Molecular Genetics and Metabolism Reports 40 (2024) 101103

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Expanded inherited metabolic diseases screening by tandem mass spectrophotometry: The first report from Iran

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ARTICLE INFO

Keywords:
Screening
Inherited metabolic disease
Genetics
IMD
Neonates

ABSTRACT

Inherited metabolic diseases (IMD) are a group of rare genetic disorders that can present with a variety of symptoms. Since these disorders are hard to treat once the symptoms occur, neonatal screening may be a logical strategy. Here we evaluate the first results of national expanded IMD screening in Iran. A total of 46 IMDs were screened in this national program. Between April 2018 and March 2022, all infants who underwent national IMD screening at Shahid Beheshti University of Medical Sciences were included in this study. History and Physical examinations of infants, screening results, recall rate, response rate, and prevalence of IMDs were evaluated. A total of 125,819 infants were screened during this period. The recall rate of the test was 0.81%. 124 cases were diagnosed with a definite IMD and the raw overall prevalence of IMDs was estimated to be 1:1015. Amino-acidopathies were the most commonly detected disorders and Hyperphenylalaninemia/PKU was the most prevalent disorder among all groups. Since IMDs vary from region even in a single country, screening for IMDs is crucial in societies with a high rate of consanguineous marriages. More studies are essential for figuring out the most efficient combination of diseases to be screened based on countries' facilities.

1. Introduction

Inherited metabolic diseases (IMD) encompass a collection of rare genetic disorders characterized by impaired metabolism of specific substances within the body. As a result, the accumulation of toxic metabolites or reduction in essential biological products can lead to disturbances in specific organs, giving rise to various clinical symptoms [1,2]. The clinical manifestations of IMDs can vary widely, ranging from an absence of symptoms to the development of progressive and potentially fatal diseases [3,4].

Given the inherent lack of symptoms during the neonatal period and the difficulty in treating organ damage once it occurs, the implementation of mass neonatal screening programs and early preventive interventions is a logical approach in many communities. This approach is particularly valuable in societies where consanguineous marriages are prevalent, as many IMDs are inherited in an autosomal recessive manner. It should be noted, however, that the distribution of IMD subtypes varies across different geographical regions due to the diverse prevalence of genetic mutations among various ethnicities and races [5–7].

Iran, being a country with a high rate of consanguineous marriages, has implemented a national neonatal screening program for hyperphenylalaninemia over several years. More recently, starting in 2018, an expanded screening program for IMDs utilizing acylcarnitine profiling with tandem mass spectrophotometry has been launched in certain provinces and remains ongoing. This article focuses on the primary outcomes of the program conducted at Shahid Beheshti University of Medical Sciences, which serves as a major operational center for this national program in Tehran Province. This study period spans from April 2018 to March 2022.

2. Materials & Methods

In the revised national screening program for IMDs, launched in April 2018, a comprehensive evaluation of newborn infants for IMD takes place during their initial visit for vaccination and checkups at local

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https://doi.org/10.1016/j.ymgmr.2024.101103
Received 17 April 2024; Received in revised form 30 May 2024; Accepted 4 June 2024

healthcare centers within the first 72 h of life. To conduct the assessment, blood samples are obtained through a heel prick technique, and these samples are then applied onto filter papers. Subsequently, the filter papers are sent for analysis using tandem mass spectrophotometry, which allows for the examination of acylcarnitine and specific amino acid profiles. Primary disorders that can be diagnosed with this screening are summarized in Table 1. In addition, 20 other disorders can also be diagnosed indirectly through this screening results. Infants will be categorized into three groups, namely Healthy (low risk), suspicious (medium risk), and high risk, based on the outcomes of screening assessments and their medical history. Infants deemed high risk, as well as those displaying borderline suspicious test results that persistently appear abnormal upon repeated testing, will be referred to specialized pediatric endocrine and metabolism experts at tertiary centers overseeing their care. (See Fig. 1.)

