REVIEW ARTICLE

Newborn Screening for inherited metabolic disorders; news and views

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Newborn screening is important for the early detection of many congenital genetic and metabolic disorders, aimed at the earliest possible recognition and management of affected newborns, to prevent the morbidity, mortality, and disabilities associated with an inherited metabolic disorder. This comprehensive system includes; testing, education, follow up, diagnosis, treatment, management, and evaluation. There are major differences among many of the disorders being considered for inclusion in newborn screening programs. In recent times, advances in laboratory technology such as tandem mass spectrometry (MS/MS), which is more specific, sensitive, reliable, and comprehensive than traditional assays, has increased the number of genetic conditions that can be diagnosed through neonatal screening programs at birth. With a single dried filter paper blood spot, MS/MS can identify more than 30 inherited metabolic disorders in around two to three minutes. Advances in the diagnosis and treatment and an increased understanding of the natural history of inborn errors of metabolism have produced pressure to implement expanded newborn screening programs in many countries. Even as many countries throughout the world have made newborn screening mandatory, in Iran, nationwide newborn screening for inherited metabolic disorders other than hypothyroidism has not been initiated, hence, there is little information about these diseases. This article aims to review the recent advances in newborn metabolic screening and its situation in Iran and other countries.

Key words: Disease prevention, metabolic disease, newborn screening, tandem mass spectrometry

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INTRODUCTION

Inborn errors of metabolism (IEMs) are a complex and heterogeneous group of monogenic disorders that exhibit clinical symptoms due to an error in a genetic code that results in a lowered or deficient activity of an enzyme in a single pathway of intermediary metabolism.[1] Due to severe clinical consequences of IEMs, they are an important cause of morbidity and mortality in clinical practice, especially in pediatrics. Delay in diagnosis and treatment of these disorders, leads to a variety of adverse outcomes, including moderate-to-severe neuropsychological dysfunction, mental retardation, and death. Each disorder is individually rare, but their cumulative incidence is relatively high, around 1 in 1500 to 1 in 5000 live births.[2,3]

Newborn screening is a vital process that identifies apparently healthy infants with serious inherited disorders, generally metabolic in origin, that are usually correctable by dietary or drug interventions before they suffer significant morbidity or mortality.[4] Carlson commented that, "newborn screening represents one of the major child health advances of this past century".[5] In expanded newborn screening, a single test allows for early detection and treatment of a large number of disorders and it can potentially prevent serious consequences.[6,7]

General aims of newborn screening and criteria for screening

The goal of newborn screening is to identify apparently healthy infants with severe congenital disorders that are relatively prevalent and treatable (or controllable). It originated with the work of Robert Guthrie, 'Guthrie test,' in the 1960s, for detecting the metabolic disorder, phenylketonuria (PKU) and used for years to justify screening programs.[8,9] With the development of immunoassays for thyroxin and the thyroid stimulating hormone (TSH) in the 1970s, it became feasible to add congenital hypothyroidism (CH) to the Newborn Screening (NBS) panel. Many conditions are potential candidates for NBS, but this is not practical for all.[10] The World Health Organization (WHO) Wilson–Jungner criteria are the gold standard criteria that are used to determine the disorders that need to be included in the newborn screening program. These criteria cover the aspects of knowledge of the disease, its treatment, the scientific validity of the tests, and cost considerations associated with the screening program.[9,11,12] According

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to the WHO Wilson–Jungner criteria, a disease which has the following properties should be screened: (1) An important health problem; (2) the natural history of the condition should be adequately understood; (3) it should be recognizable in the early stages; (4) there should be a suitable test or examination; (5) the test should be acceptable to the population; (6) intervals for repeating the test should be determined; (7) there should be an accepted treatment for patients; (8) facilities for diagnosis and treatment should be available; (9) there should be an agreed policy concerning whom to treat as patients; and (10) the costs of case finding should be economically balanced against the benefits.[12]

