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Global prevalence of classic phenylketonuria based on Neonatal Screening Program Data: systematic review and meta-analysis

Hamid Reza Shoraka, PhD[1], Ali Akbar Haghdoost, MD, PhD[2], Mohammad Reza Baneshi, PhD[3], Zohre Bagherinezhad, PhDc[4], Farzaneh Zolala, PhD[5]

[1]Health Services Management Research Center, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran; [2]HIV/STI Surveillance Research Center, and WHO Collaborating Center for HIV Surveillance, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran; [3]Modeling in health research center, Institute for futures studies in health, Kerman University of medical sciences, Kerman, Iran; [4]Department of Medical Library and Information Science, Kerman University of Medical Sciences, Kerman, Iran; [5]Social Determinants of Health Research Center, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran

Phenylketonuria is a disease caused by congenital defects in phenylalanine metabolism that leads to irreversible nerve cell damage. However, its detection in the early days of life can reduce its severity. Thus, many countries have started disease screening programs for neonates. The present study aimed to determine the worldwide prevalence of classic phenylketonuria using the data of neonatal screening studies. The PubMed, Web of Sciences, Sciences Direct, ProQuest, and Scopus databases were searched for related articles. Article quality was evaluated using the Joanna Briggs Institute Critical Appraisal Evaluation Checklist. A random effect was used to calculate the pooled prevalence, and a phenylketonuria prevalence per 100,000 neonates was reported. A total of 53 studies with 119,152,905 participants conducted in 1964–2017 were included in this systematic review. The highest prevalence (38.13) was reported in Turkey, while the lowest (0.3) in Thailand. A total of 46 studies were entered into the meta-analysis for pooled prevalence estimation. The overall worldwide prevalence of the disease is 6.002 per 100,000 neonates (95% confidence interval, 5.07–6.93). The meta-regression test showed high heterogeneity in the worldwide disease prevalence (I^2=99%). Heterogeneity in the worldwide prevalence of phenylketonuria is high, possibly due to differences in factors affecting the disease, such as consanguineous marriages and genetic reserves in different countries, study performance, diagnostic tests, cutoff points, and sample size.

Keywords: Phenylketonuria, Prevalence, Neonates, Screening, Meta-analysis

Key message

Question: What is the global prevalence of classic phenylketonuria based on Neonatal Screening Program Data?

Finding: The overall worldwide prevalence of the disease is 6.002 per 100,000 neonates. The highest prevalence (38.13) was reported in Turkey, while the lowest (0.3) in Thailand.

Meaning: This difference in the prevalence may be due to differences in the number of consanguineous marriages among the different regions, phenylalanine cutoff points, and sample sizes.

Introduction

Genetic and congenital abnormalities are the most important causes of death and malformation in the first month of life.[1] Phenylketonuria (PKU) is an inborn error of amino acid metabolism caused by phenylalanine hydroxylase gene mutations.[2,3] PKU patients experience an irreversible decrease in intelligence quotient scores, suppressed verbal function, impaired attention, and underdeveloped motor control skills.[4,5]

The early diagnosis of PKU before the end of the first month of life is critical to controlling hyperphenylalaninemia.[4,6] Children with PKU seem normal during the first days of life; however, nervous system damage progresses gradually and becomes apparent over several months.[7] The early detection of PKU in the asymptomatic period and treatment with a phenylalanine restricted diet is warranted to ensure normal development.[8-12] Therefore, neonatal screening as a fundamental public health intervention started in the mid-20th century.[4,12,13]

Since PKU has autosomal recessive inheritance, consanguineous marriage is an important risk factor[1,4]; thus, countries with a high prevalence of consanguineous marriages have high

Corresponding author: Farzaneh Zolala, PhD. Institute for Futures Studies in Health, Kerman University of Medical Sciences, Medical University Campus, Haft-Bagh Highway, Kerman, Iran
✉ E-mail: zolalafarzaneh@gmail.com, https://orcid.org/0000-0002-1848-183X
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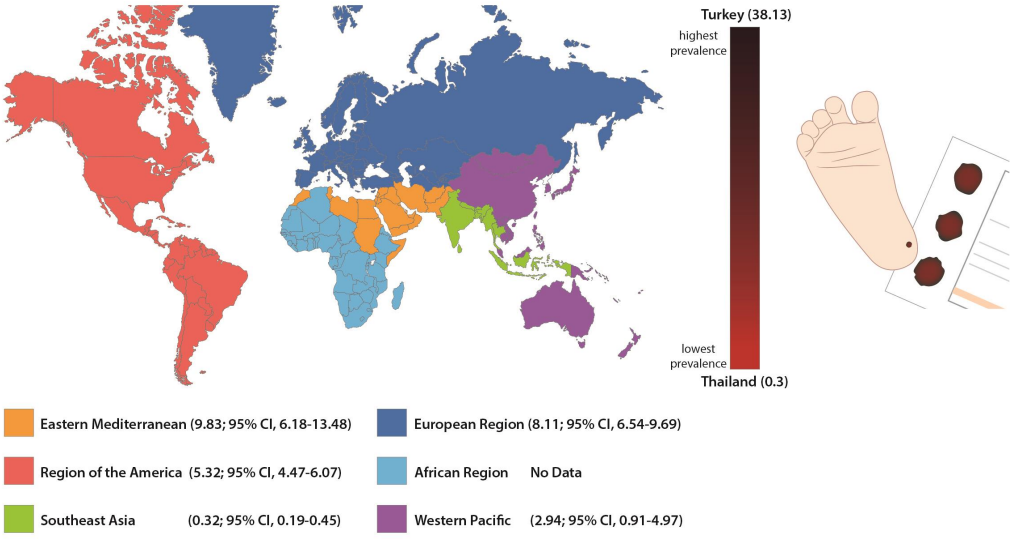
Graphical abstract