The referred infants will undergo additional supplementary examinations and essential paraclinical tests to further evaluate their condition. Depending on the abnormal parameters, these tests may include serum ammonia, lactate, pyruvate, serum aminoacids High-performance liquid chromatography (HPLC), urine organic acids, blood gas, and specific enzyme activity in special conditions like hyperphenylalaninemia. Treatment will be initiated for high-risk infants even before a definitive diagnosis is made, to ensure timely intervention. Furthermore, regular contact with parents will be maintained to provide ongoing follow-up assurance and outcomes. Genetic confirmation for diagnosed patients will be requested if parents are capable of underwriting the financial cost since genetic tests are not supported by insurance in Iran and not all families agree to undergo this financial burden.

This study focused on evaluating the initial outcomes of a nationwide newborn screening program for IMDs at Mofid Children's Hospital, which serves as the referral center for Shahid Beheshti University of Medical Sciences from initiation to March 2022. Shahid Beheshti University of Medical Sciences is among the three prominent universities in Tehran, supervising a significant portion of healthcare facilities in both the city and its surrounding urban areas. During the specified period, we collected data on the total number of infants who underwent screening. Subsequently, we tracked the progress of the referred patients and assessed various factors including medical history, family history, demographic information, physical examination findings, time of screening, time to test results, and time to diagnosis. A questionnaire was developed to capture these details along with the IMD screening profile. To examine the associations and correlations between variables, we utilized chi-square and logistic regression tests. These statistical tests allowed us to interpret the relationships between different variables. Additionally, to compare the means between different groups, we employed the *t*-test. The statistical software SPSS version 26 was utilized for conducting these analyses.

Table 1
Primary Disorders Diagnosed With National Screening Program.

Amino Acid Disorders	Fatty Acids Oxidation Defects	Organic Acid Disorders
Argininoosuccinic aciduria	Primary Carnitine Deficiency	Propionic Acidemia
Citrullinemia Type 1	MCAD	Methylmalonic Acidemia
Maple Syrup Urine Disease	VLCAD	B12 Deficiency
Homocystinuria	LCHAD	Isovaleric Acidemia
Phenylketonuria	TFP	3MCC
Tyrosinemia Type 1		OH-3-Methylglutaric Aciduria
Tyrosinemia Type 2		Holocarboxylase Deficiency
Tyrosinemia Type 3		Beta Ketothiolase Deficiency
		Glutaric Aciduria Type1

MCAD. Medium-chain acyl-coenzyme A dehydrogenase deficiency, VLCAD. Very long-chain acyl-CoA dehydrogenase deficiency, LCHAD. Long-chain hydroxy-acyl-CoA dehydrogenase deficiency, TFP. Trifunctional protein deficiency, 3MCC. 3-methylcrotonyl-CoA carboxylase deficiency.

3. Results

Between April 2018 and March 2022, a total of 125,819 newborn infants were screened for IMDs in Shahid Beheshti University of Medical Sciences affiliated health centers. Among these infants, as explained in the screening protocol, 1349 were referred for further evaluation due to either initial pathologic abnormal screening results or subsequent abnormal results upon repeat testing (Refer Rate 1.1%).

Among the referred cases, it was observed that 680 cases (50.4%) were male, while 669 cases were female. The average gestational age of the referred infants was 37.3 weeks, ranging from a minimum of 29 weeks to a maximum of 41 weeks. Of these infants, 76.1% were born following a term pregnancy, while 23.9% were born prematurely. Furthermore, 46.9% of the referred patients were the first children in their families, while twin pregnancies were identified in 6.7% of cases. Additionally, a history of previous abortion or fetal death was reported in 12.9% of cases, and 18.9% of the infants had a history of hospitalization during their first days of life. Hospital admissions were mostly due to prematurity complications (e.g. respiratory distress, low birth weight, and feeding problems), phototherapy for high-risk hyperbilirubinemia, and probable sepsis work-up. No hospitalization was attributed to IMD symptoms in the neonatal period. Also, a total of 635 infants (47.1%) in the referred cases had consanguineous parents. The mean weight, height, and head circumference of the referred infants were recorded as 3033.8 g, 49.1 cm, and 35 cm, respectively. Among the physical findings observed, the most prevalent were icterus (11.3%), vomiting (8.7%), and feeding difficulties (7.6%).

