Effectiveness of Phenylketonuria Diagnosis in The Neonatal Treatment Reference Service

Geanlucas Mendes Monteiro1; Elen Villegas Campos1; Abilio Torres dos Santos Neto1; Elenir Rose Jardim Cury Pontes2; Maria Lúcia Ivo2;Maria Angélica Marcheti2; Andréia Insabralde de Queiroz Cardoso2; Liane de Rosso Giuliani3.

1 Graduate Program in Health and Development in the West Central Region, Federal University of Mato Grosso do Sul, Campo Grande-MS-Brazil.
2 Integrated Institute of Health - INISA, Federal University of Mato Grosso do Sul, Campo Grande-MS-Brazil, CEP:79070–900.
3 Medical school - FAMED, Federal University of Mato Grosso do Sul, Campo Grande-MS-Brazil, CEP:79070–900.
*e-mail: elenvillegas@hotmail.com

ABSTRACT

Phenylketonuria is an inborn error of metabolism of autosomal recessive inheritance, with partial or total deficiency of the phenylalanine hydroxylase hepatic enzyme, which converts L-phenylalanine into tyrosine, thus causing accumulation of phenylalanine at the brain and serum level, interfering in the brain protein synthesis and entailing serious deficits. The objective of this study was to analyze the effectiveness of Phenylketonuria Diagnosis in the neonatal treatment reference service. This is a cross-sectional, analytical study with a quantitative approach, documented with retrospective data collection. Sociodemographic data, diagnosis, onset of treatment and the coverage rate analysis were grouped on a quadrennial basis. The sample consisted of 14 patients, from whom 57.1% had records of birth and collection time. In variable days of life, 28.6% were screened within the recommended period, 71.4% were diagnosed up to one month of life and 1 case at 3 years of age, for the onset of treatment (14.3%). The ideal collection would be performed up to 30 days of life. The lowest coverage rate for quadrennial was between 2014-2017 with 84.3%, with an incidence of 1:21,933. In conclusion, we highlight the need to optimize the neonatal screening service in order to make early diagnosis, begin specific treatment and minimize or eradicate irreversible sequelae.

Keywords: phenylketonuria, newborn, neonatal screening, nursing.

INTRODUCTION

In 1934, phenylketonuria (PKU) or phenylpyruvicoligophrenia was initially identified and described as *imbecilitas phenylpiruvica* by AsbjörnFolling, physician, nutritional biochemist and professor of medicine at the University of Oslo. Phenylpyruvic and phenylacetic acids were discovered in urine of

patients with mental retardation. It is the best known of all congenital amino acid diseases, occurring in about 10/100,000 live births (PANEQUE *et al*., 2013).

Also called Autosomal Recessive Phenylalanine Hydroxylase Deficiency, it is an inborn error of metabolism of genetic origin (BROWN and LICHTER-KONECKI, 2016). It is characterized by partial or total deficiency of phenylalanine hydroxylase (PAH) hepatic enzyme that converts L-phenylalanine (Phe) to tyrosine (Tyr). It is a consequence of the mutation of the gene located in chromosome 12q22.24. This results in accumulation of phenylalanine in brain and serum, whose excess interferes with brain protein synthesis and causes extensive changes in the central nervous system and serious cognitive and neurological deficiencies (LAMÔNICA; GEJÃO and ANASTÁCIO-PESSAN, 2015).

In 1963 Robert Guthrie used a few drops of dried blood on filter paper (Guthrie Bacterial Inhibition test), and created neonatal screening as a simple method of early diagnosis that allows rapid incorporation of treatment (BERNAL and EIROA, 2017).

The sample for the Newborn Blood Screening (NBS), popularly known as "heel prick" test (plus complementary examination), should be taken from a puncture of the newborn's heel, from where five drops of blood are placed on a filter paper between 48 hours and the 5th day of birth, for early diagnosis and initiation of treatment before the third month of life (BRASIL, 2016).

In the 60s, the World Health Organization (WHO) implemented the program to prevent mental disability and health problems of the newborn and advocated the importance of NBS (BRASIL, 20047). In Brazil, the screening for phenylketonuria began in 1976, in the city of São Paulo, in a specialized association for the care of children with intellectual disabilities (Associação de Pais e Amigos dos Excepcionais – APAE-SP), a pioneering initiative in Latin America (MARQUI, 2016).