The pooled prevalence of classic PKU in the World Health Organization Regions

disease prevalence.[6,14] PKU varies among ethnic groups, races, and geographic regions. For example, In Japan, the incidence is reportedly 1:108,822.[15] Turkey, with an incidence of 1:6,000, and Iran, with an incidence of 1:4,698, are among the countries with the highest PKU incidences.[16,17]

Despite numerous studies conducted in various countries on PKU prevalence using screening programs, no study has systematically compared the prevalence of PKU across regions and countries or sources of heterogeneity. To address this gap, this systematic review and meta-analysis aimed to investigate the worldwide prevalence of PKU. Moreover, many countries have acknowledged the benefits of newborn screening programs for PKU. Moreover, newborn screening programs have enabled the rapid and large-scale testing of many children with good quality control.

Methods

This systematic review adhered to the guidelines of the Joanna Briggs Institute Reviewers' Manual 2014, Systematic Review of Prevalence Data.[18] The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2009 flow diagram was used to guide the study identification and selection process.[19]

1. Search method

The PubMed, Web of Sciences, Sciences Direct, ProQuest, and Scopus databases were searched on Oct 28, 2018, without publication date restrictions. The search strategy carefully captured all potentially eligible records of PKU prevalence. A combination of medical subject headings (MeSH) and similar text words in English was used. Google Scholar was searched, as were the reference lists of the reviewed articles to identify additional relevant articles.

The key MeSH terms were as follows: (Infants OR Newborns OR Neonate) AND (Phenylketonuria OR Hyperphenylalaninemia, Non-Phenylketonuric OR BH4 Deficiency OR Tetrahydrobiopterin Deficiency OR Phenylketonuria II OR DHPR Deficiency OR Dihydropteridine Reductase Deficiency OR Atypical PKU) AND (Incidence OR Prevalence) and Screening. No time limitation was considered for the database search.

2. Inclusion criteria

All original articles that directly reported PKU prevalence based on newborn screening of populations were included. A newborn, infant, or neonate is a child younger than 28 days of age; in this review, the sampling period was limited to the first 28 days of life. All studies were included if they used a laboratory screening test for disease detection.

Reviews, comments, and letters were excluded. Moreover, studies that reported the prevalence in a selective neonatal population (congenital diseases, intellectual disability), those that included children older than 28 days, and those that indirectly estimated prevalence according to consanguinity or the incidence of another genetic disease were excluded. In some cases, studies were conducted of the same PKU prevalence in one country using different dates; in such cases, the more recent study (which also included the data from the older study) was included and the older study was omitted. Moreover, studies that detected PKU based on clinical manifestations in neonates or neural tube defects in fetuses were excluded.

3. Data collection

The title, abstract, and keywords of every identified article

were carefully scanned and relevant articles were selected by title or abstract review.

4. Data extraction and management

Two reviewers (HSH. and FZ) independently extracted the patient characteristics, study characteristics, screening test used, and incidence from the reviewed studies using a data extraction form. Any disagreements between the 2 researchers were solved by consultation of another reviewer or a clinical adviser. Abstracts not published as full texts were not included in the study. To avoid data entry errors, all results were double-entered into a data extraction form. The included studies used different units such as mmol/L, μmol/dL, and mg/dL to report phenylalanine level. However, in this study, to increase comparability, all units were converted to mg/dL. In addition, to ensure more accurate comparisons, PKU prevalence was calculated as percentage and rate per 100,000 screened neonates.

5. Assessment of methodological quality

Article quality was assessed using the Joanna Briggs Institute Critical Appraisal Checklist for studies that reported prevalence data. Each article was evaluated according to the following methodological criteria: appropriate sample, adequate sample size, valid methodology, valid measure to detect the disease, and an appropriate statistical analysis.

6. Risk of bias

Risk of bias was assessed using the risk of bias tool for studies measuring disease prevalence designed and developed by Hoy et al.[20] Based on this 10-point checklist, studies were assessed for internal and external validity and grouped as having high, moderate, or low bias risk. Studies with a score of 9–10 were considered at having low risk of bias; 6–8, as having moderate risk; and less than 6, as having high risk. Those studies with a high risk of bias were excluded from the meta-analysis.

7. Data analysis

The data were analyzed using Stata ver. 12 (StataCorp LP., College Station, TX, USA). In a meta-analysis, pooled prevalence was estimated based on World Health Organization (WHO) regions and reported as per 100,000 neonates/population with 95% confidence interval (CI).

The degrees of heterogeneity among the included studies are expressed by the I^2 heterogeneity statistic, and the random effects model was used to estimate the pooled prevalence in subgroups. A forest plot was used to display the meta-analysis results.

The mixed model test considered WHO regions as a random intercept. In this test, phenylalanine levels were modeled as independent variables, while prevalence was considered a dependent variable.

Furthermore, a meta-regression analysis was performed to investigate the impact of variables such as WHO region, phenylalanine cutoff point, study period, national or governmental screening program, and participant age on the I^2 and pooled prevalence.

