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PROPOSAL FOR ADJUSTMENT OF THE REFERENCE VALUES OF THE BASIC PANEL OF NEONATAL SCREENING IN A HOSPITAL IN GUATEMALA

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Abstract: In Guatemala, the reference values for neonatal screening tests are based on those calculated in a population different from the Guatemalan population. The aim of the study was to develop an adjustment in the reference values of the basic neonatal screening panel, in order to reduce false positives and avoid false negatives in altered tests, with a quantitative, retrospective and exploratory methodological design. A total of 1,304 live newborns were included, and the proposed reference values were calculated based on the non-Gaussian distribution of the population; percentiles 99, 99.5 and 99.9 were calculated. The proposed reference values according to the findings are neonatal TSH < 10 IUIU/mL, immunoreactive Trypsinogen < 63.54 ng/mL, total Galactose < 8.78 mg/dL, Phenylalanine < 2.17 mg/dL and 17 Hydroxyprogesterone values chosen based on weeks of gestation less than 32 SMG < 61.38 ng/mL, 33 to 34 SMG < 33.79 ng/mL, 35 to 36 < SMG 17.03 ng/mL, 37 to 38 SMG < 19.03 ng/mL 39 to 40 SMG < 14.45 ng/mL and with more than 40 SMG < 11.23 ng/mL. These results show the need to make adjustments to the reference values of the basic neonatal panel, based on the characteristics of the population where they are performed, such as sex, birth weight, type of feeding, weeks of gestation and type of delivery, since these are factors that influence these values.
Keywords: Neonatal screening; Guatemala; reference values; cut-off point.

INTRODUCTION

Neonatal Screening (NS) consists of a set of tests performed on apparently healthy newborns in search of endocrine-metabolic diseases, which over time can cause serious and irreversible damage (1).

The Roosevelt Hospital and the San Juan de Dios General Hospital are two level IV specialized hospitals in the network of facilities of the Ministry of Public Health and Social Assistance. They are currently the only centers that have specialized areas where NT is performed; together with private entities, they cover approximately 5% of newborns nationwide. In Guatemala, there is no law that guarantees the active search for diseases detected through NT, therefore, its coverage at the national level is low.

The basic panel of NT tests in Guatemala detects: Congenital Hypothyroidism, Congenital Adrenal Hyperplasia, Cystic Fibrosis, Galactosemia and Phenylketonuria. However, in the institutions where NT is performed, the reference values of the tests indicated by the manufacturer are applied, which have been calculated in a population with different characteristics from the Guatemalan population, which can generate an interpretation that is not real, representing the loss of identification of positive cases or, on the contrary, showing positive results that are not.

The objective of this research was to develop an adjustment in the reference values of the basic NT panel by means of the statistical calculation of percentiles, with which we seek to have reference values appropriate to the population that help to reduce the percentage of false positives and false negatives, achieving the timely detection of cases that need to be addressed, as well as the optimization of the use of institutional resources.

MATERIALS AND METHODS

STUDY DESIGN

Prospective exploratory cross-sectional design

POPULATION AND SAMPLE

The population consisted of 11 299 live newborns (NB) at Roosevelt Hospital during the year 2020, from which a sample of 1304 was calculated.

INCLUSION CRITERIA

Live newborns with breastfeeding or infant formula.

EXCLUSION CRITERIA

- Stillbirths.
- NBs requiring at least one blood transfusion.
- NB with treatment of corticoids, calcium chelators, anticonvulsants and/or antipsychotics.

DATA COLLECTION INSTRUMENT

Prior to sample collection, an interview was conducted with the mother/father/guardian of the newborn through which the following data were collected on the "neonatal screening" form of our own design: date of birth, weeks of gestation, date of sample collection, sex, weight, type of feeding, history of transfusions, presence of meconium ileus.

SAMPLING AND TEST PROCESSING

Samples were taken from the left foot heel of each NB with sterile green BD Microtrainer® Quikhell™ lancets with 1.0 mm depth and 2.5 mm cut, and collected with Whatman 903 filter paper.

