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Neonatal Screening for Inborn Errors of Metabolism in Mazandaran Province 2017-2020

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Abstract

Background and Purpose: The purpose of inborn metabolic screening is early diagnosis and treatment before becoming symptomatic or occurring complications. This research is based on pilot program of inborn metabolic screening of organic acid, fatty acid and amino acid disorders in Mazandaran province of Iran; and study of incidence of persistent inherited metabolic disorders divided by gender and region is done; and metabolites need changing cut-off are determined.

Method: This research is a cross-sectional descriptive type and used by census method on 33510 healthy newborns who participated in metabolic screening program used by tandem mass spectrometry method. Data put in Excel file and analyzed by SPSS v.25 software.

Results: Among 33510 healthy newborns, since the beginning of this program till March 2020, 12 cases found with persistent inborn metabolic disorders (0.04% of screened): MSUD (4 cases [0.012%]), Citrullinemia (3 cases [0.01%]), PKU (3 cases [0.01%]), Propionic acidemia (1 case [0.003%]), Tyrosinemia type II (1 case [0.003%]), 3MCC deficiency (1 case [0.003%]), MCAD deficiency (1 case [0.003%]). There were no significant relation between gender and persistent metabolic disorders.

Conclusion: It is recommended changing cut-off of metabolites that are specified in this study because of many reports of false positive; and creating accurate follow-up system to ensure that confirmatory tests are performed and record results for evaluation of transient metabolic disorders; and also recommended resolving bugs of newborn information registration system. Because of inborn metabolic disorders are rare, it is recommended continuing this kind of study in larger statistical community.

Keywords: *Transient Metabolic Disorders; Persistent Metabolic Disorders; Newborn Screening; Inborn Errors of Metabolism*

Introduction

Screening for inherited metabolic diseases aims to diagnose, control, and treat metabolic disorders in seemingly healthy neonates before the disorder gets symptomatic or causes complications. Robert Gutierrez performed the first screening test for metabolic diseases, known as the Guthrie test or the PKU test, in the 1960s to diagnose phenylketonuria [1,2]. Since the 1970s, technological advances in

laboratory methods for measuring thyroglobulin (Tg) and TSH have allowed screening for neonatal hypothyroidism [2]. The World Health Organization (WHO) has proposed a screening model for disorders based on the Wilson and Jungner Criteria, which includes aspects such as diagnosis, treatment possibility, scientific validity of laboratory tests, and cost-benefit considerations [1,3,4]. Since 2000, the use of tandem mass spectrometry, also known as MS/MS, has significantly increased the number of tests that can diagnose a wide range of diseases. This served as the foundation for the national development of a neonatal screening program in the United States [5-7].

The following are the main categories of inherited metabolic disorders that are typically screened at birth:

1. Organic acidemia: Isovaleric acidemia, glutaric aciduria type 1 (GA1), 3-hydroxy-3-methylglutaric aciduria, multiple carboxylase deficiency (MCD), methylmalonic acidemia (cbIA and cbIB deficiency), 3-methylcrotonyl-CoA carboxylase deficiency (also known as 3-MCC deficiency), propionic acidemia, and beta-ketothiolase deficiency.
2. Fatty acid disorders: Medium-chain acyl-coA dehydrogenase deficiency (MCADD), very long-chain acyl-CoA dehydrogenase deficiency (VLCADD), long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency, trifunctional protein deficiency (TFP), and carnitine deficiency.
3. Aminoacidopathies: Phenylketonuria, maple syrup urine disease (MSUD), homocystinuria, citrullinemia, argininosuccinic aciduria, and tyrosinemia type-I [8].

Early diagnosis of inherited metabolic diseases can prevent death or complications caused by conditions such as mental disability, as well as reduce the financial and emotional burden placed on families. For example, studies show that patients who were identified through PKU screening and then received necessary treatments grew up to be healthy children with normal education, whereas those who were screened never or very late were admitted to special centers due to mental disability complications [9-11].