Through Ordinance GM/MS Nº 22, January 15th, 1992, the heel prick test (neonatal screening) was incorporated into the Unified Health System and became compulsory for all live newborns[5]. In 2001, the Ministry of Health published the Ministerial Ordinance (Ordinance GM/MS Nº 822, of June 6th, 2001) that created the *Programa Nacional de Triagem Neonatal (PNTN)* (National Neonatal Screening Program) (BRASIL, 2001).

In order to organize the program and its reference services, and with a view to a better coverage of the percentage oscillation of live births, the National Neonatal Screening Program created three phases. Phase I included screening for phenylketonuria and congenital hypothyroidism; in phase II, sickle cell diseases and other hemoglobinopathies were complemented; and in phase III, cystic fibrosis was added (BRASIL, 2001). Giving continuity to the commitment in 2012, through Ordinance Nº. 2,829 / 2012, the Ministry of Health culminated phase IV in the Neonatal Screening, and added two more pathologies: biotinidase deficiency and congenital adrenal hyperplasia (BRASIL, 2012).

In Brazil, phenylketonuria occurs from 1:16,300 to 1:34,500 births (JAHJA *et al*., 2016). NBS samples showed different results in different regions of the country, such as prevalence of 1:19,409 in Ribeirão Preto (SP); 1:25,313 in Rio de Janeiro (RJ); 1:16,334 in the Recôncavo Baiano (BA); 1:8,690 in Sergipe (SE); 1:28,309 in Tocantins (TO); 1:33,068 in Mato Grosso (MT); and 1:28,862 in Santa Catarina (SC) (CARVALHO *et al*., 2017) .

Regarding incidence, Turkey presented 1:4000 because of the large number of consanguineous marriages; and the disease affects most commonly Caucasian populations from 1 in 10,000 births. PKU is

not curable, so that the individual will lifelong control the blood levels of phenylalanine, follow a restrictive treatment of food with phenylalanine and supplement the diet with specific dietary formulas in order to reduce symptoms and prevent sequelae (SAAD *et al*., 2015).

The first clinical manifestations in the newborn are irritability and "mousy-odoured" urine; as phenylalanine accumulates in the brain, the symptoms appear from 4 to 6 months of age: hypotonia and neuropsychomotor developmental delay. If left untreated, the disease may develop with severe motor delay, microcephaly, epilepsy, autistic symptoms, hyperactivity, and severe intellectual disability, psychoses that evolve with self-harm and eczema (MARQUI, 2016; BERNAL and EIROA, 2017).

The work performed by the multidisciplinary team and the commitment of each professional interfere with the entire process of diagnosis, treatment and follow-up of patients with phenylketonuria. This reflects in deeper knowledge of the disease, its carriers and their individualities, thus reducing treatment dropout, irreversible sequelae, expenses and morbidity. Regarding nursing practice, quality care implies greater coverage of live births with neonatal screening and continuity in the follow-up of diagnosed cases (LUZ *et al*., 2008; JURECKI *et al*., 2017).

Prenatal care is the most opportune period to provide guidance to pregnant women regarding screened diseases through the foot test. Because nurses assume a large part of this process, they have a great responsibility, because this is the ideal moment for the understanding, reflection and learning of pregnant women about neonatal care. After birth, the active search for the patients with diagnostic confirmation, the filling out of the medical records as accurately as possible, and the closer ties with the patient's family will result in improved adherence to the treatment and reduction of infant morbidity and mortality (MENEZES *et al*., 2016).

Therefore, an effective neonatal screening for phenylketonuria requires the functioning of an integrated and multidisciplinary system, that begins in a collection at the appropriate time, a correct procedure, suitable transport, and laboratory providing analysis results in a reasonable time, which will allow the localization and contact with families with altered results for early diagnostic confirmation and timely case management. Conversely, delays at any stage of this screening and diagnostic process may jeopardize the program's desired outcomes.

MATERIALS AND METHODS

This is a cross-sectional analytical study with a quantitative, documentary approach with retrospective data collection. The research was conducted from 2006-2017, involving all patients with phenylketonuria diagnosed at the Neonatal Screening Reference Service of Mato Grosso do Sul State and followed up by the outpatient clinic (IPED APAE). Patients excluded from the sample were those with other metabolic diseases diagnosed during neonatal screening, and those with phenylketonuria in active follow-up at the outpatient clinic of the institution but diagnosed in another state.