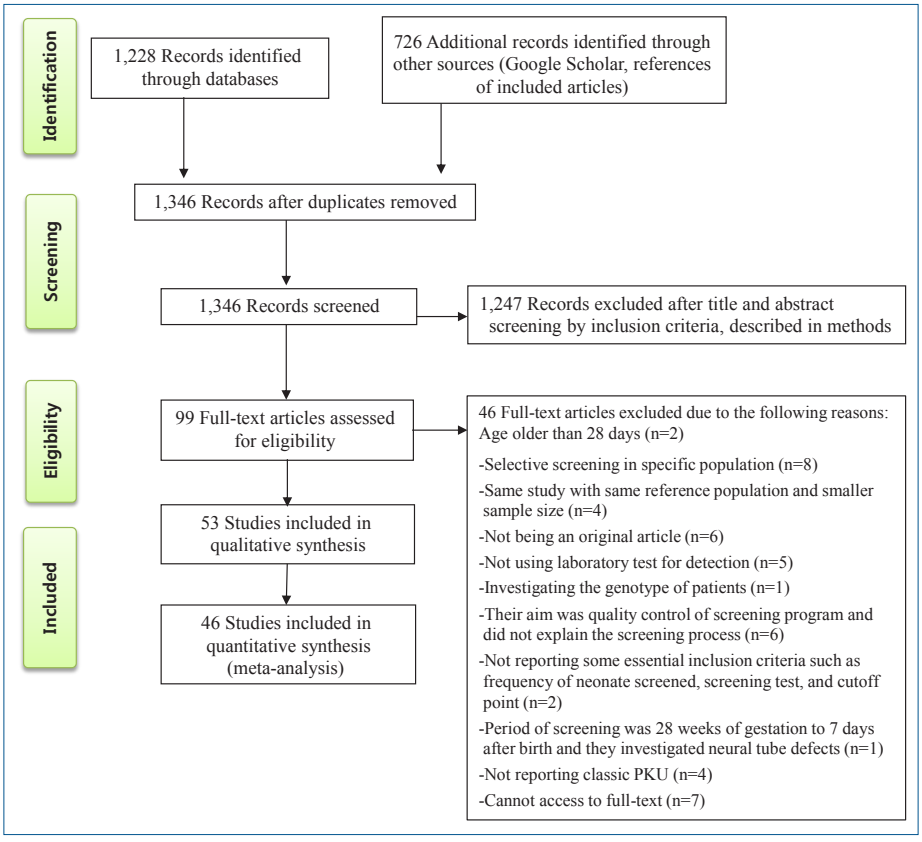


Fig. 1. Flow diagram of the literature search and study selection process. PKU, phenylketonuria.

Results

1. Study selection

After a comprehensive search, 1,228 relevant articles were identified, 608 duplicates were removed. The relevance of the remaining 1,346 studies was evaluated based on the titles/abstracts alone; of them, 99 studies were subjected to full-text review, which eliminated another 46 studies according to the inclusion and exclusion criteria. Finally, 53 studies with 119,152,905 participants were included in the present systematic review; of them, 46 were included in the meta-analysis (Fig. 1).

2. Risk of bias

In the bias assessment, 7 studies scored below 6 (high risk) and were excluded from the meta-analysis; thus, the pooled global prevalence was estimated without them.[11-13,21-24] Moreover, 33 of the studies scored 6–8 (moderate risk),[1,9,16,17,25-53] while the other 13 studies had low risk.[2,10,54-64]

3. Study characteristics

In this section, the systematic review results are presented based on different characteristics, including region, test, and participant characteristics.

4. Region characteristics

The included studies (1964–2017) are presented in Table 1. The longest study, conducted in France, examined 35 years of screening data from 1966 to 2001.[56] The largest screening population was in China (35,795,550 newborns for 30 years from the start of the screening project); the largest number of cases was detected (3,082 patients) in this study.[60] However, the smallest population belonged to a study conducted in Iraq in 2015, and only 8,255 newborns were screened.[13]

5. Participants' characteristics

Sampling age at screening was below 5 days in 30 studies,[1,2,9,10,16,17,21,23,27,29,31-33,35,39,41,43-46,48,50-53,56-58,60,61] 5–10 days in 15 studies,[13,22,28,30,34,37,42,47,49,54,55,59,62,63,65] and over 10 days in 3 studies.[25,26,38] Five studies did not report newborn age at screening.[11,12,24,36,64]