Thus far, no extensive screening program has been implemented in Iran to diagnose the most common metabolic disorders [1,12-14]. This pilot study began on March 15, 2017 in some Mazandaran Province cities, including Sari, Qaemshahr, Neka, Juybar, and Amol. After screening over 33,500 seemingly healthy neonates over a three-year period, it is necessary to investigate the prevalence of identified disorders as well as the predictive value of screening in order to determine the relative frequency of disorders in the studied population for national planning and to make sound decisions about the plan's future. Hence, the purpose of this study is to assess the frequency and geographical distribution of inherited metabolic disorders registered during this plan by gender, as well as the number of false positives and negatives screening results for each metabolic disorder.

Methodology

All seemingly healthy neonates born in Mazandaran Province (Sari, Qaemshahr, Neka, Juybar, and Amol) who were screened in this pilot plan (n = 33510) were chosen as the sample in this cross-sectional descriptive study using total enumeration sampling.

The sample consisted of seemingly healthy neonates who were taken to selected health centers for hypothyroidism, PKU, and G6PD deficiency screening on days 3 to 5 of birth (which was the golden time to perform the intended tests in this study). The study excluded preterm, clinically ill, and hospitalized neonates. Blood was drawn from the soles of neonates and placed on two filter papers with five positions: three drops of blood on one filter paper (one drop in each position) and five drops of blood on the other filter paper (one drop on each position). The filter paper containing 5 drops of blood was sent to a reference laboratory (located in Mazandaran University of Medical Sciences' Central Complex) for TSH, G6PD, and PKU analysis (National Neonatal Screening Program). The filter paper containing three drops of blood, along with Form No. 1 of the National Neonatal Screening Program, was sent to Fajr Laboratory (a reference laboratory certified by Iran's Ministry of Health and Medical Education) for metabolic analysis using tandem mass spectrometry. All test results were recorded in an Excel file, and any suspicious results were reported to the appropriate health center. The neonates' families were no-

tified of the suspicious results, which were then sent to the emergency room (E/R) of Sari's Bu Ali Hospital for confirmation tests (blood and urine). The results of confirmatory tests were used to make appropriate decisions about therapeutic and follow-up measures. To achieve the research objectives, the results of each neonate's primary screening laboratory and conformation tests were recorded on a questionnaire and analyzed. The national screening program for inherited metabolic diseases was carried out in a number of laboratories across Iran, including the Fajr Medical Pathobiology and Genetics Laboratory in Sari. In this study, an electrospray ionization device made by Shimadzu Corporation in Japan was used to determine the relative frequency of ions with a specific charge/mass ratio. The laboratory kits used in this study were purchased from Chromsystems Instruments and Chemicals. Peptide levels for each of them could be reported using specific threshold values. If the neonate's amino acid and acylcarnitine levels were between the maximum normal and the minimum cut-off, the case was considered suspicious. Furthermore, the ranges above and below the cut-off, as well as the ranges between the minimum normal and the minimum cut-off, were labeled as pathologic and gray zone, respectively. Confirmatory tests for the pathologic range were developed and carried out using High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry techniques (GS-MS). For those suspected of having a metabolic disease, the screening test was repeated. The above algorithm was developed based on the opinions of the selected physician.

Findings

The analysis of the collected files yielded the following results. In Mazandaran Province, neonatal screening for inherited metabolic diseases began on March 11, 2017 in Sari, Aug. 9, 2017 in Qaemshahr, October 12, 2017 in Juybar and Neka, and December 10, 2018 in Amol. The total number of screened neonates in these five cities from the start of the screening program to early 2020 is as follows:

- A total of 16,430 neonates in Sari, including 7942 girls (48.34%) and 8488 boys (51.66%)
- A total of 6,624 neonates in Qaemshahr, including 3171 girls (47.87%) and 3453 boys (52.13%)
- A total of 2,480 neonates in Juybar, including 1198 girls (48.31%) and 1282 boys (51.69%)
- A total of 3,780 neonates in Neka, including 1871 girls (49.5%) and 1909 boys (50.5%)
- A total of 4,196 neonates in Amol, including 2023 girls (48.21%) and 2173 boys (51.79%).