Brief history of expanded newborn screening

Historically, most NBS programs such as PKU were established without evidence-based evaluation. PKU screening was based on a prediction that dietary treatment would prevent mental retardation.[8] In the late 1960s, the WHO criteria established by Wilson and Jungner have served to justify NBS programs. Screening for PKU successfully fulfilled these criteria. Then programs were extended, with success, to other conditions, such as CH. Yet, an increasing number of conditions that can be detected by multiplex technologies do not fulfill all the Wilson and Jungner criteria. Since 2000, the progressive inclusion of tests for several diseases has been driven by the tandem mass spectrometry (MS/MS) technology, hence, national NBS programs differ widely now. In 2005, the American College of Medical Genetics (ACMG) proposed 29 core conditions that are considered appropriate for newborn screening, because they have a screening test, an efficacious treatment, and an adequate knowledge of natural history.[8,13,14]

Newborn screening as a public health system

An NBS program is not just a panel of screening tests to identify whether a metabolite is found in abnormally high or low concentrations in a particular blood spot. Ideally, it also integrates five parts as follows: (1) Testing of newborn infants, (2) follow-up of an abnormal screening result, (3) diagnostic testing and confirmation by specialized laboratory testing, interpretation, and treatment, (4) lifelong disease management (5) the NBS system's evaluation, which makes regular and timely communication between nurseries, screening laboratories, health authorities, pediatricians, and subspecialists.[15,16] Today, recommendations for the screening process also vary, for example, the time of blood sampling ranges from 24 hours (USA) and 48 to 72 hours (Germany) to 120 hours (UK).[14] In one cohort screened with MS/MS in Australia, involving 362,000 newborns, the prevalence of inborn errors, excluding phenylketonuria, was 15.7 per 100,000 births, as compared with the adjusted rates of 8.6-9.5 per 100,000 births before the MS/MS screening.[17] Follow-up reports provide a better outcome for patients at six years of age, by MS/MS screening, with fewer deaths and fewer clinically significant disabilities.[18] This strategy represents diagnosis and treatment before the onset of symptoms.[19] Economically, each Euro spent on the screening program saved more than 25 Euros in health and social costs.[20] Results of many studies reflect a minimum burden and a better outcome for patients, with fewer deaths and fewer clinically significant disabilities achieved by expanded newborn screening.[18,21-23] However, other types of screening such as carrier state, lethal conditions, disorders with delayed onset, and susceptibility testing are controversial. They need an extensive and ethical considerations approach, to allow us to find an optimal equilibrium between the potential benefits and the possible damages derived from neonatal screening.[24]

Tandem mass spectrometry in newborn screening

In recent times, advances in laboratory technology with MS/MS has enhanced the identification of newborns with an inherited metabolic disease using a single sample of a dried blood spot on filter paper.[11,25,26] Tandem mass spectrometry is a powerful technology used for rapid identification and quantification of a large number of different analytes from a single sample, by separating the ions based on their molecular mass-to-charge ratio and measuring their intensities.[27] A tandem mass spectrometer comprises of five basic components: An ion source, a mass analyzer (MS1), a collision chamber, a second mass analyzer (MS2) that separates the ions and fragments produced in the collision chamber, and a detector.[27,28] Samples are introduced into the instrument and then ionized to generate charged molecules. These molecular ions are extracted into the first analyzer (MS1) and separated by their mass-to-charge ratio. These molecular ions then enter the collision cell one at a time, where they collide with an inert gas and fragments. These fragments are separated by the second analyzer (MS2) and are detected as they emerge from the analyzer. The results are presented as a graphical mass spectrum, showing each ion by its mass-to-charge ratio and its relative intensity. MS/MS can be used for the rapid detection of a large number of IEMs, including some diseases that can not be readily detected by other methods, such as, Medium-chain Acyl-CoA Dehydrogenase (MCAD) deficiency. MS/MS has a number of advantages compared with the currently available analytical methods and in recent years its use for newborn screening has universally expanded. Some advantages of using MS/MS for newborn screening include the following: (a) The analysis can be performed on very small quantities of blood or other body fluids; (b) the analysis uses two mass spectrometers concurrently. The first is used to separate the components of the mixture, hence eliminating or minimizing prior chromatographic separation; (c) the time required for the analysis of each sample is therefore reduced to around two-to-three minutes;

and (d) the process can be automated, permitting analysis of around 600 samples per 24 hours, with a very modest cost per sample.[11,29]