The sociodemographic variables studied were: gender, date of birth, time of birth, date of first collection of neonatal screening, date of confirmatory examination, date of onset of treatment, source of collection of neonatal screening, parents´ schooling, neonatal screening coverage rate in 2006-2009, 2010-2013, 2014-2017. The diagnostic variables were: age at diagnosis, diagnostic classification, blood

phenylalanine value at the "heel prick" test (first test, confirmatory phenylalanine/ tyrosine) and presence of symptoms.

For data statistical analysis, an electronic spreadsheet was prepared. The previous information was analyzed by the SPSS version 19.0 software. Data were analyzed by means of statistics with averages, medians, minimum and maximum, and percentage.

The research project was approved by the Research Ethics Committee Involving Human Beings of the Federal University of Mato Grosso do Sul, number 3,354,977 and CAAE 13745619.4.0000.0021. As information was collected from a database, a Data Handling Term was used for access with authorization from the Neonatal Screening Reference Service of Mato Grosso do Sul State.

All stages of the study followed the recommendations of the National Health Council, through Resolution 466/2012, that presents the guidelines and regulatory standards for research involving human beings.

RESULTS

From the system of the Neonatal Screening Reference Service of Mato Grosso do SulState, it was initially known that 30 patients were being followed up at the outpatient clinic of the institution. Of these, 16 patients (53.3%) were excluded from the study because they had not met the pre-established criteria: 09 had PKU diagnosed in another state, and the dates of birth of 07 of them were out of the defined period. Thus, the sample consisted of 14 individuals.

SOCIO-DEMOGRAPHIC VARIABLES

Table 1 - Number and percentage of patients with phenylketonuria according to the sociodemographic variables, Neonatal Screening Service of Mato Grosso do Sul State - 2006-2017.

Variables	Nº.	%
Gender		
Male	9	64,3
Female	5	35,7
Days of life		
≥ 48h to 05	4	28,6
06 to 15	7	50,0
16 to 30	2	14,3
≥ 31	1	7,1
Period between heel prick test and confirmatory in days		
≤ 07	1	7,1
08 a 15	3	21,4
16 a 30	7	50,0
31 a 50	2	14,3
≥ 51	1	7,1

Period between confirmatory and onset of treatment in days		
≤ 07	3	21,4
08 to 15	7	50,0
16 to 30	2	14,3
31to 50	-	-
≥ 51	2	14,3
Origin		
Campo grande	2	14,3
Hinterland of Mato Grosso do Sul	12	85,7

Source: Research data / elaborated by the authors.

From the 14 individuals, 09 were males (64.3%) and 05, females (35.7%). Regarding the time of birth and time of test collection, only 57.1% (n = 8) in both were recorded. Although the sum was the same, the values are not from the same individuals: some children had only the time of birth recorded while others presented only the time of "heel prick" test collection.

As for the days of life of the "heel prick" test collection, 50% of participants were within the range of 6 to 15 days of life, followed by 28.6% who presented the interval of ≥ 48h to 05 days of life. The average age of the "heel prick" test collection was 8.4 days and the median was 07 (minimum 2, maximum 24). One case was out of the estimate because the first collection for the "heel prick" test was made at 03 years of age (Table 1).

In the variable period between "heel prick" test and confirmatory exam for PKU, 50% of the individuals presented interception of 16 to 30 days, followed by 08 to 15 days (21.4%). The average of days was 23.2 and the median 19,5 (minimum 7, maximum 52). Regarding the interval between the confirmatory exam and onset of treatment, 50% of the individuals showed a period of 08 to 15 days, followed by 21.4% with a period ≤ 07 days.

Concerning origin, 85.7% of the individuals came from the hinterland of Mato Grosso do Sul (Table 1), and in relation to the division by mesoregions 71.4% (n = 10) of the patients diagnosed in the state are from the southeast mesoregions.

The parents' schooling was not registered in the patients´ medical records.

DIAGNOSTIC VARIABLES AND ONSET OF TREATMENT

Table 2 - Number and percentage of patients with phenylketonuria according to diagnosis and onset of treatment, Mato Grosso do Sul State Neonatal Screening Service - 2006-2017.