The samples were processed in the Endocrinology and Specialties Laboratory of the Roosevelt Hospital, using the semi-automated methodology of Perkin Elmer®. Fluoroimmunoassays DELFIA® Neonatal hTSH (neonatal TSH), DELFIA® Neonatal 17α-OH-progesterone kit (17 hydroxyprogesterone), DELFIA® Neonatal IRT kit (immunoreactive trypsinogen); and enzymatic assays by colorimetry Neonatal Total Galactose Kit (total galactose), Neonatal Phenylalanine (and phenylalanine) were performed.

DATA COLLECTION AND PROCESSING

The data collected in the "neonatal screening" report card and the test results were transcribed in a Microsoft Office Excel 2013 sheet, which was adequate to contain the dependent variable: reference value, and the independent variables; sex, birth weight, type of feeding, weeks of gestation, type of delivery, time of sampling, neonatal thyroid stimulating hormone (TSH), 17 hydroxyprogesterone, immunoreactive trypsinogen, total galactose and phenylalanine. Each variable was coded for subsequent analysis.

STATISTICAL ANALYSIS

The data were analyzed in the statistical program Epi Info 7.2.5, which included 1304 newborns, a number calculated with a confidence level of 95% and an error limit of 3.0% from the total population born during the corresponding period. The characteristics are shown in frequencies and percentages. The mean and median of each test of the basic NT panel were calculated. The 99th, 99.5th, and 99.9th percentiles were calculated for each of the values of the 5 tests of the basic panel taking each of the characteristics of interest as a reference.

ETHICAL CONSIDERATIONS

The mother/father/guardian signed the Informed Consent to participate in the neonatal screening. Throughout the study process, compliance with the four basic principles of ethics was guaranteed: *respect for persons, beneficence, non-maleficence and justice.* Confidentiality was maintained at all times. The protocol was approved by the Ethics Committee of the European University of the Atlantic, with approval recorded in Act CE-63 dated May 7, 2021.

RESULTS

A total of 1304 newborns were screened for this study, of whom 709 (54.37%) were male and 595 (42.63%) were female, all born at the Roosevelt Hospital in Guatemala City. Table 1 describes the main characteristics of the population according to sex, birth weight and type of feeding.

The weight ranges in which most of the NBs are found are from 6.3 to 7.2 pounds with a total of 481 (36.89%), then from 5.3 to 6.2 with 305 (23.39%) and finally from 7.3 to 8.2 pounds with 282 (21.63%). If we unify a range from 5.3 to 8.2 pounds, the total number of newborns is 1068 (81.90%). 1.38% of this study were sieved below 3.3 pounds [< 2.2 pounds (0.23%), 2.3 to 3.2 pounds (1.15%)]. Regarding the type of feeding at the time of sampling, 1165 (89.34%) NBs were breastfed, 83 (6.36%) were receiving mixed feeding, and 19 (1.46%) were receiving parenteral feeding.

Features	Total n (%)
Sex	
Male	709 (54.37)
Female	595 (45.63)
Birth weight	
< 2.2 lbs.	3 (0.23)
2.3 a 3.2	15 (1.15)
3.3 a 4.2	45 (3.45)
4.3 a 5.2	93 (7.13)
5.3 a 6.2	305 (23.39)
6.3 a 7.2	481 (36.89)
7.3 a 8.2	282 (21.63)
> 8.3	80 (6.13)
Type of power supply	
Maternal	1165 (89.34)
Artificial	37 (2.84)
Parenteral	19 (1.46)
Mixed	83 (6.36)

Table 1. Characteristics of newborns screened at Roosevelt Hospital.

Regarding the characteristics related to pregnancy and delivery. Regarding gestational age, 698 (53.53%) were born in weeks 39-40, 371 (28.45%) were born in weeks 37-38, together representing 81.98% and 11.65% were born under 36 weeks of gestation. Regarding the type of delivery 788 (60.43%) were born by cesarean section and 516 (39.57%) by normal delivery.