The neonatal participation rate was reported in 23 studies

Table 1. Description of studies included in the study

ID	Study	Study location	Population size	Study period	Age taking a blood sample	Screening test/cutoff level (mg/dL)	No. of cases in screening test (incidence per 100,000)	Diagnostic test/cutoff level for classic PKU (mg/dL)	No. of classic PKU cases (incidence per 100,000)	Consanguinity	Neonatal participation rate	Rist of bias Score	Remarks	WHO regions
1	MacCready,[25] 1964	USA/Massachusetts	134,580	1962–1964	28 Days	Guthrie/4	NA	Guthrie/≥4	14 (10.4)	NA	NA	7		Pan American
2	Peterson,[26] 1968	USA/California	311,953	1966	30 Days	Guthrie and Fluorometric/20	NA	Guthrie and Fluorometric/≥20	16 (5.12)	NA	NA	6		Pan American
3	Fox,[27] 1971	Canada/Manitoba	85,868	1966–1970	4–5 Days	Guthrie test/20	NA	Guthrie test/≥20	5 (5.82)	NA	95.7%	8		Pan American
4	Alm,[54] 1981	Sweden	1,362,497	1965–1979	4–6 Days	Ion exchange chromatography/4.12	312 (22.89)	Ion exchange chromatography/≥4.12	43 (3.15)	NA	86%	9	The phenylalanine cut point reported 0.25 mmol/L, this is equal to 4.12 mg/dL	Europe
5	Antonozzi,[28] 1982	Italy/3 regions	220,000	1974–1981	Median of 7.6 days	Ion exchange chromatography/1.65	211 (95.9)	Ion exchange chromatography/≥1.65	23 (10.45)	NA	42%	6	The phenyl alanine cut point reported 100 μmol/dL, this is equal to 1.65 mg/dL	Europe
6	Farhud,[22] 1982	Iran/Tehran	8,633	1982	4–8 Days	Guthrie/20	NA	Guthrie/≥ 20	1 (11.58)	NA	NA	5		Eastern Mediterranean
7	Liu,[29] 1986	china/11 province	198,320	1982–1985	1–3 Days	Guthrie/4	225 (113.4)	Guthrie/≥15	9 (4.53)	NA	NA	8	Reports overall incidence 1:16500 but the incidence is 1: 22035	Western Pacific
8	Mathias,[30] 1986	West Germany	940,369	1969–1984	5–7 Days	Guthrie/15	170 (18.07)	Ion exchange chromatography/≥15	94 (10)	NA	NA	8		Europe
9	Özalp,[31] 1986	Turkey/Ankara	20,979	1983–1985	>24 Hr	Guthrie/4	NA	Fluorometric/≥20	8 (38.13)	NA	NA	8		Europe
10	Aoki,[55] 1988	Japan	12,168,645	1977–1985	5–7 Days	Guthrie/20	NA	Guthrie/≥20	102 (0.83)	NA	87%	10		Western Pacific
11	Missiou-Tsagaraki,[32] 1988	Greece	1,042,000	1974–1986	>24 Hr	Thin-layer chromatography/4	776 (74.47)	Thin-layer chromatography/≥20	43 (4.12)	NA	NA	8		Europe
12	Chen,[33] 1989	China/Shanghai	358,767	1981–1989	>3 Days	Guthrie/4	72 (20.06)	Fluorometric/≥ 20	21 (5.85)	NA	33%	6		Western Pacific
13	Smith,[34] 1991	United Kingdom	3,796,645	1984–1988	6–7 Days	9 Laboratories Guthrie, 6 used fluorometric and 11 used thin layer or paper chromatography/4	NA	9 Laboratories Guthrie, 6 used fluorometric and 11 used layer or paper chromatography/≥20	273 (7.19)		100%	8	The phenylalanine cut point reported 240 μmol/dL, this is equal to 4 mg/dL	Europe
14	Gerasimova,[35] 1992	Russia, Moscow	139,664	1990–1991	4–5 Days	Fluorometric/3	529 (378)	Fluorometric/≥20	21 (15.03)	NA	NA	7	The phenylalanine cut point reported 180 μmol/dL, this is equal to 3 mg/dL	Europe

Table 1. Description of studies included in the study (Continued)