Variables	**Nº.**	**%**
Age of diagnosis		
< 1 month –Earlier	10	71,4
>01 to 03 months	3	21,4
>03 to 06 months – Late	-	-
>06 months to 2years	-	-

> 2 years	1	7,1
Classification of diagnosis		
Classical Fenilcetonúria	3	21,4
Mild Fenilcetonúria	9	64,3
Benign persistentHiperfenilalaninemia	1	7,1
Transitory Hyperfhenylalaninemia	1	7,1
Deficiency of cofactor tetrahydrobiopterin (BH4)	-	-
Onset of treatment		
< 1 month	2	14,3
>1 to3 months	10	71,4
>3 to6 months	-	-
>6 months to2years	1	7,1
> 2 years	1	7,1
Symptomatic		
Yes	5	35,7
No	9	64,2
Value of bloodphenylalanine		
< 4mg/dl	-	-
5-10mgl/dl	3	21,4
11-20mg/dl	8	57,1
>20mg/dl	3	21,4
Relationphenylalanine/tyrosine		
Yes	8	57,1
No	6	42,9

Source: Research data / elaborated by the authors.

Regarding the age of diagnosis, it was found that 71.4% of patients were diagnosed at up to one month of life, performed early. One case was seen of late diagnosis, for not collecting the "heel prick" test in a suitable time, made at three years of age. Of the individuals (n = 14), 64.3% were diagnosed with mild phenylketonuria, followed by 21.4% with diagnosis of classic phenylketonuria (Table 2).

In relation to the onset of treatment, 71.4% of participants were within the range (> 1 to 3 months), 14.3% started up to 30 days of life, but two subjects started treatment excessively late, one at 279 days old and another at 03 years old. As for symptoms, 35.7% (n = 5) presented some characteristic manifestation of the disease at the beginning of treatment. The blood phenylalanine value was between 11-20mg/dl in 57.1% of the diagnostic tests. Finally, the phenylalanine/tyrosine ratio was performed in 57.1% of patients, and 42.9% had not the material collected (Table 2).

ANALYSIS OF THE NEONATAL SCREENING TEST FOR PHENYLETONURIA ON A QUADRENNIAL BASIS

Table 3 - Distribution of variables related to the comparison by 4-year periods 2006-2009, 2010-2013 and 2014-2017, number of newborn screening per quadrennium, coverage rate, number of diagnosed cases and incidence of patients with phenylketonuria followed at the neonatal screening service in Mato Grosso do Sul State.

Period	Nº. of screenings	Rate of coverage %	Nº. of cases diagnosticated	Incidence
2006 - 2009	146.495	91,7	3	1: 53.251
2010 - 2013	143.451	86,0	3	1: 57.332
2014 - 2017	147.918	84,3	8	1: 21.933

Source: Research data / elaborated by the authors.

Comparing the quadrennia, the one that stands out with the highest coverage rate of the "heel prick" test collection is the period 2006-2009 with 91.7%, where 03 cases of phenylketonuria were diagnosed with an incidence rate of 1:53.251. On the other hand, the 2014-2017 period presented a decreased screening rate of the screening test, 84.3%, but the number of diagnosed cases increased to 08, with an incidence of 1:21,933.

DISCUSSION

From this study, it was possible to be aware of the sociodemographic variables, diagnosis, onset of treatment and analysis of the neonatal screening test for phenylketonuria on a quadrennial basis of patients of a reference service in neonatal screening. Regarding the sociodemographic variables of the 14 patients included in the sample, the male gender prevails, which diverges from a study in which individuals diagnosed with phenylketonuria comprised 43 male and 33 female patients, with no significant difference between the sexes (FREEHAULF *et al*., 2013). In Mato Grosso do Sul, prevalence among males was observed.

Regarding the registration of the time of birth and the collection of the neonatal screening exam, some children had only the birth time record and others, the time of the collection of the foot test in medical records, and in conference cards were not completed correctly. According to the internal protocol of the referral service, the exam collection cards are stored for up to 5 years and after that are discarded, making it impossible to know if they were not filled in correctly. It is of utmost importance the complete record with date and time of the two information not to risk collecting the exam before the minimum recommended time. Bernal and Eiroa (2017) in their research show that the test result can be false negative in 10% of cases if collected up to 24 hours of life and 2.4% between 24 and 48 hours, thus being undiagnosed, because that the newborn must have received protein feeding (breast milk) for at least 48 hours of life to present alteration of the exam to PKU.