In relation to the hour ranges corresponding to the time of sampling. Of which 842 (64.57%) were screened in the range of 24 to 72 hours after birth. A total of 206 (15.80%) were screened before 24 hours of birth.

The reference values of the laboratory tests were calculated based on the distribution of the data. The mean and median of each test of the basic NT panel were calculated: Neonatal TSH, 1.92 and 1.39, 17 hydroxyprogesterone, 5.61 and 4.84, Immunoreactive Trypsinogen, 19.54 and 16.40, Total Galactose, 1.89 and 1.70, and Phenylalanine, 1.14 and 1.10, respectively. The data were different, so it is inferred that the population has a non-Gaussian distribution, as shown in Figure 1, where the graph is not symmetrical.

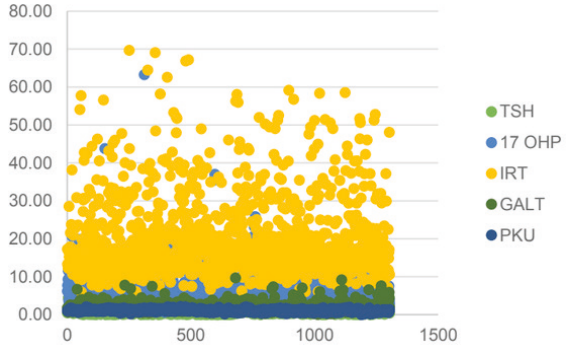

Figure 1. Scatter plot of basic Neonatal Screening panel tests.

TSH: thyroid stimulating hormone, 17 OHP: 17 hydroxyprogesterone, IRT: immunoreactive trypsinogen, GALT: total galactose, PKU: phenylalanine, phenylketonuria screening test.

The following tables show the statistical calculation of the mean and percentiles. The 99th, 99.5th and 99.9th percentiles were calculated for each of the values of the 5 basic panel tests taking as reference each of the characteristics presented in the previous tables.

Features	Media	99th percentile	99.5th percentile	Percentile 99.9*.
	1.92	7.69	8.58	9.93*
Sex				
Male	2.04	7.65	7.96	9.59
Female	1.78	7.66	9.57	10.08
Birth weight				
< 2.2 lbs.	1.85	2.37	2.38	2.38
2.3 a 3.2	1.52	7.22	7.60	7.90
3.3 a 4.2	1.34	5.10	5.13	5.15
4.3 a 5.2	1.79	6.23	8.21	9.80
5.3 a 6.2	1.95	7.94	9.54	9.72
6.3 a 7.2	1.96	7.40	7.73	8.76
7.3 a 8.2	1.97	7.68	8.02	9.48
> 8.3	1.92	6.88	7.94	8.80
Type of power supply				
Maternal	1.94	7.81	9.10	9.96
Artificial	1.71	5.71	5.80	5.87
Parenteral	1.59	3.77	3.84	3.90
Mixed	1.77	5.54	6.32	6.94
Weeks of gestation				
< 28	2.09	2.09	2.09	2.09
29-30	1.60	2.56	2.57	2.58
31-32	1.13	4.25	4.33	4.39
33-34	1.41	7.63	8.71	9.57
35-36	1.75	7.25	7.56	7.81
37-38	1.95	6.40	7.86	7.97
39-40	1.94	7.81	9.54	10.05
> 40	2.11	7.40	7.48	7.54
Type of delivery				
Cesarean section	1.56	6.13	7.53	9.13
Normal	2.47	8.14	9.66	10.09
Time of sampling				
< 24 hours	3.47	9.48	9.57	10.07
24 to 72 hours	1.84	6.55	7.46	9.81
3 to 7 days	0.92	3.03	3.26	3.68
> 7 days	0.94	3.07	3.56	4.02

Percentiles for the reference value of **Neonatal TSH** (IUI/mL)

*99.9 percentile chosen because of its similarity to the current reference value (12.4uIUI/mL).