ID	Study	Study location	Population size	Study period	Age taking a blood sample	Screening test/ cutoff level (mg/dL)	No. of cases in screening test (incidence per 100,000)	Diagnostic test/ cutoff level for classic PKU (mg/dL)	No. of classic PKU cases (incidence per 100,000)	Consanguinity	Neonatal participation rate	Rist of bias Score	Remarks	WHO regions
15	Cabalska,[36] 1993	Poland	2,861,504	1965–1990	NA	Guthrie/4	NA	Guthrie/≥20	368 (12.86)	NA	40%–100%	8	They just presented data from the National Research Institute for Mother and Child	Europe
16	Fernandez-Iglesias,[37] 1995	Spain/Principado	75,488	1982–1993	5–8 Days	Thin-layer chromatography/4	NA	High performance liquid chromatography/≥ 4	5 (6.62)	NA	NA	7	The phenylalanine cut point reported 240 μmol/dL, this is equal to 4 mg/dL	Europe
17	Hitzeroth,[23] 1995	South Africa/ Pretoria	59,600	1979–1986	3–5 Days	Thin-layer chromatography/NA	NA	Thin-layer chromatography/NA	1 (1.67)	NA	NA	5		Africa
18	Özalp,[16] 1995	Turkey	576,122	1987–1994	>24 Hr	Fluorometric/20	NA	Fluorometric/≥ 20	96 (16.66)	45%	NA	7	The phenylalanine cut point reported 1,200 μmol/dL, this is equal to 20 mg/dL)	Europe
19	Kucinskas,[38] 1996	Lithuania	907,168	1975–1993	21 Days	Fluorometric/2.5	NA	Fluorometric/≥ 2.5	85 (9.36)	NA	NA	8	The phenylalanine cut point reported 150 μmol/dL, this is equal to 2.5 mg/dL	Europe
20	Ounap,[66] 1998	Estonia	36,074	1993–1995	3–5 Days	Fluorometric/3	NA	Fluorometric /≥3	6 (16.63)	NA	85%	7	The phenyl alanine cut point reported 180 μmol/dL, this is equal to 3 mg/dL	Europe
21	Abadie,[56] 2001	France	21,500,000	1966–2001	3–5 Days	Until 1990 (Guthrie) 1991–2001 (Fluorometric)/10	1,426 (6.63)	Until 1990 (Guthrie), 1991–2001 (Fluorometric)/≥10	1,164 (5.41)	NA	3%–65%	9	The phenylalanine cut point reported 600 μmol/dL, this is equal to 10 mg/dL	Europe
22	Zytkovicz,[39] 2001	England	257,000	1999–2001	1–3 Days	MS/MS/2.29	92 (35.79)	MS/MS/≥2.29	7 (2.72)	NA	NA	8	The phenylalanine cut point reported 139 μmol/dL, this is equal to 2.29 mg/dL	Europe
23	Schulze,[40] 2002	Germany	423,773	1994–1999	5 Days (1–10 days)	MS/MS/2.5	NA	MS/MS/≥10	41 (9.67)	NA	NA	8	The phenylalanine cut point reported 150 μmol/dL, this is equal to 2.5 mg/dl	Europe
24	Zaffanello,[57] 2002	Northeastern Italy	1,142,338	1978–1997	3–5 Days	Guthrie/2	NA	High performance liquid chromatography/≥20	25 (2.18)	NA	97%	9		Europe
25	Capistrano-Estrada,[41] 2003	Philippines	189,720	1996–2001	>24 Hr	Guthrie/3.3	75 (39.53)	Guthrie/≥3.3	3 (1.58)	NA	NA	6	The phenylalanine cut point reported 200 μmol/dL, this is equal to 3.3 mg/dL	Western Pacific
26	Charoensiriwatana,[42] 2003	Thailand	1,425,025	1992–2001	2–7 Days	Guthrie/4	321 (22.52)	Fluorometric/≥4	5 (0.35)	NA	NA	7		South-East Asia
27	Jiang,[43] 2003	China/Guangdong	461,805	NA	3 Days	Guthrie and Fluorometric/2	NA	Guthrie and Fluorometric/≥ 20	6 (1.29)	NA	NA	7	The phenylalanine cut point reported 120 μmol/dL, this is equal to 2 mg/dL	Western Pacific
28	Yoon,[44] 2005	South Korea	5,243,841	1996–2006	NA	Guthrie and Fluorometric/4	NA	High performance liquid chromatography/≥20	16 (0.3)	NA	NA	8		Western Pacific
29	Pangkanon,[67] 2009	Thailand	79,179	2001–2004	2–3 Days	MS/MS/2.29	NA	MS/MS/≥20	5 (6.31)	NA	5.40%	9	The phenylalanine cut point reported 139 μmol/dL, this is equal to 2.29 mg/dL	South-East Asia
30	Senemar,[17] 2009	Iran/ Fars	70,477	2000–2005	3 Days	Fluorometric/4	NA	Fluorometric/≥4	15 (21.28)	86.60%	NA	7		Eastern Mediterranean
31	Cornejo,[58] 2010	Chile	2,478,123	1992–2008	3.6 Mean	Fluorometric/20	NA	1998–2002 Fluorometric-2002–2008 MS/MS/≥20	131 (5.28)	NA	48–98%	9		Pan American
32	Habib,[45] 2010	Iran /Fars	175,235	2004–2007	3–5 Days	Enzymatic colorimetric method/4	30 (17.11)	High performance liquid chromatography/≥10	28 (15.97)	NA	NA	8		Eastern Mediterranean
33	Karamifar,[9] 2010	Iran /Fars	76,966	2007–2008	3–5 Days	Enzymatic colorimetric method/2	9 (11.69)	High performance liquid chromatography/≥20	8 (10.39)	NA	NA	8		Eastern Mediterranean
34	Niu,[46] 2010	Taiwan	1,495,132	2000–2009	2–3 Days	MS/MS/4	NA	MS/MS/≥20	5 (0.33)	NA	>99%	8	The phenylalanine cut point reported 240 μmol/dL, this is equal to 4 mg/dL	Western Pacific
35	Vilarinho,[59] 2010	Portugal	316,243	2004–2008	3–6 Days	MS/MS/2.5	NA	MS/MS/≥6	26 (8.22)	NA	99.80%	9	The phenylalanine cut point reported 150 μmol/dL, this is equal to 2.5 mg/dL	Europe
36	Sutivijit,[47] 2011	Thailand/Southern Region	1,118,676	2000–2009	>2 Days	Guthrie/4	120 (10.72)	Fluorometric/≥4	5 (0.44)	NA	near 100%	8		South-East Asia
37	Botler,[10] 2012	Brazil	541,248	2005–2007	2–5 Days	Fluorometric/4	64 (11.82)	Thin layer amino acid chromatography≥ 10	26 (4.8)	NA	71-80%	9		Pan American
38	Shi,[60] 2012	China	35,795,550	1981–2011	2–3 Days	Guthrie/2	NA	Fluorometric: ≥2 Guthrie: ≥4	3,082 (8.6)	NA	3.86% in 2003 and 59.01% in 2009	9	The phenylalanine cut point reported 120 μmol/dL, this is equal to 2 mg/dL	Western Pacific
39	Yang,[61] 2012	China/Zhejiang	3,791,538	1999–2010	3–5 Days	Fluorescent ninhydrine method/2	NA	Fluorescent ninhydrine method/≥2	143 (3.77)	NA	NA	9	The phenylalanine cut point reported 120 μmol/dL, this is equal to 2 mg/dL	Western Pacific

Table 1. Description of studies included in the study (Continued)