Metabolic disorders detected by MS/MS

The application of MS/MS to newborn screening currently involves measuring mainly two groups of analytes, amino acids and acylcarnitine species, for the detection of aminoacidopathies, organic acidurias, and fatty acid oxidation defects,[30] although other disease markers may also be added.[26] Metabolic disorders detected by MS/MS can be categorized into four groups: Amino acid disorders, urea cycle disorders, organic acid disorders, and disorders of fatty acid oxidation [Table 1]. Around 40 disorders can be detected through the aforementioned analytes. The severity of metabolic disorders can range from mild to severe.[31]

In view of the advances in the technology, recently, the American College of Medical Genetics (ACMG) has considered IEMs that are suitable for inclusion into newborn metabolic screening programs. Based on criteria such as: (1) Clinical characteristics (e.g., incidence, burden of disease if not treated, and phenotype in the newborn); (2) analytical characteristics of the screening test (e.g., availability and features of the platform); and (3) diagnosis, treatment, and management of the condition in acute and chronic forms, each disorder was assigned a score. Conditions with scores of >1200 met the key criteria and were preliminarily considered appropriate for inclusion in a core newborn screening panel. Conditions scoring <1000 were not considered appropriate for inclusion in the core newborn screening panel. Conditions with intermediate scores (1000-1199) were part of the differential diagnosis of a high-scoring core condition, but without an efficacious treatment or without a well-understood natural history.[8,13,14] Based on the ACMG recommendations, 29 core conditions that are detectable by MS/MS are considered appropriate for newborn screening [Table 1]. In the following section three representatives of these conditions, that is, PKU, MCAD deficiency, and methylmalonic acidemia (MMA) will be briefly described (characteristics of all these disorders may be found in general pediatric texts and further details on the rarer disorders are available).[32]

Phenylketonuria (PKU), one of the most commonly screened metabolic disorders, can readily and reliably be detected by MS/MS. It is an autosomal recessive disorder, most commonly caused by a mutation in the gene coding for phenylalanine hydroxylase. Classic symptoms of untreated PKU include; mental retardation, learning difficulties, spasticity, seizures, developmental delay, and congenital heart disease. Figure 1 illustrates the blood amino acid profile from a patient with PKU analyzed by MS/MS, essentially as described elsewhere.[33,34] It is now clear that if Phenylketonuria is diagnosed immediately after birth, the irreversible mental retardation can be prevented. This requires comprehensive newborn screening in all populations around the world. Infants diagnosed with PKU are treated with a special low-phenylalanine formula and it is recommended that strict dietary therapy be continued during their entire lifetime.[35-37]

It is noteworthy that MCAD deficiency scores higher than PKU for inclusion into newborn screening program [Table 1]. MCAD deficiency is the most common disorder of fatty acid oxidation, affecting 1 in 10,000 babies born in England.[38] The disorder occurs when one of the enzymes required for converting fat to energy is missing or not working properly. Infants with MCAD deficiency appear to develop normally, but secondary to acute vomiting or fasting, present with rapidly severe hypoglycemia, which can lead to lethargy, seizures, mental retardation/intellectual disability, motor deficits or death. The treatment includes avoidance of fasting and L-carnitine supplementation when deemed necessary. MCAD deficiency can reliably be detected by MS/MS analysis[38] and is currently included in most expanded newborn screening programs worldwide. Newborn screening has been effective in substantially reducing the risk of disability or death resulting from MCAD.[3,39,40] Figure 2 illustrates the blood acylcarnitine profile from a patient with MCAD deficiency analyzed by MS/MS essentially, as described elsewhere.[33,34] Other disorders of fatty acid oxidation with relatively high scores, for example, deficiencies of very-long chain acyl-CoA dehydrogenase, 3-hydroxyacyl-CoA dehydrogenase, trifunctional protein, and riboflavin responsive multiple acyl-CoA dehydrogenase deficiency can also reliably be detected by MS/MS analysis.[41-43]