The screened metabolites were classified into Gray Zone and Pathologic Zone by gender based on laboratory reports provided in pdf files. The frequency of screened metabolites confirmed metabolites in stable metabolic diseases by gender, Pathologic Zone (marked with two positive or negative signs), and Gray Zone (marked with a positive or negative sign), percentage of people reported from those screened for each area, and the distribution of individuals reported in each zone for each metabolite by gender are presented in the table below.

Distribution among reported individuals			Percentage among screened individuals			Number of reported cases in screening program			
Total	**Boy**	**Girl**	**Total**	**Boy**	**Girl**	**Total**	**Boy**	**Girl**	
40	20	20	0.006	0.003	0.003	2	1	1	Ala++
60	20	40	0.009	0.003	0.006	3	1	2	Ala+
0	0	0	0	0	0	0	0	0	Ala-
							No pathological lower limit		Ala--
38.2	21.7	16.5	0.17	0.1	0.07	58	33	25	Arg++
23.7	12.5	11.2	0.11	0.06	0.05	36	19	17	Arg+
9.9	5.9	4	0.045	0.027	0.018	15	9	6	Arg-
28.3	14.5	13.8	0.13	0.067	0.063	43	22	21	Arg--

6.2	2.3	3.9	0.024	0.01	0.014	8	3	5	Asp++
93.8	51.5	42.3	0.36	0.2	0.16	122	67	55	Asp+
0	0	0	0	0	0	0	0	0	Asp-
							No pathological lower limit		Asp--
29.2	16.9	12.3	0.057	0.033	0.024	19	11	8	Cit++
36.9	20	16.9	0.072	0.039	0.033	24	13	11	Cit+
7.7	6.15	1.55	0.015	0.012	0.003	5	4	1	Cit-
26.2	9.2	17	0.051	0.018	0.033	17	6	11	Cit--
35.3	19.3	16	0.46	0.25	0.21	154	84	70	Glu++
64.7	32	32.7	0.84	0.41	0.43	282	139	143	Glu+
0	0	0	0	0	0	0	0	0	Glu-
							No pathological lower limit		Glu--
23.7	10.2	13.5	0.042	0.018	0.024	14	6	8	Gly++
76.3	42.4	33.9	0.13	0.07	0.06	45	25	20	Gly+
0	0	0	0	0	0	0	0	0	Gly-
							No pathological lower limit		Gly--
70.6	41.2	29.4	0.036	0.021	0.015	12	7	5	Leu++
29.4	23.5	5.9	0.015	0.012	0.003	5	4	1	Leu+
0	0	0	0	0	0	0	0	0	Leu-
							No pathological lower limit		Leu--
2.4	1.8	0.6	0.012	0.009	0.003	4	3	1	Met++
1.2	1.2	0	0.006	0.006	0	2	2	0	Met+
40	20	20	0.2	0.1	0.1	68	34	34	Met-
56.5	31.8	24.7	0.28	0.16	0.12	96	54	42	Met--
7.1	4.1	3	0.036	0.021	0.015	12	7	5	Orn++
92.3	43.4	48.9	0.46	0.22	0.24	155	73	82	Orn+
0.6	0	0.6	0.003	0	0.003	1	0	1	Orn-
							No pathological lower limit		Orn--
33.3	5.5	27.8	0.018	0.003	0.015	6	1	5	Phe++
66.7	50	16.7	0.036	0.026	0.01	12	9	3	Phe+
0	0	0	0	0	0	0	0	0	Phe-
							No pathological lower limit		Phe--
5	5	0	0.006	0.006	0	2	2	0	Pro++
92.5	50	42.5	0.11	0.06	0.05	37	20	17	Pro+
2.5	2.5	0	0.003	0.003	0	1	1	0	Pro-
							No pathological lower limit		Pro--
97.5	41.7	55.8	1.26	0.54	0.72	423	181	242	Tyr++
2.5	1.4	1.1	0.033	0.018	0.015	11	6	5	Tyr+
0	0	0	0	0	0	0	0	0	Tyr-