Features	Media	99th percentile	Percentile 99.5*.	99.9th percentile
	5.61	17.79	20.70	41.74
Sex				
Male	5.87	20.08	24.44	44.68
Female	5.29	14.52	16.82	28.36
Birth weight				
< 2.2 lbs.	18.80	35.01	35.20	35.36
2.3 a 3.2	11.94	34.72	35.86	36.77
3.3 a 4.2	9.12	46.84	55.07	61.65
4.3 a 5.2	6.28	17.61	18.26	18.77
5.3 a 6.2	5.42	14.60	15.95	18.00
6.3 a 7.2	5.50	15.82	20.46	33.91
7.3 a 8.2	5.05	12.06	12.42	13.72
> 8.3	4.44	11.28	11.44	11.57
Type of power supply				
Maternal	5.73	18.52	21.15	42.68
Artificial	4.44	10.45	10.57	10.67
Parenteral	4.08	9.92	9.96	9.99
Mixed	4.71	13.09	13.30	13.46
Time of sampling				
< 24 hours	8.52	23.08	25.83	40.13
24 to 72 hours	5.26	14.56	16.36	21.27
3 to 7 days	4.42	20.18	25.11	33.34
> 7 days	4.39	14.46	35.65	57.77
Weeks of gestation				
< 28	15.80	15.80	15.80*	15.80
29-30	22.52	36.71	36.86*	36.97
31-32	16.82	59.47	61.38*	62.92
33-34	9.84	32.17	33.79*	35.08
35-36	6.41	16.46	17.13*	17.67
37-38	5.65	15.61	19.03*	36.18
39-40	5.16	13.21	14.45*	20.28
> 40	4.46	9.37	11.23*	12.73
Type of delivery				
Cesarean section	4.89	14.50	15.85	21.94
Normal	6.69	20.70	30.62	53.26

Percentiles for the reference value of **17-hydroxyprogesterone** (ng/mL)

*99.5 percentile chosen because of its similarity to current reference values and selected by week of gestation.

Features	Media	99th percentile	Percentile 99.5*.	99.9th percentile
	19.63	57.79	63.54*	70.40
Sex				
Male	19.74	57.70	67.04	73.47
Female	19.49	56.90	58.76	67.03
Birth weight				
< 2.2 lbs.	15.10	21.72	21.81	21.88
2.3 a 3.2	18.28	46.50	47.80	48.84
3.3 a 4.2	17.12	41.18	41.84	42.37
4.3 a 5.2	19.33	45.88	52.54	57.87
5.3 a 6.2	20.21	66.80	68.11	69.52
6.3 a 7.2	19.55	54.60	58.44	73.00
7.3 a 8.2	19.93	56.06	57.55	67.24
> 8.3	18.97	57.07	57.59	58.00
Type of power supply				
Maternal	19.57	57.91	62.96	69.60
Artificial	18.62	47.11	48.85	50.25
Parenteral	15.58	23.43	23.46	23.49
Mixed	21.80	55.86	63.28	69.22
Time of sampling				
< 24 hours	21.55	62.43	68.94	79.83
24 to 72 hours	19.26	54.89	58.34	67.50
3 to 7 days	18.97	46.92	48.91	50.98
> 7 days	19.66	66.07	68.30	69.42
Weeks of gestation				
< 28	12.90	12.90	12.90	12.90
29-30	14.30	16.65	16.68	16.70
31-32	18.86	33.51	33.61	33.68
33-34	17.49	37.59	38.54	39.31
35-36	18.07	51.23	55.13	58.39
37-38	19.20	61.17	67.49	77.62
39-40	20.12	56.61	58.25	70.00
> 40	20.45	55.32	59.91	63.58
Type of delivery				
Cesarean section	19.12	56.08	58.41	69.91
Normal	20.40	59.06	67.03	75.66

Percentiles for the reference value of **immunoreactive Trypsinogen** (ng/mL)

*99.5 percentile chosen because of its similarity to the current reference value (60.0 ng/mL).