In the variable days of life to collect the neonatal screening exam, 28.6% of patients underwent the toe test within the period recommended by the Technical Manual for Biological Neonatal Screening (BRASIL, 2016). In this study, 50.0% of patients performed the collection after the deadline, between 06 to 15 days of life, with an average of 8.4 days (minimum 2, maximum 24). One of the patients in the

research sample was removed from the average calculation because he did not collect the heel test. After birth, he was hospitalized 16 days after performing surgical repair of the omphalocele and after 3 years performed the first examination for clinical suspicion. of neuropsychomotor developmental delay.

Regarding the time interval between the foot test and the confirmatory exam, it was observed that 50.0% were in the 16 to 30 days interval through the active search of the cases with altered result for diagnostic confirmation, and in relation to the space. of time between confirmatory examination and treatment initiation 21.4% had active search less than or equal to 07 days, which would be ideal for the earliest possible initiation of treatment.

These values may be justified by the fact that 85.7% of the patients lived in the state, before the active search was done with the transfer of information from the reference service to the collection units responsible for the patient and after that each unit sought their patient. for the measures to be taken. In 2019 there was a change in the flow in the neonatal screening service and the referral center began to directly search the patient for an altered result to confirm the diagnosis and follow-up in order to optimize care (2004).

The diagnosed cases werefrom 11 citiesof Mato Grosso do Sul: Campo Grande, Três Lagoas, Paranhos, Itaquiraí, Rio Brilhante, Fátima do Sul, Dourados, Ivinhema, Naviraí, Mundo Novo and Bodoquena. Two patients (14.2%) are from Paranhos, located in the southeastern mesoregion of the state and presenting the highest incidence of the disease, considering that the population of the municipality was 13,852 inhabitants in 2017 IBGE, 2017).

PKU is a genetic disease and thus its frequency is expected to remain stable over the years. In 2017 the population of Mato Grosso do Sul was 2,713,147 inhabitants and, in the period involving the research (2006–2017), 507,218 live births were reported and 14 patients were diagnosed. The incidence was 1:36,229, different from what is shown in the technical manual of biological newborn screening, that varies from 1:15,000 to 1:25,000 in Brazil (BRASIL, 2016).

None of the medical records of patients in the sample reported the parents´ schooling level, even though the information is crucial for getting to know their level of education and commitment to a follow-up therapy to manage the disease. The information would enable the medical team to closely monitor those parents with scarce understanding of the child's pathology and of the sequelae that may occur when a strict treatment is neglected.The family’s lack of knowledge of phenylketonuria and its important characteristics reflects directly on the dietary therapy management, since the individual's schooling is a significant social determinant of health: the higher the social and economic levels, the better the quality of life and health (WITALIS *et al*., 2016).

Of the 14 patients in the sample, 71.4% were diagnosed within 30 days of life and only one case was considered serious since the diagnosis occurred at 3 years of age because the neonatal screening test was not performed. This may represent a failure in the collection flow and active search following the program, in which case it already had irreversible sequelae (BRASIL, 2016).

At the beginning of treatment 35.7% had some characteristic sign and symptom of the disease: severe developmental delay (1 case); irritability (2 cases); abnormal urine odor characteristic of rat's nest (2 cases). Of the total sample 14.3% presented signs and symptoms up to 30 days of life, of these 71.4% had more than 1 month of life up to 3 months, and only two cases presented late signs, one of which

collected the foot test and confirmatory examination up to 13 days old and the treatment began at 279 days after birth and the other did not perform the examination. This demonstrates that late examination or lack of examination favors the disease, so treatment should start within the first 30 days of life, being ideal up to 21 days (MARQUI, 2017).

The symptoms of an undiagnosed child may appear between the third and fourth month of birth with developmental delay and loss of interest for things surrounding him/her. One should suspect PKU when the child has blue eyes, fair skin and light hair, although dark eyes and dark hair do not exclude suspicion. Other signs and symptoms are marked irritability in the form of constant crying and/or autistic pattern behavior, persistent vomiting, eczema and seborrheic lesions, urine with "mousy, mold and bioterium odor", striated muscle hypertonia, hyperactivity of deep tendon reflexes, flat feet and association with other anomalies characterized by the presence of very separated incisor teeth (RAMALHO, 2011).

Other findings reveal that, even when referring to milder forms of the disease without treatment, complications are seen: behavioral disorders, learning disabilities, impaired communication and reduced emotional well-being. Without the "heel prick" test, PKU may be misdiagnosed as hyperactivity or autistic spectrum, and medication will be prescribed with no improvement in the quality of life (YILDIZ *et al*., 2016).