The referred infants had a mean age of 7.1 days at the time of sample collection for further evaluation. The average age at which they visited the referral center was 10.12 days, while the mean age at which they were examined by the expert specialists was 15.6 days. The mean time required for a definitive disorder diagnosis or to determine the infant's healthy status was 51.13 days and 71.85 days, respectively.

Among the 125,819 screened infants, a total of 1031 cases (recall rate of 0.81%) were recalled for repeat sampling due to initial suspicious results in local healthcare centers. The initial response rate, which is defined as the proportion of first-time examined referred infants to the total number of referred infants to tertiary centers (Mofid Hospital), was found to be 82.95% (1119 out of 1349 infants). Out of the referred infants, a total of 520 infants reached a definite diagnosis status, indicating whether they were determined to be healthy or diagnosed with a specific disease. Meanwhile, 335 cases were still undergoing follow-up to determine the appropriate diagnosis. However, 264 infants abandoned the follow-up process without receiving a proper diagnosis. The secondary response rate was defined as the proportion of patients who did not withdraw from the diagnosis course after the first visit at Mofid Hospital, whether they reached a definite result or remained in a follow-up process (855 of 1119 cases, 76.4%). Taking these figures into account, the overall response rate was calculated to be 63.38%.

Out of the referred infants, a total of 124 were diagnosed with definite IMDs. Among all the diagnosed IMDs, aminoacidopathies were the most prevalent, accounting for 61.29% (76 cases) of the total. This was followed by organic acidemias (30 cases, 24.19%), fatty acid oxidation defects (15 cases, 12.09%), and urea cycle disorders (3 cases, 2.4%). Within the subgroup of diseases, hyperphenylalaninemia/phenylketonuria (PKU) was the most commonly diagnosed disorder with 70 cases. The next most frequent diagnoses were 3-methylcrotonyl-CoA carboxylase deficiency (3MCC), short-chain Acyl-CoA dehydrogenase deficiency (SCAD), and primary carnitine deficiency. The prevalence rates of aminoacidopathies, organic acidemias, and fatty acid oxidation defects were estimated as 1: 6667, 1: 17,392, and 1: 34,784,