Features	Media	99th percentile	99.5th percentile	Percentile 99.9*.
	1.89	6.20	7.00	8.78*
Sex				
Male	1.89	5.28	6.38	7.16
Female	1.89	6.91	7.50	9.40
Birth weight				
< 2.2 lbs.	1.87	3.46	3.48	3.50
2.3 a 3.2	1.39	2.24	2.27	2.29
3.3 a 4.2	1.90	5.40	5.75	6.03
4.3 a 5.2	1.91	6.31	6.96	7.47
5.3 a 6.2	1.91	4.88	6.08	6.81
6.3 a 7.2	1.95	7.16	7.68	9.46
7.3 a 8.2	1.74	4.42	4.50	5.08
> 8.3	2.06	5.48	6.19	6.76
Type of power supply				
Maternal	1.87	6.15	7.15	8.97
Artificial	2.52	5.62	5.91	6.14
Parenteral	2.76	6.51	6.56	6.59
Mixed	1.76	4.17	4.34	4.47
Time of sampling				
< 24 hours	1.72	4.49	4.89	6.49
24 to 72 hours	1.74	4.56	4.98	6.73
3 to 7 days	2.49	7.68	8.37	9.43
> 7 days	2.53	7.57	8.29	9.02
Weeks of gestation				
< 28	0.70	0.70	0.70	0.70
29-30	1.75	2.49	2.49	2.50
31-32	1.46	2.27	2.29	2.30
33-34	1.80	4.16	4.33	4.47
35-36	1.77	3.69	4.19	4.60
37-38	1.96	6.84	7.52	8.92
39-40	1.87	6.60	6.90	8.22
> 40	2.00	5.19	5.40	5.56
Type of delivery				
Cesarean section	1.92	6.60	6.94	8.10
Normal	1.84	5.56	6.98	8.52

Percentiles for the reference value for **total Galactose** (mg/dL)

*99.9 percentile chosen because of its similarity to the current reference value (10.0 mg/dL).

Features	Media	99th percentile	99.5th percentile	Percentile 99.9*.
	1.14	1.90	2.00	2.17*
Sex				
Male	1.14	1.90	2.10	4.11
Female	1.13	1.90	1.90	2.08
Birth weight				
< 2.2 lbs.	0.93	1.29	1.30	1.30
2.3 a 3.2	1.10	1.79	1.79	1.80
3.3 a 4.2	1.08	2.01	2.06	2.09
4.3 a 5.2	1.10	1.62	1.71	1.78
5.3 a 6.2	1.17	2.00	2.05	6.90
6.3 a 7.2	1.14	1.90	2.02	2.15
7.3 a 8.2	1.16	1.80	1.80	1.80
> 8.3	1.07	1.92	1.96	1.99
Type of power supply				
Maternal	1.15	1.90	2.00	2.10
Artificial	1.13	6.26	7.63	8.73
Parenteral	0.89	1.38	1.39	1.40
Mixed	1.03	1.82	1.86	1.89
Time of sampling				
< 24 hours	1.27	1.90	1.90	2.06
24 to 72 hours	1.15	1.90	2.00	2.12
3 to 7 days	1.06	1.80	3.96	7.99
> 7 days	0.94	1.60	1.64	1.69
Weeks of gestation				
< 28	1.30	1.30	1.30	1.30
29-30	1.45	2.09	2.09	2.10
31-32	1.16	1.87	1.89	1.90
33-34	1.13	2.03	2.07	2.09
35-36	1.18	1.80	5.29	8.26
37-38	1.11	1.83	1.93	2.10
39-40	1.15	1.90	1.95	2.06
> 40	1.14	1.80	1.80	1.80
Type of delivery				
Cesarean section	1.11	1.90	1.90	3.57
Normal	1.18	1.99	2.10	2.15

Table 6. Percentiles for the reference value of **Phenylalanine** (mg/dL)

*99.9 percentile chosen because of its similarity to the current reference value (5.0 mg/dL).