Methylmalonic acidemia (MMA) is a heterogeneous metabolic disorder characterized by the accumulation of methylmalonic acid in body fluids. This autosomal recessive disorder results from the inability to convert L-methylmalonyl-CoA to succinyl-CoA in the metabolic pathway of propionate. Results of both neonatal mass screening and selective screening programs indicate that MMA is one of the most common organic acidurias worldwide.[44]

Methylmalonic acidemia results either from a deficiency of methylmalonyl-CoA mutase or a defect in the intracellular processing of its active cofactor deoxyadenosylcobalamin from cobalamin. Clinically, it presents with acute or chronic neurological signs, mental retardation, osteoporosis, and progressive renal failure, due to accumulation of toxic

Table 1: MS/MS detectable disorders (name, abbreviation and score) for the newborn screening panel

Disorders	Abbreviation	Score	Disorders	Abbreviation	Score
Amino acid disorders			**Organic acid disorders**		
Phenylketonuria	PKU	1663	Methylmalonic acidemia (Cbl A, B)	Cbl A, B	1343
Maple syrup urine disease	MSUD	1493	Propionic acidemia	PROP	1333
Benign hyperphenylalaninemia	H-PHE	1365	beta-Ketothiolase deficiency	BKT	1282
Homocystinuria (CBS deficiency)	HCY	1357	Glutaric acidemia type II	GAII (MAD)	1224
Tyrosinaemia type I	TYRI	1257	Methylmalonic acidemia (Cbl C,D)	Cbl C,D	1166
Tyrosinaemia type II	TYRII	1249	Malonic acidemia	MAL	1143
Defects of biopterin cofactor biosynthesis	BIOPT(BS)	1174	Isobutyryl-CoA dehydrogenase deficiency	IBG	1134
Tyrosinaemia type III	TYRIII	1149	2-Methyl-3-hydroxybutyric aciduria	2M3HBA	1132
Defects of biopterin cofactor regeneration	BIOPT(REG)	1146	3-Methylglutaconic aciduria	3MGA	1057
Hypermethioninemia (Methionine adenosyl transferase deficiency)	MET	1121	2-Methylbutyryl-CoA dehydrogenase deficiency	2MBG	1124
Urea cycle disorders			**Fatty acid oxidation disorders**		
Citrullinaemia type I	CIT(I)	1266	Medium-chain acyl-CoA dehydrogenase deficiency	MCAD	1799
Argininosuccinic acidemia	ASA	1263	Very Long-chain acyl-CoA dehydrogenase deficiency	VLCAD	1493
Argininemia	ARG	1151	Long-chain 3-OH acyl-CoA dehydrogenase deficiency	LCHAD	1445
Citrullinaemia type II	CIT(II)	1001	Trifunctional protein deficiency	TFP	1418
Ornithine transcarbamylase deficiency	OTC	942	Carnitine uptake defect	CUD	1309
Organic acid disorders			Short-chain acyl-CoA dehydrogenase deficiency	SCAD	1252
Biotinidase deficiency	BIOT	1566	Medium / short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency	M / SCHAD	1223
Isovaleric acidemia	IVA	1493	Medium-chain ketoacyl-CoA thiolase deficiency	MCKAT	1170
Glutaric acidemia type I	GAI	1435	Carnitine palmitoyltransferase II deficiency	CPT II	1169
3-Hydroxy-3-methyglutaric aciduria	HMG	1420	Carnitine / acylcarnitine translocase deficiency	CACT	1141
Multiple carboxylase deficiency	MCD	1386	Carnitine palmitoyltransferase I deficiency (liver)	CPT IA	1131
Methylmalonic acidemia (mutase deficiency)	MUT	1358	Dienoyl-CoA reductase deficiency	DERED	1119
3-Methylcrotonyl-CoA carboxylase deficiency	3MCC	1355	Carnitine palmitoyltransferase I deficiency (muscle)	CPT IB	1009