							No pathological lower limit		Tyr--
46.2	30.7	15.5	0.018	0.012	0.006	6	4	2	Val++
53.8	30.7	23.1	0.021	0.012	0.009	7	4	3	Val+
0	0	0	0	0	0	0	0	0	Val-
							No pathological lower limit		Val--
5.8	4.5	1.3	0.027	0.021	0.006	9	7	2	C0++
3.2	1.3	1.9	0.015	0.006	0.009	5	2	3	C0+
53.2	26.28	26.92	0.25	0.12	0.13	83	41	42	C0-
37.8	16.7	21.1	0.18	0.08	0.1	59	26	33	C0--
							No pathological lower limit		C2++
2.9	1.9	1	0.009	0.006	0.003	3	2	1	C2+
69.5	40	29.5	0.22	0.12	0.1	73	42	31	C2-
27.6	17.1	10.5	0.09	0.055	0.035	29	18	11	C2--
41.1	21.4	19.7	0.14	0.072	0.068	46	24	22	C3++
11.6	7.1	4.5	0.04	0.025	0.015	13	8	5	C3+
7.1	3.55	3.55	0.024	0.012	0.012	8	4	4	C3-
40.2	17.9	22.3	0.13	0.06	0.07	45	20	25	C3--
38.5	27	11.5	0.03	0.02	0.01	10	7	3	C4++
61.5	42.3	19.2	0.048	0.033	0.015	16	11	5	C4+
0	0	0	0	0	0	0	0	0	C4-
							No pathological lower limit		C4--
0	0	0	0	0	0	0	0	0	C4OH+C3DC++
100	72.2	27.8	0.055	0.04	0.015	18	13	5	C4OH+C3DC+
0	0	0	0	0	0	0	0	0	C4OH+C3DC-
							No pathological lower limit		C4OH+C3DC--
29.4	5.9	23.5	0.015	0.003	0.012	5	1	4	C5OH+C4DC++
64.7	47.1	17.6	0.033	0.023	0.01	11	8	3	C5OH+C4DC+
5.9	0	5.9	0.003	0	0.003	1	0	1	C5OH+C4DC-
							No pathological lower limit		C5OH+C4DC--
73.5	47	26.5	0.075	0.048	0.027	25	16	9	C5++
23.5	8.8	14.7	0.025	0.01	0.015	8	3	5	C5+
2.9	0	2.9	0.003	0	0.003	1	0	1	C5-
							No pathological lower limit		C5--
76.9	38.45	38.45	0.03	0.015	0.015	10	5	5	C5:1++
23.1	7.7	15.4	0.009	0.003	0.006	3	1	2	C5:1+
0	0	0	0	0	0	0	0	0	C5:1-
							No pathological lower limit		C5:1--
55.3	28.9	26.4	1.05	0.55	0.5	352	184	168	C5DC+C6OH++
44.7	22.8	21.9	0.85	0.43	0.42	284	145	139	C5DC+C6OH+