Neonatal screening test	Original reference value	Proposed reference value
TSH	<12.4 IUI/mL	< 10 uIU/mL
17 hydroxyprogesterone		> 40 SMG - < 11.23 ng/mL
< 3.3 lbs	< 57.7 ng/mL	39 - 40 SMG - < 14.45 ng/mL
3.3 to 5.5 lbs	< 34.5 ng/mL	37 - 38 SMG - < 19.03 ng/mL
> 5.5 lbs	< 19.7 ng/mL	35 - 36 SMG - < 17.03 ng/mL
		33 - 34 SMG - < 33.79 ng/mL
		< 32 SMG - < 61.38 ng/mL
Immunoreactive trypsinogen	< 60.0 ng/mL	< 63.54 ng/mL
Total galactose	< 10.0 mg/dL	< 8.78 mg/dL
Phenylalanine	< 5 mg/dL	< 2.17 mg/dL

Table 7. Original reference values and proposed reference values

SMG = Week of gestation

DISCUSSION

The general objective of the study was to develop a proposal for the reference values of the basic neonatal screening panel in order to optimize resources at the Roosevelt Hospital in Guatemala City, since the laboratories that currently process neonatal screening tests in Guatemala use the reference values proposed by the parent company for the reagents used, but they are calculated for a population different from the Guatemalan population.

The reference values are calculated based on the distribution of the population, in the case of the NBs screened at Roosevelt Hospital had a non-Gaussian distribution, so the reference values were calculated based on the percentiles.

Factors influencing NT outcomes are diverse, including gestational age, birth weight and mode of nutrition. Preterm NBs (birth with a gestational age less than 36 weeks and birth weight below 3.3 pounds) are metabolically different from healthy term NBs, 1.38% of this study were screened below 3.3 pounds (Table 1) and 11.65% were born below 36 weeks of gestation, their stratification is important since they are more predisposed to suffer from metabolic diseases (2).

Nutrition is a factor to be considered in the measurement of total phenylalanine and galactose. 89.34% have breastfeeding, 2.84% and 1.46% have artificial and parenteral feeding, respectively (Table 1); nutrition is a dynamic interaction in which it provides carbohydrates, amino acids, among others; the way it is provided is important, as it is evident how it is used for energy and growth, and how each nutritional component is metabolized (2).

Regarding the time of sampling, 64.57% were screened between 24 and 72 hours, NT should be performed between 24 and 48 hours, according to international regulations. A 15.80% were screened before 24 hours after birth, so the sensitivity of the tests may be reduced at this time of sampling (3).

Neonatal screening tests are not designed to be diagnostic, so all results that are altered or outside the reference value should be confirmed with diagnostic tests, confirming the suspected disease and considered a true positive. If diagnostic tests are found to be normal, it excludes the suspected disease from screening and is considered a false positive. False negative is considered when an individual has the disease and their screening results are normal (4).

In neonatal screening, reference values indicate that the test is normal (no confirmatory testing is required) or abnormal (confirmatory testing is required). For obtaining the reference value for neonatal screening tests, the

99th percentile is used to set the target high value in order to obtain low false positive and negative rates (4).

In several countries the reference values for the TN of Congenital Hypothyrodism (CH) have been reestablished from 20-25 IUI/mL to 6-10 IUI/mL, thus doubling the incidence of CH (5). In this study, the 99.9th percentile was chosen as it is the closest to the upper limit of the proposed range. The reference value was established without taking into account the factors that influence the test, 9.93 IUIU/mL (Table 2), which is lower than the current value of 12.4 IUI/mL.

Regarding sex, it is 9.59 IUIU/mL for males and 10.08 IUI/mL for females. Heather et al. (2019) indicate that TSH values in NBs present differences with respect to sex, males present lower TSH values compared to females (6).

Table 2 also shows a difference between the reference value for preterm NBs weighing less than 3.3 lb and those weighing more than 4.3 lb. Preterm NBs below 3.3 lb, their reference value was 2.38 to 7.90 IUI/mL, compared to NBs with birth weight above 4.3 lb which was between 8.76 to 9.80 IUI/mL. The reference values for births below 36 weeks gestation were 2.09 to 9.57 IUI/mL, and above 36 weeks were 7.97 to 10.05 IUI/mL.