compounds proximal to the metabolic block. Pernicious anemia and metabolic ketoacidosis are the clinical hallmarks of MMA. The therapy consists of restriction of methylmalonate precursors, protein restriction, L-carnitine, and vitamin B12 supplementation.[45,46]

Figure 2 illustrates the blood acylcarnitine profile from a patient with MMA analyzed by MS/MS showing the accumulation of propionylcarnitine

Status of newborn screening

Newborn screening, as a routine part of the care of most newborns, is expanding considerably throughout the world. Many countries screen all newborns for PKU and hypothyroidism, while in some of the more developed countries, the routine screening panels include 20 or more conditions. In other parts of the world, NBS is only just getting started. Some countries have initiated nationwide screening only within the last several years. In the following

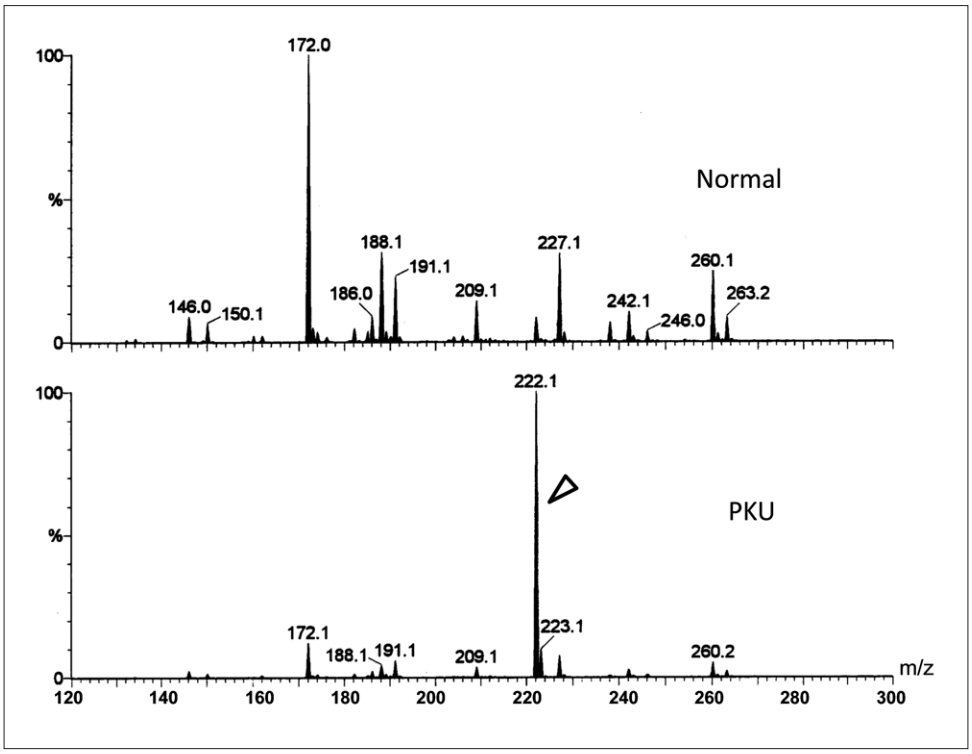

Figure 1: Blood amino acid profiles from a control subject and a patient with PKU analyzed by tandem mass spectrometry (neutral loss of m/z 102). Ion signals at representative m/z values correspond to butyl esters of phenylalanine (m/z 222), phenylalanine internal standard (m/z 227), tyrosine (m/z 238), tyrosine internal standard (m/z 242). The key diagnostic peaks are identified by arrows