ID	Study	Study location	Population size	Study period	Age taking a blood sample	Screening test/ cutoff level (mg/dL)	No. of cases in screening test (incidence per 100,000)	Diagnostic test/ cutoff level for classic PKU (mg/dL)	No. of classic PKU cases (incidence per 100,000)	Consanguinity	Neonatal participation rate	Rist of bias Score	Remarks	WHO regions
40	Dluholucký,[11] 2013	Slovakia	927,524	1995–2012	NA	Guthrie/NA	NA	Fluorometric (NA)	157 (16.92)	NA	98%	5		Europe
41	Al Hosani,[62] 2014	United Arab Emirates	750,365	1995–2011	>2 Days	Time-resolved fluorescence/4	57 (7.59)	1995-2001 time-resolved fluorescence application 2011 MS/MS/≥20	51 (6.79)	NA	1995 -50%, 2010-95%	10		Eastern Mediterranean
42	Dluholucký,[12] 2014	Slovakia	82,892	2013–2014	NA	MS/MS/NA	NA	MS/MS (NA)	5 (6.03)	NA	NA	5		Europe
43	Ramalho,[63] 2014	Brazil/Sergipe	43,449	2007–2008	2–6 Days	Enzymatic colorimetric method/ 5	NA	Enzymatic colorimetric method/≥20	4 (9.2)	NA	78.93%	9		Pan American
44	Hamawandi,[13] 2015	Iraq/Sulaimani	8,255	2013–2014	3–10 Days	ELISA/4	11 (133.25)	High performance liquid chromatography/≥4	1 (12.11)	100%	NA	5		Eastern Mediterranean
45	Šmon,[2] 2015	Slovenia	385,831	1993–2012	3–5 Days	Fluorometric/3.3	NA	Fluorometric/≥20	38 (9.84)	NA	NA	9	The phenylalanine cut point reported 0.2 mmol/l, this is equal to 3.3 mg/dL	Europe
46	Hassan,[49] 2016	Egypt	25,276	2008	3–7 Days	MS/MS/2.5	NA	MS/MS/≥1.69	5 (19.78)	NA	NA	6	The phenylalanine cut point reported 150 μmol/dL, this is equal to 2.5 mg/dL	Eastern Mediterranean
47	Zhong,[24] 2016	China	13,187,196	2013	NA	NA/NA	NA	NA	1,123 (8.51)	NA	10%-85%	4		Western Pacific
48	Al-Jasmi,[21] 2016	United Arab Emirates	136,049	2011–2014	3–5 Days	MS/MS/NA	NA	MS/MS (NA)	11 (8.08)	81%	NA	5		Eastern Mediterranean
49	Alkhazraji,[48] 2016	Iraq/Baghdad	80,409	2014	3–5 Days	MS/MS/2.5	NA	MS/MS/≥1.69	6 (7.46)	NA	66%	6	Self-calculated Prevalence. Article did not report prevalence of PKU. The phenylalanine cut point reported 150 μmol/dL, this is equal to 2.5 mg/dL	Eastern Mediterranean
50	Saadatpour, [50] 2016	Iran/Hormogan	71,677	2014–2016	3–5 Days	ELISA/2	15 20.92)	High performance liquid chromatography≥4 mg/dL	3 (4.18)	66%	88%	8	Consanguinity Reported 53 % but from 3 positive case 2 had Consanguinity marriage and should correct 66%	Eastern Mediterranean
51	Alfadhel,[52] 2017	Saudi Arabia	775,000	2005–2012	After 24 hr of birth	MS/MS/3	NA	MS/MS≥2.03	53 (6.83)	NA	NA	7		Eastern Mediterranean
52	Abbaskhanian, [51] 2017	Iran/Mazandaran	407,244	2007–2015	3–5 Days	ELISA/4	465 (114.18)	High performance liquid chromatography>20	6 (1.47)	NA	NA	8		Eastern Mediterranean
53	Motamedi,[1] 2017	Iran/Lorestan	384,993	2006–2016	3–5 Days	High performance liquid chromatography/4	NA	High performance liquid chromatography≥4	74 (19.22)	82%	53.60%	7		Eastern Mediterranean

PKU, phenylketonuria; WHO, World Health Organization; MS/MS, tandem mass spectrometry; ELISA, enzyme-linked immunosorbent assay; NA, not announced.

[1,10,11,24,27,28,33,34,44,46-48,50,53-60,62,63]; of them, it was above 90% in 7 studies.[11,27,34,46,47,57,59] The participation rate increased with the progression of the screening process in 8 studies.[1,24,27,36,56,58,60,62]

Moreover, 6 studies reported the percentage of consanguineous marriages among the parents of newborns with PKU.[1,13,16,17,21,50] The percentage of consanguineous marriages varied from 45% in Turkey[16] to 100% in Iraq.[13]

6. Test characteristics

In the included studies, 2 stages were used to diagnose infants with classical PKU.

7. Screening tests

A total of 19 studies reported the number of positive cases in the first stage of screening. The phenylalanine cutoff point for separating positive cases and referrals for diagnostic testing ranged from 1.65 mg/dL to 20 mg/dL. The highest recall rate in the first stage of screening was 378 per 100,000 neonates in a study conducted in Russia.[35]

8. Diagnostic tests

In the diagnostic stage, the phenylalanine cutoff point for diagnosing classic PKU patients ranged from 1.65 mg/dL to 20 mg/dL. Moreover, 22 studies selected 20 mg/dL as the positive cutoff point and 5 studies did not report a cutoff point.[2,9,16,22,25,26,31-36,43,44,46,51,55,57,58,62-64]

9. Pooled global prevalence of classic PKU

Among the included studies, the highest prevalence was found in Turkey (38.13), followed by Iran, with a prevalence of 21.28 per 100,000 neonates,[17,31] while the lowest prevalence was reported in studies conducted in Thailand (0.3) and Taiwan (0.44).[42,46 47,64]

A subgroup estimation of the polled prevalence showed that the pooled prevalence of classic PKU in the included studies was 6.002 (95% CI, 5.07–6.93). The highest prevalence was seen in Eastern Mediterranean (9.83; 95% CI, 6.18–13.48), Europe (8.11; 95% CI, 6.54–9.69), Pan America (5.32; 95% CI, 4.47–6.07), Western Pacific (2.94; 95% CI, 0.91–4.97), and Southeast Asia (0.32; 95% CI, 0.19–0.45) per 100,000 neonates (Fig. 2).