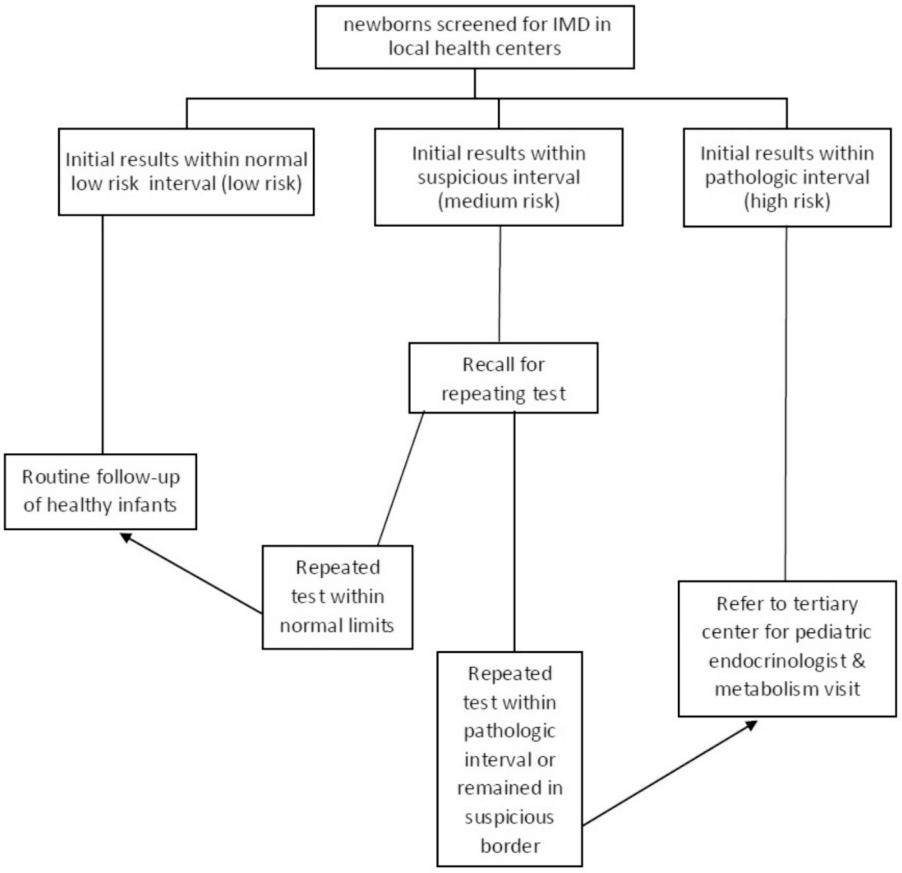

Fig. 1. Fellow chart of the screening process.

respectively. The overall raw estimation of IMD prevalence in this population was 1 in 1015 newborns. Meanwhile, regarding omitting the withdrawn cases and patients who were still in the diagnosis process, the adjusted overall prevalence of IMD was estimated at 1:1008. Fig. 2 shows the prevalence of each diagnosed IMD specifically.

Among the measured metabolites in the screening profile, C0-carnitine had the highest rate of false-positive abnormal amounts, accounting for 76% of cases. This was followed by elevated levels of tyrosine (73%) and C2-carnitine (70%). However, it is important to note that in the last evaluated year, there has been a change in this pattern due to laboratory optimization. During the last year of the study, the most common false-positive abnormal measures were observed in the C5OH value, the Arginine/Alanine, C5DC/C8, and C5DC/C16 ratios. The screening test was estimated to cost between 20 and 600 USD per person depending on the need for further evaluation. The positive predictive value for this screening program, with the available data described above, was determined to be 23.84%. It should be noted that the actual positive predictive value would be different if the 335 cases who were still undergoing follow-up reached the final determination of disease state. Unfortunately, the lack of available data prevented the

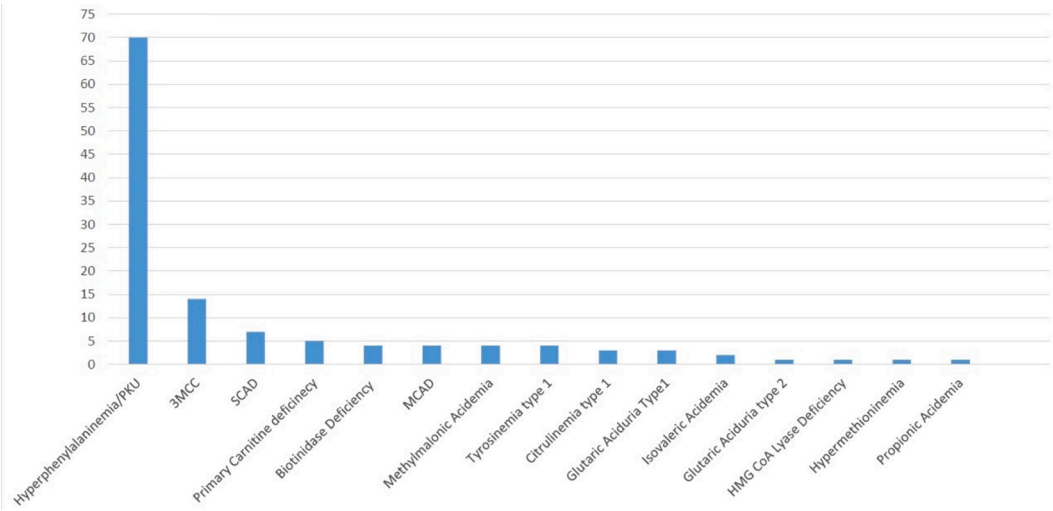

Fig. 2. Total number of diagnosed IMD.