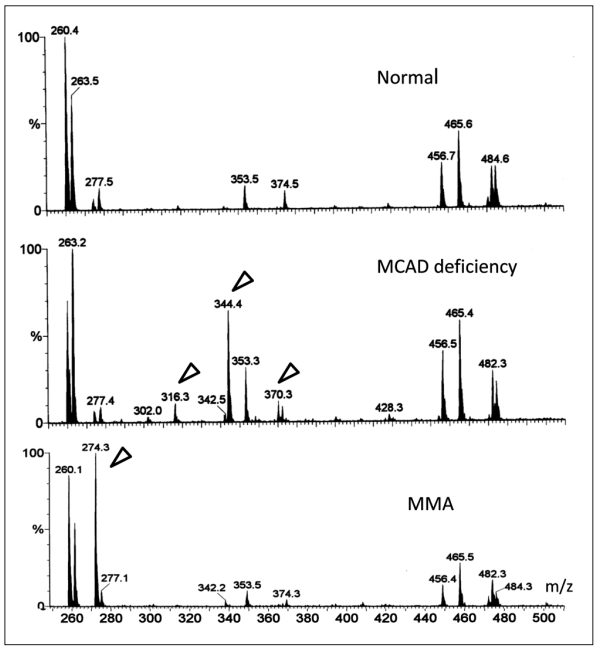

Figure 2: Blood acylcarnitine profiles from a control subject and patients with medium chain acyl CoA dehydrogenase deficiency (MCAD) and Methylmalonic acidemia (MMA) analyzed by tandem mass spectrometry (parents of m/z 85). Ion signals at representative m/z values correspond to butyl esters of C2 carnitine (m/z 260), C2 carnitine internal standard (m/z 263), C3 carnitine (m/z 274), C3 carnitine internal standard (m/z 277), C4 carnitine (m/z 288), C6 carnitine (m/z 316), C8 carnitine (m/z 344), C8 carnitine internal standard (m/z 353), C10:1 carnitine (m/z 370), C10 carnitine (m/z 372), C14 carnitine (m/z 428), C16:1 carnitine (m/z 454), C16 carnitine(m/z 456), C16 carnitine internal standard (m/z 456), C18:2 carnitine (m/z 480), C18:1 carnitine(m/z 482), C18 carnitine (m/z 484). The key diagnostic peaks are identified by arrows

section the status of newborn screening in different parts of the world and Iran is briefly reviewed.

Status of newborn screening in developed countries

Many developed countries have expanded their national newborn screening programs using MS/MS technology. However, the panel of disorders screened for, varies widely. For example in Australia all disorders detectable by MS/MS analysis are screened; in the United States nearly all of the 4.3 million newborns are screened for more than 30 metabolic conditions; in the Netherlands 17 disorders are screened; in Denmark the panel includes 13 metabolic disorders; in Germany 12 disorders; and the United Kingdom (UK) screens only for PKU and MCAD deficiency.[14,47,48]

Status of newborn screening in the Middle East and Northern Africa

The region of the Middle East and Northern Africa (MENA) consists of 21 countries with a population of about 400 million, with a high birth rate, and an estimated 10 million newborns per year. The population is characterized by a high consanguinity (25-70%) and a high percentage of first-cousin marriages. There are several pilot studies that highlight the high incidence of genetic defects in these countries and the need for newborn screening programs. As the bulk of inherited metabolic disorders are autosomal recessive, it is not surprising that the MENA region, with a high rate of consanguineous marriages, has a high incidence

of these diseases. Even as the developed countries, where consanguinity is <1%, are proceeding to expand their screening panels, the developing countries where parental consanguinity is common and inherited disorders are more prevalent, are still in the initial step of this preventive health care.[49] Less than half of the MENA countries have a national newborn screening program, which primarily covers only CH. Lebanon and Saudi Arabia use MS/MS for a large panel of metabolic conditions. In 2004, Qatar has partnered with the University of Heidelberg in Germany to establish MS/MS screening for metabolic conditions, by sending them dried blood spot specimens for analysis.[50]