In addition, the study states that the main purpose for the diagnosis to be performed before the first month of life is because of the estimated loss of 1.9 to 4.1 of intelligence quotient (IQ) at each increase of 1.67mg/dl of blood phenylalanine (YILDIZ *et al*., 2016).

In another study conducted in the northeast of the country, 74.3% of individuals were diagnosed at more than one month of age. There is a need to increasingly improve the guidelines and dissemination of information about the ideal time to collect the "heel prick" test for PKU diagnosis, and to explain the reason why time is crucial for the disease prognosis (MARQUI, 2016).

According to the classification of the patients' disease, 64.3% (n = 9) have mild phenylketonuria and present partial deficiency of the phenylalanine hydroxylase hepatic enzyme requiring treatment, but the complications are milder (BONDY; ROSENBERG, 1974).

Analyzing the coverage rate for neonatal screening, the quadrennium that stands out with the highest coverage rate of the foot test collection is the period 2006-2009 with 91.7%. In this case 03 cases of phenylketonuria were diagnosed with an incidence rate of 1: 53.251 and in relation to the 2014-2017 quadrennium, although 57.2% of the patients in the sample were diagnosed, there was a decrease in the number of collections and this period had an incidence of 1: 21.933. A study by Carvalho *et al* (2017) states that the low number of collections may be justified by the fact that the examinations were performed by medical agreement or private institution and these values are not added to the total coverage rate made by the health service. reference for neonatal screening.

A more reliable coverage rate of total state examinations would be possible if these amounts were passed on to the referral service. Another hypothesis that may be related is the low coverage of the family health strategy proven by the increase in the maternal and child mortality rate from 2014, being an indicator of health quality (BRASIL, 2014; MALTA *et al*., 2016). For this reason, this study performed the division of quadrenniums to evaluate the periods.

This research contributed to analyze the effectiveness of the diagnosis of Phenylketonuria and

concluded that the knowledge about the pathology and neonatal screening by professionals involved in the process advocated by the neonatal screening program favors the diagnosis and treatment of the disease in a timely manner.

REFERENCE

Bernal, AC.; Eiroa, H (2017) Fenilcetonuria de diagnóstico tardio. vol.22 no.6 Salud(i)ciência. Ciudad autonoma de Buenos Aires set.

Brasil (2001) Ministério da Saúde. Secretaria de Atenção à Saúde. Portaria GM/MS n.º 822/GM. 06 de junho de 2001. Brasília (Brasil): Ministério da Saúde.

Brasil (2004) Ministério da Saúde; Secretaria de Atenção à Saúde, Departamento de Atenção Especializada. Manual de normas técnicas e rotinas operacionais do programa nacional de triagem neonatal / Brasil. 2ª ed. Brasília: Ministério da Saúde.

Brasil (2012) Ministério da Saúde. Portaria nº2.829, de 14 de dezembro de 2012 - Inclusão da fase IV no Programa Nacional de Triagem Neonatal. Brasília (Brasil): Ministério da Saúde.

Brasil (2016) Ministério da Saúde. Secretaria de Atenção à Saúde. Departamento de Atenção Especializada e Temática.Triagem neonatal biológica: manual técnico/ Ministério da Saúde, Secretaria de Atenção à Saúde, Departamento de Atenção Especializada e Temática. – Brasília: Ministério da Saúde.

Bondy, PK., Rosenberg, LE.(1974) Duncan's diseases of metabolism.6ª ed. Philadelphia.

Brown, CS.; Lichter- konecki, U. (2016) Phenylketonuria (PKU): A problem solved? Molecular Genetics and Metabolism Reports.Disponível online em http://www.sciencedirect.com/science/article/pii/S2214426915300604

Carvalho, DCSN., Macêdo, TCC., Moreno, M., Figueiredo, FWS., Sales, IB., Christofolini, J., Bianco, B., Barbosa, CP. e Christofolini, D.M (2017)Evolução do Programa de Triagem Neonatal em hospital de referência no Ceará: 11 anos de observação. ABCS Health Sci. 42(3):143-146.