calculation of the negative predictive value, sensitivity, and specificity, which would have provided a more comprehensive assessment of the screening program's performance in identifying false-negative patients. Genetic confirmation was performed in only 7 patients due to lack of financial support in most families.

In long-term follow-up, 7 deaths occurred in the definite IMD group. Three patients were diagnosed with PKU, and four others had tyrosinemia, Citrullinemia type 1, Glutaric aciduria type 1 or 2. Patients with PKU had poor compliance and unfavorable commitment to dietary and medical treatment, nevertheless, in other conditions, despite early preventive treatments, symptoms developed and progressed to a fatal condition.

Among the group of infants diagnosed with IMDs, it was found that 19 cases (19%) had consanguineous parents. Interestingly, no significant association was observed between IMDs and factors such as parental consanguinity, and pregnancy complications of the infants. Moreover, twin pregnancies, gravidity, and history of hospitalization were not associated with IMDs. However, a significant correlation was identified between IMDs and premature birth (P-value = 0.024). Also, analysis of Covariance with hospitalization confirmed the mentioned significance. In this case, 7.2% of children with IMD had a premature birth, and the prevalence of IMD in premature groups was 2.09%.

4. Discussion

In this study screening outcomes of 125,819 infants from specific regions of Tehran and its urban areas were evaluated. Among the screened infants, 1349 were referred for further evaluation, and 124 IMDs were diagnosed through this national screening program. Aminoacidopathies were found to be the most prevalent metabolic disorders. Specifically, elevated phenylalanine levels and phenylketonuria (PKU) were the most common subgroups diagnosed among the screened infants. The raw overall incidence of IMDs in the population studied was estimated to be 1 in 1015, which is approximately comparable to the findings reported in a study conducted by Alfadhel et al. in Saudi Arabia. Interestingly, both of these studies show that the incidence rate of IMDs in this specific population is higher than most reported rates worldwide. However, It should be noted that with the exclusion of endocrine disorders (e.g. congenital adrenal hyperplasia) the incidence rate of other IMDs would diluted to 1:1443 in the Alfadhel et al. study, potentially leading to a higher overall incidence of IMDs in the population studied in Iran [8]. The inclusion of galactosemia screening and lower coverage of some fatty acids oxidation disorders were other noticeable differences between Saudi Arabia screening programs and our protocols. This highlights the significance of considering the specific conditions included in screening programs when interpreting and comparing the incidence rates of IMDs across different studies and populations. As depicted in Table 2, the incidence of IMD varies not only between different countries but also within different regions of the same country [9–11]. In a study from the northeast region of Iran conducted by Keyfi et al. in a referral inherited metabolic laboratory, organic acidemias were the most commonly diagnosed disorders. To explain this difference, it should be considered that although 89 PKU/hyperphenylalaninemia cases were diagnosed in the mentioned study, in the former national screening program, most of the hyperphenylalaninemia cases were likely identified and screened in the first few days of life, allowing for early detection and management. As a result, most of these cases might not have been captured in the referral laboratory study conducted by Keyfi et al. Moreover, in contrast to our work the population of the latter study was much lower and selective which can lead to the observed discrepancy [12].