0	0	0	0	0	0	0	0	0	C5DC+C6OH-
							No pathological lower limit		C5DC+C6OH--
42.9	28.6	14.3	0.009	0.006	0.003	3	2	1	C6++
42.9	14.3	28.6	0.009	0.003	0.006	3	1	2	C6+
14.3	0	14.3	0.003	0	0.003	1	0	1	C6-
							No pathological lower limit		C6--
5.3	0	5.3	0.003	0	0.003	1	0	1	C8++
94.7	68.4	26.3	0.055	0.04	0.015	18	13	5	C8+
0	0	0	0	0	0	0	0	0	C8-
							No pathological lower limit		C8--
5.6	0	5.6	0.003	0	0.003	1	0	1	C8:1++
88.9	55.6	33.3	0.048	0.03	0.018	16	10	6	C8:1+
5.6	0	5.6	0.003	0	0.003	1	0	1	C8:1-
							No pathological lower limit		C8:1--
92.3	46.15	46.15	0.036	0.018	0.018	12	6	6	C10++
7.7	0	7.7	0.003	0	0.003	1	0	1	C10+
0	0	0	0	0	0	0	0	0	C10-
							No pathological lower limit		C10--
50	50	0	0.003	0.003	0	1	1	0	C10:1++
50	50	0	0.003	0.003	0	1	1	0	C10:1+
0	0	0	0	0	0	0	0	0	C10:1-
							No pathological lower limit		C10:1--
25	6.25	18.75	0.012	0.003	0.009	4	1	3	C12++
75	43.5	31.25	0.036	0.021	0.015	12	7	5	C12+
0	0	0	0	0	0	0	0	0	C12-
							No pathological lower limit		C12--
13	8.7	4.3	0.009	0.006	0.003	3	2	1	C14++
87	60.9	26.1	0.06	0.042	0.018	20	14	6	C14+
0	0	0	0	0	0	0	0	0	C14-
							No pathological lower limit		C14--
25	10	15	0.015	0.006	0.009	5	2	3	C14:1++
75	45	30	0.045	0.027	0.018	15	9	6	C14:1+
0	0	0	0	0	0	0	0	0	C14:1-
							No pathological lower limit		C14:1--
10.5	5.25	5.25	0.006	0.003	0.003	2	1	1	C14:2++
84.2	42.1	42.1	0.048	0.024	0.024	16	8	8	C14:2+
5.3	5.3	0	0.003	0.003	0	1	1	0	C14:2-
							No pathological lower limit		C14:2--
100	54.5	45.5	0.033	0.018	0.015	11	6	5	C14OH++

0	0	0	0	0	0	0	0	0	C14OH+
0	0	0	0	0	0	0	0	0	C14OH-
							No pathological lower limit		C14OH--
21.3	13.3	8	0.048	0.03	0.018	16	10	6	C16++
33.3	25.3	8	0.075	0.057	0.018	25	19	6	C16+
1.4	0	1.4	0.003	0	0.003	1	0	1	C16-
44	32	12	0.1	0.072	0.028	33	24	9	C16--
6	4.1	1.9	0.048	0.033	0.015	16	11	5	C16:1++
93.7	52.3	41.4	0.75	0.42	0.33	251	140	111	C16:1+
0.4	0	0.4	0.003	0	0.003	1	0	1	C16:1-
							No pathological lower limit		C16:1--
50	25	25	0.018	0.009	0.009	6	3	3	C16:1OH++
41.7	41.7	0	0.015	0.015	0	5	5	0	C16:1OH+
8.3	0	8.3	0.003	0	0.003	1	0	1	C16:1OH-
							No pathological lower limit		C16:1OH--
0	0	0	0	0	0	0	0	0	C16OH++
100	58.8	41.2	0.051	0.03	0.021	17	10	7	C16OH+
0	0	0	0	0	0	0	0	0	C16OH-
							No pathological lower limit		C16OH--
26.8	14.3	12.5	0.045	0.024	0.021	15	8	7	C18++
23.2	8.9	14.3	0.039	0.015	0.024	13	5	8	C18+
0	0	0	0	0	0	0	0	0	C18-
50	32.1	17.9	0.084	0.054	0.03	28	18	10	C18--
25	9.1	15.9	0.033	0.012	0.021	11	4	7	C18:1++
22.7	15.9	6.8	0.03	0.021	0.009	10	7	3	C18:1+
0	0	0	0	0	0	0	0	0	C18:1-
52.3	27.3	25	0.069	0.036	0.033	23	12	11	C18:1--
100	66.7	33.3	0.009	0.006	0.003	3	2	1	C18:1OH++
0	0	0	0	0	0	0	0	0	C18:1OH+
0	0	0	0	0	0	0	0	0	C18:1OH-
							No pathological lower limit		C18:1OH--
75	25	50	0.009	0.003	0.006	3	1	2	C18:2OH++
25	0	25	0.003	0	0.003	1	0	1	C18:2OH+
0	0	0	0	0	0	0	0	0	C18:2OH-
							No pathological lower limit		C18:2OH--
0	0	0	0	0	0	0	0	0	C18OH++
100	50	50	0.012	0.006	0.006	4	2	2	C18OH+
0	0	0	0	0	0	0	0	0	C18OH-
							No pathological lower limit		C18OH--