10. Statistical analysis

According to the results of mixed model, cutoff point selection had no effect on prevalence. The p value obtained from the likelihood ratio test in the mixed model test suggested that the random intercept model was appropriate. Moreover, based on the intraclass coefficient, 29% of the PKU prevalence changes in different countries were justified by consideration of the WHO regions (Table 2).

A meta-regression test was used to assess the effect of year, phenylalanine cutoff point, region, neonate age at screening, and screening level (national or regional) on heterogeneity.

In the naïve model without any variables, I^2 was 99%. Several models with different variables were created in which I^2 ranged was 97%–99%, and the input of different variables did not decrease the heterogeneity.

Among the variables included in the model, only some WHO regions were significant. In the meta-regression model, the European region was selected as a reference. In the Eastern Mediterranean region, the prevalence was 1.01 greater than that in the European region, but the difference was not significant. The pooled prevalence of the different regions is reported in Table 3.

According to I^2 by region and overall, the studies had high heterogeneity (Table 4).

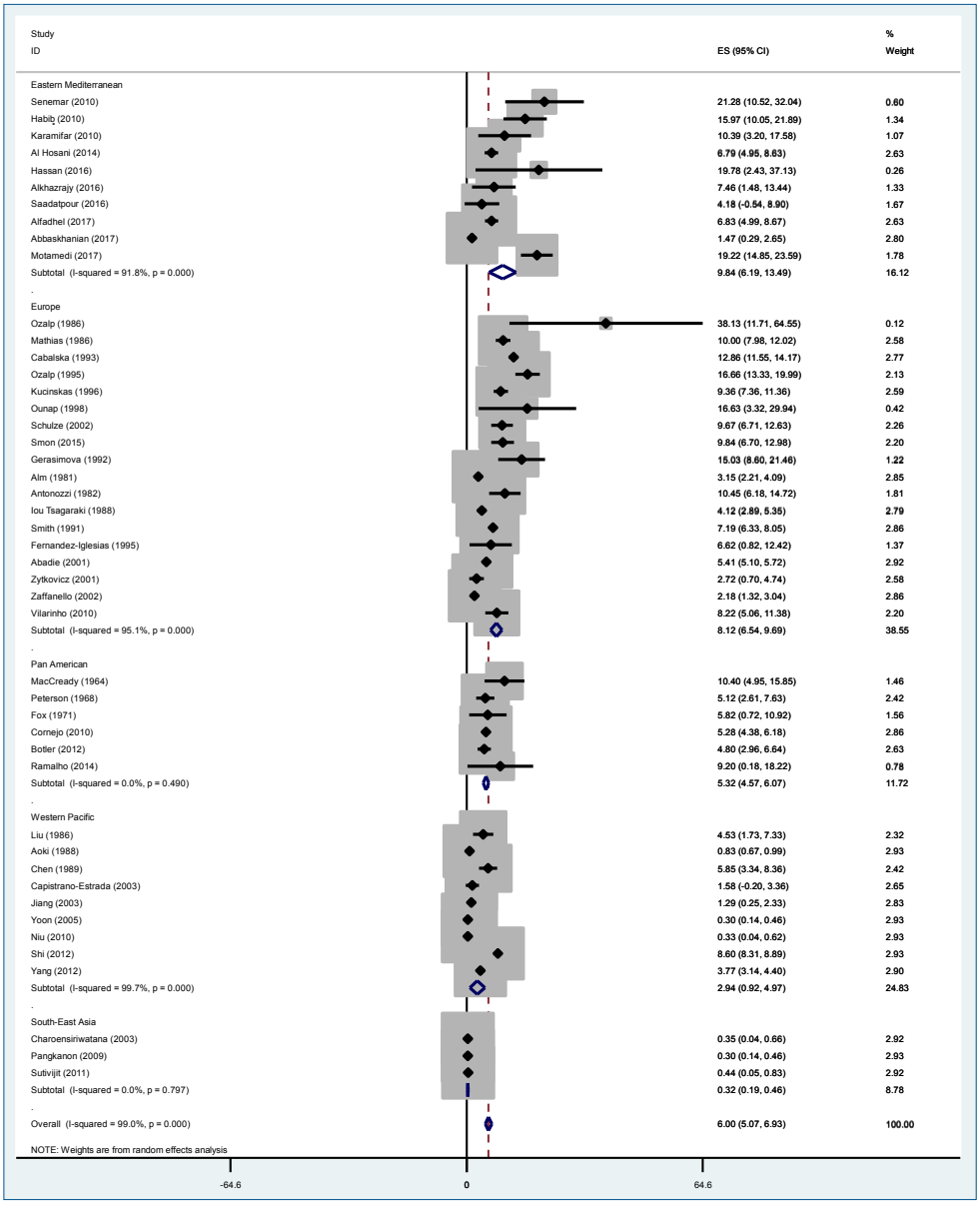


Fig. 2. Forest plot of pooled global prevalence of phenylketonuria. ES, estimated; CI, confidence interval.