Primary and secondary response rates were 83.69% and 76.4% respectively. These measures were higher in comparison with some previous studies in the Middle East area and eastern Asia [8,11]. However, it should be noted that despite some studies worldwide, only a few patients were confirmed through genetic studies due to the expensive nature of these tests and lack of support from insurance services [10,14–16]. Except for hyperalaninemia/PKU cases in which enzyme activity was performed to diagnose patients with tetrahydrobiopterin deficiency, other specific enzyme activity tests were not available in conditions such as primary carnitine deficiency. Moreover, the unavailability of a comprehensive genetic study and a loss of follow-up in approximately 36% of referred infants resulted in a lack of information to calculate false negative and negative predictive values for this study. Genetic assessment in populations with a high prevalence of inherited disorders is essential to provide an overview of predominant mutations and polymorphisms. In addition, genetic assessments can play a crucial role in indefinite cases and deliver proper diagnostic status to either start or abandon treatment. Genetic confirmation is also crucial for later screening planning in the next siblings of the affected child and prevents further complications diagnosis and treatment of patients with IMD. Attending and commitment of the undercover screening population is one of the most important aspects of mass screening programs to obtain a more reliable overview of IMD prevalence in societies and examine the quality of screening tests. Limitations in data availability highlight the need for improved access to genetic testing and better follow-up protocols through cultural education and improvement of communication facilities. Another crucial issue that our study revealed is that the mean age of patients in screening and referring time is higher than the targeted time (within the first three days of life). Some parts of this condition can be due to a history of hospitalization or complications during the initial days of life, however, better follow-up tools should be administered to improve these values.

The consanguineous marriage rate observed in this study was 47.1%, which surpassed the rates reported in previous studies conducted in Iran [17–19]. This rate was also higher than that of certain countries in the Middle East region, such as Jordan and Egypt, but lower than Saudi Arabia [20–22]. The high prevalence of consanguineous parents, present in the healthy group, might be a contributing factor to the lack of a significant association between IMD prevalence and parental

Table 2
Summarized Comparison Between IMD Screening Results In Different Regions.

	Shakiba et al. 2022	Alfadhel et al. 2017 [8]	Lin et al. 2019 [10]	Yang et al. 2020 [11]	Lee et al. 2011 [13]
Country/ Region	Iran/Tehran	Saudi Arabia	China/Guangzhou	China/Beijing	Hong Kong
Study Population	125,819	775,000	364,545	58,659	177,248
Primary Response Rate	83.69%	51%	98.2%	76.34%	N/A
Prevalence of IMDs	Aminoacidopathies> Organic Acidemias> Fatty Acids Oxidation Defects	Organic Acidemias> Aminoacidopathies> Fatty Acids Oxidation Defects	Fatty Acids Oxidation Defects> Aminoacidopathies> Organic Acidemias	Organic Acidemias> Aminoacidopathies> Fatty Acids Oxidation Defects	Aminoacidopathies> Fatty Acids Oxidation Defects> Organic Acidemias
Overall IMDs Prevalence	1:1015	1:1443	1:2804	1:3666	1:4122

consanguinity in our study. In addition, the high prevalence of specific mutations in certain populations and races may play a considerable role in the incidence of genetic disorders apart from parental consanguinity. However, this statement, regarding our study, should be studied in several population-based studies.