Results from expanded newborn screening programs using tandem mass spectrometry have revealed that the incidence of inherited metabolic disorders varies considerably among countries. For example the combined incidence of amino acidemias, organic acidemias, and fatty acid oxidation defects is 1 in 5800 in the mainland of China,[51] 1 in 5882 in Taiwan, in China,[52] 1 in 2000 in Korea,[53] 1 in 9330 in Japan,[54] 1 in 6369 (excluding hyperphenylalaninemias) in Australia,[17] 1 in 4900 in Denmark,[55] 1 in 2517 in Germany,[20] 1 in 4000 in North America,[56,57] 1 in 4122 in Hong Kong[21] and 1 in 1327 in Qatar.[20] The high incidence of metabolic disorders in Qatar, with a high rate of consanguinity, is noteworthy. For example, the incidence of homocystinuria in Qatar is 1 in 1800[58] compared with its incidence of 1 in 200,000 in USA,[59] 1 in 230,750 in Australia,[18] and 1 in 316,243 in Portugal.[60] The estimated incidence of MCAD deficiency in Qatar is 1 in 4000,[61] whereas, in populations derived from Europe it ranges from 1 in 10,000 to 20,000.[38]

Status of newborn screening in Iran

Iran is a large country with different ethnicities in the northern, southern, and central provinces, and more than 70 million people, with a birth rate of around 1.4 million per year. In Iran, newborn screening is mandatory only for CH and covers approximately 84% of the neonates. CH was selected for inclusion in the newborn screening programs in many countries because of its high prevalence, availability of screening methods, and cost-effective intervention.[62] The prevalence of permanent CH in one study in Isfahan was 1 in 748 live births during 2002-2005. This is approximately five to six times higher than the worldwide incidence of CH. According to this study, permanent CH in Isfahan was nearly two to three times more frequent than the prevalence of permanent CH in Greece (1 in 1800), Saudi Arabia (1 in 1400), and Turkey (1 in 2354).[63] For PKU and Glucose-6-phosphate dehydrogenase only limited local screening programs were performed in Iran. The largest study for evaluating the incidence of PKU was performed in the Fars province by Habib and colleagues. They have reported an incidence of 1.6 in 10,000 for PKU, which is one of the highest values reported for similar populations around the world.[64] In another study performed by Farhad and Kabiri on 8633 newborns in Tehran, the incidence rate was reported as 1.1 in 10,000.[65]

DISCUSSION AND CONCLUSION

Newborn blood spot screening started in the 1960s for detection of PKU. Over the years, screening programs have expanded to include other inherited diseases. Newborn screening is carried out to prevent significant morbidity, mortality, and mental handicap in infants. Newborns are tested to identify serious or life-threatening conditions before the symptoms begin.[66] Natural history and the prevalence of each disorder shows remarkable variation among neonates from one country to another. Therefore, the data cannot be applicable directly to another locality. In developing countries like Iran, where a high rate of consanguineous marriages may lead to increased incidence of metabolic conditions, MS/MS screening for metabolic disorders will likely prove cost-effective. Furthermore, many families feel relieved that they are aware of their child's condition in early infancy, although there might not be a specific treatment.[67] On account of the importance of early diagnosis and treatment of inherited metabolic diseases and with respect to that little available information about the frequency and natural history of these disorders in Iran, establishment of the MS/MS neonatal screening program seems to be a major health priority in this country. In order to minimize morbidity, mortality, and disabilities associated with the inherited metabolic disorders, we suggest a national pilot study, to evaluate the establishment of the expanded newborn screening, in this country.

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