Freehauf, C., Hove, J.L.K.V., Gao, D., Bernstein, L. e Thomas, J.A. (2013) Impact of geographic access to care on compliance and metabolic control in phenylketonuria. Molecular Genetics and Metabolism Reports. Disponível online em http://www.mgmjournal.com/retrieve/pii/S1096719212003678

IBGE – Instituto Brasileiro de Geografia e Estatistica (2017) Senso Demográfico. Resultados preliminares. Mato Grosso do Sul. Disponível online em https://www.ibge.gov.br/estatisticas/sociais/populacao/9103-estimativas-de-populacao.html?edicao=16985&t=resultados

Jahja, R., Van Spronsen, FJ., De Sonneville, LM., VanDermeere, JJ., Bosch, AM., Hollak, CE., *et al*. (2016) Social-cognitive functioning and social skills in patients with early treated phenylketonuria: a PKU-COBESO study. J InheriMetabDis. Disponível online em https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4851698/pdf/10545_2016_Article_9918.pdf

Jurecki, ER.,Cederbaum, S., Kopesky, J., Perry, K., Rohr, F., Sanchez-Valle, A. (2019) Adherence to clinic recommendations among patients with phenylketonuria in the United States. Molecular Genetics and Metabolism Reports. Disponível online em http://www.mgmjournal.com/article/S1096-7192(17)30005-7/pdf

Lamônica, DAC., Gejão, MG. e Anastácio-Pessan, FL. (2015). Fenilcetonúria e habilidades de leitura e escrita. Rev. CEFAC vol.17 no.1 São Paulo, Brasil. Jan./Feb.

Luz, GS., Carvalho, MDB., Pelloso, SM. eHigarashi, IH. (2008) Prevalência das doenças diagnosticadas pelo Programa de Triagem Neonatal em Maringá, Paraná, Brasil: 2001-2006. Rev Gaucha Enferm. V.29(3):446-43.

Malta, DC, Santos, MAS, Stopa, SR, Vieira, JEB, Melo, EA e Dos Reis, AAC. (2016) A Cobertura da Estratégia de Saúde da Família (ESF) no Brasil, segundo a Pesquisa Nacional de Saúde, 2013. Ciência & Saúde Coletiva, 21(2):327-338.

Marqui, ABT. (2016) Panorama da triagem neonatal para fenilcetonúria no Brasil. Medicina (Ribeirão Preto. Online) 49(6): 517-525. Disponível online em http://revistas.usp.br/rmrp/article/view/127440/124597.

Marqui, ABT. (2017) Fenilcetonúria: aspectos genéticos, diagnóstico e tratamento. Rev Soc Bras Clin Med. out-dez;15(4):282-8. Disponível online emhttp://docs.bvsalud.org/biblioref/2018/01/877193/154282-288.pdf

Menezes, FL., Gracioli, MAS., Freitas, HMB., Diaz,CMG., Rocha, BD., Gomes, IEM. (2016). Conhecimento das mães acerca do teste do pezinho. Rev Saude Publica do Paraná.17(2):220-228.

Paneque, OA., Msc. Chacón,GG., Galcerán,TZ., Peña, DYV., Ramírez, R.M. e Roca, TZO. (2013). Evaluación del estado de salud en pacientes con fenilcetonúria. Rev Cubana Pediatr vol.85 no.3 Ciudad de la Habana jul.-set.

Saad, K., Elserogy, Y., Abdel Rahman, A.A., Al-Atram, A.A., Mohamad, I.L., Elmelegy, T.T. (2015). ADHD, autism and neuroradiological complications among phenylketonuric children in Upper Egypt. Acta neurologica Belgica. 115(4): 657-63.

Ramalho, ARO. (2011). Avaliação do Programa de Triagem Neonatal para a Fenilcetonúria no Estado de Sergipe. Dissertação de Mestrado em Ciências da Saúde. Núcleo de Pós-Graduação em Medicina, UFS, Aracaju, Sergipe.

Yildiz, Y., Dursun, A., Tokatli, A., Coskun, T., Sivri, HS. (2016). Late- diagnosed phenylketonuria in an eight-year-old boy with dyslexia and attention- deficit hyperactivity disorder.Turk J Pediatr. 58(1): 94-96. Disponível online em https://www.ncbi.nlm.nih.gov/pubmed/27922243.

Witalis, E., Mikoluc, B., Motkowski, R., Sawicka-Powierza,J., Chrobot, A., Didycz, B. (2017). Phenylketonuria patients' and their parents' knowledge and attitudes to the daily diet – multi-centre study. NutrMetab. Disponível online em https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5559804/pdf/12986_2017_Articl e_207.pdf