Some decision-analytic models in some studies estimated that combined screening programs are more beneficial than individual or no screening. For instance, Appelberg et al. developed a model to appraise the cost-effectiveness of PKU and hypothyroidism in a long-term period in Sweden. They demonstrated that combined screening of PKU and hypothyroidism saves noticeable resources and significantly improves quality-adjusted life years (QALYs) in Swedish society [23]. However, It is evident from previous studies that the most cost-effective screening patterns for IMDs can vary depending on the country, its population, available healthcare resources, and the prevalence of specific conditions. Implementing context-specific screening programs allows for better utilization of resources and the identification of affected infants at the earliest stage possible, thus improving their overall health outcomes. The study conducted by Cipriano et al. in Canada highlighted that a screening program encompassing phenylketonuria (PKU) along with nine other IMDs was the most cost-effective approach compared to testing each condition individually. In contrast, some conditions, such as glutaric aciduria type 2, were found to have a poor prognosis even with early diagnosis. Hence, screening for such conditions may not provide significant benefits, and resources can be better directed toward detecting conditions with more favorable prognoses [24]. Similarly, the study by Yu et al. in China demonstrated that the combination of PKU, primary carnitine deficiency (PCD), maple syrup urine disease (MSUD), isovaleric acidemia (IVA), and methylmalonic acidemia (MMA) constituted the most cost-effective pattern for IMD screening in that region [25]. In a study by Bessey et al., a prospective economic model was designed to evaluate the cost-effectiveness of MSUD, IVA, glutaric aciduria type 1, homocystinuria, and long-chain hydroxyacyl CoA dehydrogenase deficiency screening inclusion to the existing tandem mass spectroscopy in United Kingdom. Based on the expected incremental costs and (QALYs), this decision tree model indicated that screening for all of these diseases is more beneficial compared with not screening for these conditions [26].

In our national IMD screening program, as previously mentioned, a total of 46 metabolic disorders can be diagnosed directly or indirectly. While the initial screening test is provided free of charge, families may incur expenses for additional confirmatory tests and physician visits. Moreover, the lack of treatment facilities, including preventive regimens and medications, can render early diagnosis ineffective. Additionally, some prevalent diagnosed conditions, such as 3MCC and SCAD, may not present with symptoms throughout a person's lifetime [27,28]. In such cases, the economic and psychological burden of these diagnoses on families may be substantial, and unnecessary treatment could be a concern.

Considering these potential issues, it becomes evident that further studies should be conducted in different geographical and cultural regions to comprehensively assess the challenges and possible drawbacks of the screening program. By evaluating the program's effectiveness, economic impact, and the overall well-being of affected families, policymakers can make informed decisions to optimize the screening process and ensure that it leads to meaningful health outcomes for infants with IMDs. Moreover, tailored interventions and support systems may be developed to address the specific needs of affected families, mitigating the potential challenges posed by the program and enhancing its overall effectiveness.

The conclusion of this study emphasizes the importance of a nationwide screening program for inherited metabolic disorders (IMDs) to detect these conditions early and provide timely interventions. Although the screening program demonstrated promising response rates, challenges were identified in terms of cost-effectiveness, the impact of consanguineous marriages, and limitations in genetic testing and follow-up data. To improve the screening program's efficiency and efficacy, further research is needed to evaluate long-term benefits and limitations, prognosis of specific IMDs, and appropriateness of early diagnosis and treatment. Overall, this study serves as a foundation for future research in different regions to assess the generalizability and potential challenges of implementing similar screening programs. By addressing these concerns, we can enhance the health outcomes of infants with IMDs, reduce the burden on affected families, and optimize the allocation of healthcare resources for the betterment of public health.

Declaration of Using Generative AI

During the preparation of this work, the authors used Grammarly in order to improve language and readability. After using this tool/service, the author(s) reviewed and edited the content as needed and took full responsibility for the content of the publication.

All authors confirm the revised version of the manuscript.

CRediT authorship contribution statement

Marjan Shakiba: Validation, Supervision, Project administration, Methodology, Conceptualization. **Mehrdad Yasaei:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hedyeh Saneifard:** Writing – original draft, Validation, Resources, Formal analysis. **Asieh Mosallanejad:** Validation, Resources, Project administration, Investigation. **Mohammad Reza Alaei:** Writing – review & editing, Formal analysis, Conceptualization. **Farzad Kobarfard:** Resources, Project administration, Methodology. **Marjan Esfahanizadeh:** Resources, Project administration. **Narges Anousheh:** Writing – review & editing.

Declaration of competing interest

The authors of this manuscript declare no conflicts of interest.

Data availability

Data will be made available on request.

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