Table 2. Result of mixed model test

Variable	Coefficient	SE	*P* value	Estimate	95% CI
Cut-point level	-0.03	0.11	0.792		
Random-effect parameter					
WHO regions (var constant)		12.07		15.61	3.43–71.08
Var (residual)		8.09		36.27	23.43–56.16

SE, standard error; CI, confidence interval; WHO, World Health Organization.
Intraclass correlation coefficient=0.29. Likelihood-ratio test=0.0019.

Table 3. Result of meta-regression test

WHO region	Coefficient	SE	*P* value	I^2
Europe	Reference			
Eastern Mediterranean	1.01	1.89	0.593	
Pan American	-2.03	2.15	0.350	98.69%
Western Pacific	-5.37	1.72	0.003	
Southeast Asia	-7.97	2.54	0.003	

WHO, World Health Organization; SE, standard error.

Table 4. Prevalence rate and heterogeneity in regions

WHO region	*P* value	I^2	Prevalence in 100 000 neonates (range)	Pooled prevalence in 100,000 neonates
Pan American	0.49	0	4.8–10.4	5.32
Europe	<0.0001	95.1%	2.18–38.13	8.12
Western Pacific	<0.0001	99.7%	0.3–8.6	2.94
Southeast Asia	0.79	0	0.3–0.44	0.32
Eastern Mediterranean	<0.0001	91.8%	1.47–21.28	9.84
Overall	<0.0001	99%	1.47–38.13	6.002

WHO, World Health Organization.

Discussion

This systematic review aimed to investigate the worldwide prevalence of PKU and provided a general picture of its status. The results of this study demonstrate that the worldwide prevalence of the disease is 0.3–38.13 per 100,000 newborns. However, the meta-analysis revealed that the I2 index, which indicates heterogeneity, was reported for all regions except Southeast Asia (91.8%) and Pan America (99.7%), indicating high heterogeneity among countries and regions.

The uni- and multivariate models in the meta-regression showed that phenylalanine level, geographical area, neonate age at screening, screening level (national or regional), after the control for year of study, did not change heterogeneity.

However, the differences in prevalence can be attributed to 2 factors: (1) variability of the factors affecting disease worldwide; and (2) differences in the methods used in the studies.

PKU is a c0ngenital genetic disease; thus, factors such as culture, customs, consanguineous marriage, and genetics are expected to affect its incidence but among included studies only 6 studies in Iran, Iraq, Turkey, and the United Arab Emirates[1,13,16,17,21,50] reported consanguineous marriages among parents of children with PKU.

Therefore, a lack of information about the prevalence of PKU in many countries in which consanguineous marriage is prevalent and a lack of reporting consanguineous marriage status in parents of children with PKU in many studies prevented us from controlling the effect of this variable on prevalence.

The next important determinant of prevalence is study performance; factors such as diagnostic tests, cutoff point, and sample size can affect the pooled prevalence in prevalence studies.

However, in the mixed model test, there was no significant relationship between cutoff point and disease prevalence, which might have been due to the effect of the confounding variables. However, the difference was noticeable when the cutoff point differed in the same population and within the same country. For example, in 3 studies conducted during 2000–2008 in Fars province (Iran), a different cutoff point was found. Moreover, Senemar chose a phenylalanine level of ≤4 mg/dL to define classical PKU and reported a prevalence of 21.28.[17] Habib et al.[45] considered a phenylalanine ≤10 mg/dL cutoff value and reported a prevalence of 15.97. Furthermore, in the study of Karamifar et al.,[9] phenylalanine levels above 20 mg/dL were considered positive and a prevalence of 10.39 was reported.

Sample size is the other factor involved in the difference in prevalence among studies. In a meta-analysis, the pooled prevalence is estimated according to the sample size, and larger studies have greater impact on prevalence. Thus, although studies conducted in the Eastern Mediterranean region reported higher prevalence than those in the Western Pacific region,

since most studies in the latter had a larger sample size and the weighted sample size in that region was 24.83%, higher than that in the Eastern Mediterranean region (16.2%), the pooled prevalence in studies conducted in the Eastern Mediterranean region was close to that of the Western Pacific region.

Although this study addressed an important concern in genetic diseases, its findings may not be highly accurate, as there were many sources of heterogeneity in the reviewed studies that could have affected their pooled prevalence. Moreover, some heterogeneous sources might not have been identified. However, the standardization of study methods can partly solve this problem.

One of the limitations of this study was the failure to report consanguineous marriage in parents of newborns with PKU. Thus, it was not possible to answer the following question:

Is the difference in PKU prevalence among different countries due to differences in the number of consanguineous marriages?

Thus, we suggest that consanguineous marriages be recorded and reported in screening programs designed to identify patients with PKU and other congenital metabolic diseases.

In conclusion, all relevant studies conducted in 1964–2017 were included in this review. The highest PKU prevalence was observed in Turkey (38.13), while the lowest was seen in Thailand (0.3). Among the WHO regions, the highest prevalence belonged to Eastern Mediterranean Regional Office, while the lowest was in Southeast Asia. This difference in the prevalence may be due to differences in the number of consanguineous marriages among the different regions, phenylalanine cutoff points, and sample sizes.

Conflicts of interest

No potential conflicts of interest relevant to this article are reported.

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