Percentage of false positives			Confirmed metabolites in stable disorders			
Total	Boys	Girls	Total	Boys	Girls	
84.2	81.8	87.5	3	2	1	Cit++
76.47	57.14	80	4	3	1	Leu++
50	100	40	3	0	3	Phe++
99.75	99.45	100	1	1	0	Tyr++
33.3	25	50	4	3	1	Val++
80	0	100	1	1	0	C5OH+C4DC++

Percentage of false negatives			Unreported metabolites (false negatives)			
Total	Boys	Girls	Total	Boys	Girls	
0.003	0	0.006	1	0	1	C8++

From March 9, 2019, four other metabolites were added to the neonatal screening program. As a result, 13,178 neonates, including 6349 girls and 6829 boys, were screened for these four new metabolites until early 2020. The details about these four metabolites are presented in the table below.

Distribution among reported neonates			Percentage among screened neonates			Number of reported cases in screening program			
Total	Boy	Girl	Total	Boy	Girl	Total	Boy	Girl	
93	34.9	58.1	0.3	0.11	0.19	40	15	25	C6DC++
7	2.3	4.7	0.023	0.008	0.015	3	1	2	C6DC+
0	0	0	0	0	0	0	0	0	C6DC-
							No pathological lower limit		C6DC--
50	0	0	0.008	0.008	0	1	1	0	C10:2++
50	0	0	0.008	0.008	0	1	1	0	C10:2+
0	0	0	0	0	0	0	0	0	C10:2-
							No pathological lower limit		C10:2--
50	25	25	0.045	0.0225	0.0225	6	3	3	C12:1++
50	16.7	33.3	0.045	0.015	0.03	6	2	4	C12:1+
0	0	0	0	0	0	0	0	0	C12:1-
							No pathological lower limit		C12:1--
0	0	0	0	0	0	0	0	0	C18:2++
25	15	10	0.038	0.023	0.015	5	3	2	C18:2+
10	10	0	0.015	0.015	0	2	2	0	C18:2-
65	10	55	0.1	0.015	0.085	13	2	11	C18:2--

The 12 cases of stable metabolic disorders were as follows: 4 cases of MSUD, 3 cases of citrullinemia, 3 cases of PKU, 1 case of propionic acidemia, 1 case of tyrosinemia (it was diagnosed as type III based on the genetic test), 1 case of 3-MCC deficiency, and 1 case of MCADD.

Discussion

In this section, the prevalence of stable inherited metabolic disorders discovered in this study is compared with the findings of previous studies.

Prevalence of PKU

The prevalence of PKU in this study was 9 per 100,000 neonates; 0.018% among girls and zero among boys. The prevalence of this disorder per 100,000 neonates reported in previous studies is shown in the table below.

Place	Year	Sample size	Confirmed cases	Prevalence
Mazandaran Province	2017-2019	33510	3	9
Fars Province	2004-2007	175234	28	16
Saudi Arabia	1983-2008	165530	12	7
United Arab Emirates	1995-2000	138718	7	5
Bahrain	2008-2011	66565	3	4.5
Egypt	2008	25276	5	20
India	2000	18300	1	5.5
Thailand	1996-2006	5243841	16	0.3
Taiwan	2000-2009	1495132	25	1.7
China (Guangzhou)	2014-2018	364545	14	4
South Korea	2001-2003	37817	3	8
Germany (Baden-Württemberg)	1998-2001	250000	24	9.6
Germany (Bavaria)	1999	87000	9	10.3
Australia (South Newell)	1998	137120	17	12.5
US (Different states)	1992-1999	476337	37	5
US (New England)	1999	257000	7	2.7

Prevalence of MSUD

The prevalence of MSUD in this study was 12 per 100,000 neonates; 0.006% among girls and 0.017% among boys. The following table shows the prevalence of this disorder per 100,000 neonates reported in previous studies.

