seems to have the largest mutual economical screening differences which have resulted into large differences in the way neonatal, screening has been developed [76]. Countries in this region with fewer resources, therefore, have limited NBS programs with some just at the point of initiating a viable policy or they are just initiating it. Moreover, for some countries like Bangladesh, newborn screening activities are sadly unknown [74,77]. However, countries like Japan have an advanced newborn system with an expanded capacity for various types of metabolic disorders using state-of-the-art mass spectrometry techniques [74].Unlike many countries, Japan has a well-developed economy and has been able to scale through all the hurdles for quite a long time ago that are required in having an advanced NBS program, when compared with many others with depressed economies and corresponding struggle in the implementation or development of NBS. Down the years, countries with developed NBS has been collaborating with those that have not yet had a developed NBS so as to be able to reduce the rate of morbidity and mortality due to IEMs [74].

Furthermore factors such as the economic, government instability, cultural differences, geography location, health priority, and literacy level are some of the factors affecting the screening for neonatal disorder in many of the countries in the Asian region. The cultural factors include religious, ethnic, regional, and migratory, while the geography location could be related to the remoteness of large landmasses in the region like the Philippines, China, India, and Mongolia [69]. The literacy level, on the other hand, includes political, professional, and parental lack of awareness about the screening program, while the health priorities include region who are urgently facing other health problems such as epidemics of various infections and malnutrition [71]. Adding to this is the lack of awareness on the benefit of the screening or nursing mother most especially the rural dwellers which have resulted in many births outside the confinement of hospitals. Countries like the Philippines,

Bangladesh, Laos, Pakistan, and India have the largest number of children born out of the hospital and this accounts for a whopping 62 %, 80%, 86%, and 80% of the total births [69,71]. The problem of inadequate financing and workable policy is the clog in the wheel of progress for newborn screening in the aforementioned Asian countries and this was carefully grouped into lack of planning, professional expertise, policy expansion, literacy, logistic sustenance, organization, assessment and continuity [78,79].

In six decades of NBS, babies with inborn errors are identified at the early stage of infancy, thereby saving them from permanent disability and death. Rapid future intervention and improvement are therefore essential since the Asia continent accounts for almost half of the world's birth and thus contributing to the higher number of neonatal disorders [80]. The need then arise for the Asian continent to focus on building an enduring NBS policy that will stand the test of time [77]. In recent times, two important workshops were organized and the formation of Asia Newborn Screening Collaborative efforts was facilitated to draw a template for Asian countries to develop and share experience on screening program in their country to come out with a framework for its implementation [71,78,79]. This collaboration is such that the developed programs can assist the developing ones; especially by being judicious implementation of training programs that contribute to the creation of infrastructure such that resources can be transferred from the existing system to the development of infrastructure. Also, developing programs can take advantage of already ongoing screening efforts to expand their programs [71]. Despite the difficulties encountered from this collaborative effort, there is great potential for its success over the years in nations that constitute the region. It is crucial to note that many Asian countries have not ceased to forge ahead on their future goals of having a developed NBS that will be available for disorder and will be sustainable as prominent in most European countries [76].

7 CONCLUSION

In recent times, concern has been raised by many researchers on the need for a special integration of newborn screening into the public health system of Asia countries. Newborn screening is very crucial in this region and since the births in this region account for approximately half of the world's birth. UNICEF report revealed that about 85% of the newborn in five countries namely China, India, Malaysia, Indonesia, Bangladesh, and Pakistan do not have screening for half or more than half of their newborn population. This prompted the need for a sustainable or improved policy to encourage neonatal screening in this region. Countries in this region can be categorized into four groups based on their screening programs. Firstly, some of the countries run the new born screening programs which included the expanded new born metabolic screening as routine for all newborn. However, the second category offers the expanded new born metabolic screening as an option to families at an extra cost. The third category offers only congenital hypothyroidism plus another disorder in their screening program, while the third category has no screening program yet. The study carefully analyzed the different IEM prevalence, the diagnostic advances, historical perspectives, limiting factors and future projection of NBS in selected Asian countries. The result of this review shows a discouraging statistic of NBS in many parts of Asian continents and this is partly due or economic, cultural or geographical spread.

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