Place	Year	Sample size	Confirmed cases	Prevalence
Mazandaran Province	2017-2019	33510	4	12
Saudi Arabia	1983-2008	165530	12	7
Bahrain	2008-2011	66565	4	6
Egypt	2008	25276	1	4
Taiwan	2000-2009	1495132	13	1
South Korea	2001-2003	37817	2	5.3
Germany (Baden-Württemberg)	1998-2001	250000	2	1
Australia (South Newell)	1998	137120	1	0.7
US (Different states)	1992-1999	476337	9	1.2
US (New England)	1999	257000	1	0.4

Prevalence of tyrosinemia

The prevalence of tyrosinemia in this study was 3 per 100,000 neonates; 0.006% among girls and zero among boys. The following table shows the prevalence of this disorder per 100,000 neonates reported in previous studies.

Place	Year	Sample size	Confirmed cases	Prevalence
Mazandaran Province	2017-2019	33510	1	3
Saudi Arabia	1983-2008	165530	5	3
Bahrain	2008-2011	66565	2	3
South Korea	2001-2003	37817	2	5.3
Germany (Baden-Württemberg)	1998-2001	250000	1	0.4
Australia (South Newell)	1998	137120	1	0.7

Prevalence of citrullinemia

The prevalence of citrullinemia in this study was 3 per 100,000 neonates; 0.006% among girls and 0.012% among boys. Six cases of citrullinemia were reported in a study of 165,530 neonates in Saudi Arabia from 1983 to 2008, with a prevalence of 4 per 100,000 neonates [15]. Another study on 592,717 neonates in Thailand from 2000 to 2009 reported 15 cases of citrullinemia, with a prevalence of 2.5 per 100,000 neonates [20]. Furthermore, from 2014 to 2018, 12 cases of this disorder were reported in a study of 364,545 neonates in Guangzhou, China, and its prevalence was determined to be 3.3 per 100,000 neonates [21]. Another study, conducted on 37,817 neonates in South Korea from 2001 to 2003, reported two cases of citrullinemia with a prevalence of 5.3 per 100,000 neonates [22]. Six cases of citrullinemia were reported in a study of 250,000 neonates in the German state of Baden-Württemberg from 1998 to 2001, and its prevalence was determined to be 2.4 per 100,000 neonates [23]. Another study on 746,637 neonates in different states of the United States from 1992 to 1999 found 4 cases of citrullinemia with a prevalence of 0.5 per 100,000 neonates [26]. The prevalence of this disorder per 100,000 neonates reported in previous studies is shown in the table below.

Place	Year	Sample size	Confirmed cases	Prevalence
Mazandaran Province	2017-2019	33510	3	9
Saudi Arabia	1983-2008	165530	6	4
Taiwan	2000-2009	592717	15	2.5
China (Guangzhou)	2014-2018	364545	12	3.3
South Korea	2001-2003	37817	2	5.3
Germany (Baden-Württemberg)	1998-2001	250000	6	5.4
US (Different states)	1992-1999	746337	4	0.5

Prevalence of propionic acidemia

The prevalence of propionic acidemia in this study was 3 per 100,000 neonates; 0.006% among girls and zero among boys. The table below shows the prevalence of this disorder per 100,000 neonates as reported in previous studies.

Place	Year	Sample size	Confirmed cases	Prevalence
Mazandaran Province	2017-2019	33510	1	3
Saudi Arabia	1983-2008	165530	6	4
Bahrain	2008-2011	66565	4	6
Egypt	2008	25276	1	4
China (Guangzhou)	2014-2018	364545	2	0.5
South Korea	2001-2003	37817	2	5.3
Germany (Baden-Württemberg)	1998-2001	250000	1	0.4
US (Different states)	1992-1999	687630	6	1
US (New England)	1999	164000	2	1.2

Prevalence of 3-MCC deficiency

The prevalence of 3-MCC deficiency in this study was 3 per 100,000 neonates; zero among girls and 0.006% among boys. The following table shows the prevalence of this disorder per 100,000 neonates reported in previous studies.

Place	Year	Sample size	Confirmed cases	Prevalence
Mazandaran Province	2017-2019	33510	1	3
Saudi Arabia	1983-2008	165530	3	2
Taiwan	2000-2009	592717	14	2.4
China (Guangzhou)	2014-2018	364545	5	1.4
South Korea	2001-2003	37817	1	2.6
Germany (Baden-Württemberg)	1998-2001	250000	6	2.4
US (Different states)	1992-1999	687630	9	1.3
US (New England)	1999	164000	1	0.6

Prevalence of MCADD

The prevalence of MCADD in this study was 3 per 100,000 neonates; 0.006% among girls and zero among boys. The following table shows the prevalence of this disorder per 100,000 neonates reported in previous studies.

Place	Year	Sample size	Confirmed cases	Prevalence
Mazandaran Province	2017-2019	33510	1	3
Saudi Arabia	1983-2008	165530	2	1
Bahrain	2008-2011	66565	2	3
Taiwan	2000-2009	1321123	2	0.15
Hong Kong	2013-2016	30488	2	6.6
China (Guangzhou)	2014-2018	364545	5	1.4
South Korea	2001-2003	37817	1	2.6
Germany (Baden-Württemberg)	1998-2001	250000	16	6.4
Germany (Bavaria)	1999	87000	6	7
Australia (South Newell)	1998	137120	2	1.5
US (Different states)	1992-1999	687630	39	5.7
US (New England)	1999	184000	10	5.5

Conclusion, Limitations, and Suggestions

It is suggested that the metabolite cut-off, from which many false-positive cases have been reported, be reviewed (the correction of the blood sampling method seems to be effective for aspartic acid and glutamine). These metabolites include aspartic acid (upper limit of the gray zone), glutamine (upper limit of the gray zone), glycine (upper limit of the gray zone), ornithine (upper limit of the gray zone), tyrosine (above the normal range), C0 (lower limit of gray zone and pathological zone lower than the normal), C5DC + C6OH (pathological zone above the normal and upper limit of the gray zone), C16: 1 (upper limit of the gray zone), and C6DC (pathological zone above the normal).

It is suggested that an accurate follow-up system for confirmatory tests be established in order to check the false positives or true initial screening rate for each metabolite and the frequency of transient metabolic disorders, as well as to resolve problems with the neonatal basic information registration system. Due to the rarity of inherited metabolic disorders, it is also suggested that the same studies be conducted on larger populations.

The tables below show the final approved cases by gender and city.

	Citrullinemia (3 cases)		
	Girl	**Boy**	**Total**
Sari	0	1	1
Qaemshahr	1	0	1
Juybar	0	0	0
Neka	0	1	1
Amol	0	0	0
Total	1	2	

	MSUD (4 cases)		
	Girl	**Boy**	**Total**
Sari	1	0	1
Qaemshahr	0	0	0
Juybar	0	1	1
Neka	0	2	2
Amol	0	0	0
Total	1	3	

	PKU (3 cases)		
	Girl	**Boy**	**Total**
Sari	3	0	3
Qaemshahr	0	0	0
Juybar	0	0	0
Neka	0	0	0
Amol	0	0	0
Total	3	0	

	Propionic acidemia (1 case)		
	Girl	**Boy**	**Total**
Sari	0	0	0
Qaemshahr	0	0	0
Juybar	0	0	0
Neka	1	0	1
Amol	0	0	0
Total	1	0	

	MCAD (1 case)		
	Girl	**Boy**	**Total**
Sari	0	0	0
Qaemshahr	0	0	0
Juybar	0	0	0
Neka	0	0	0
Amol	1	0	1
Total	1	0	

	Tyrosinemia (type-III) (1 case)		
	Girl	**Boy**	**Total**
Sari	0	1	1
Qaemshahr	0	0	0
Juybar	0	0	0
Neka	0	0	0
Amol	0	0	0
Total	0	1	

	3MCC (1 case)		
	Girl	**Boy**	**Total**
Sari	0	0	0
Qaemshahr	0	0	0
Juybar	0	1	1
Neka	0	0	0
Amol	0	0	0
Total	0	0	

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