Screening for CH in premature newborns and newborns from twin births has a high risk of false positives and false negatives, since in this population there is an immaturity of the hypothalamic-pituitary-thyroid axis, a lower capacity for synthesis and metabolism of thyroid hormones, high and low levels of iodine, presence of critical illnesses and use of medications due to being in intensive care units, resulting in a late elevation of TSH (7).

Extreme preterm newborns, considered as newborns born under 2.2 pounds and under 27 weeks of gestation, resolve their thyroid immaturity after the 4th week of life. The reference value calculated for this population was 2.38 uIU/mL and 2.09 uIU/mL, respectively, which allows false negatives if the sample is taken between 24 and 48 hours (8).

For the type of delivery, the TSH reference values were 9.13 IUIU/mL in the case of cesarean delivery and 10.09 IUI/mL in normal delivery. In some hospital centers iodine antiseptics are used in cesarean deliveries and may be a cause of elevated TSH in the NB; however, in this research it was not established whether iodine antiseptics are used in the hospital (9).

In the type of feeding, there was a difference between maternal and artificial and parenteral feeding. The reference value for maternal feeding was 9.96 IUI/mL, 5.87 IUI/mL for artificial feeding and 3.90 for parenteral feeding; mixed feeding, maternal and artificial, was 6.94 IUI/mL. Iodine is found in a variety of foods, one of the richest sources being breast milk; in 2004, Governmental Agreement 29-2004 was approved, regulating the fortification of salt with iodine and salt with iodine and fluoride, reducing iodine deficiency in the population (10, 11).

Regarding the time of sampling, the reference value when the sample is obtained before 24 hours is 10.07 IUI/mL, between 24 and 72 hours is 9.81 IUI/mL and over 72 hours is between 3.68 to 4.02 IUI/mL. During the first 24 hours there is a marked peak of TSH, but it can persist up to 72 hours, so there is a difference in the time of sampling (12).

The 17 hydroxyprogesterone (17 OHP) is another test of the basic NT panel used for the suspicion of Congenital Adrenal Hyperplasia, however there are several challenges in the calculation of the reference value due to its high false positive rate and low positive predictive value, the test is influenced by various perinatal factors, so international standards dictate establishing the reference value in terms of weeks of gestation or birth weight, thus improving the detection of CAH, the values in terms of percentiles 99, 99.5 and 99.9 are 17.79 ng/mL, 20.70 ng/mL and 41.74

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ng/mL, respectively (Table 3) establishing that the 99.5 percentile is selected as the reference standard, since it is similar to the current reference values (13).

For birth weight, the reference value less than 3.2 lbs is 35.86 ng/mL, from 3.3 to 4.2 lbs is 55.07 ng/mL, from 4.3 to 7.2 lbs is 15.95 to 20.46 ng/mL and greater than 7.3 lbs is 11.44 ng/mL. For newborns born at less than 28 weeks gestation (WGS) the reference value was 15.80 ng/mL, from 29 to 30 WGS was 36.86 ng/mL, from 31 to 32 WGS was 61.38 ng/mL, from 33 to 34 WGS was 33.79 ng/mL, from 35 to 38 WGS was 17.13 ng/mL and greater than 40 WGS was 11.23 ng/mL.

Preterm NBs have high 17 OHP concentrations due to immature adrenal function and delayed maturation of enzymes involved in the glucocorticoid pathway, as well as renal and hepatic failure, fetal stress and amniotic fluid infections. As for the reference value of 3.3 to 4.2 lb it is higher compared to NBs less than 3.2 lb, likewise those born between 31 to 32 SMG are higher than those born under 30 SMG and 33-34 SMG, which may occur because most of the preterm newborns at the time of sampling were hospitalized in different services of the HR, seriously ill newborns or those with severe postnatal cardiopulmonary disease raise the values of 17 OHP, however, this cause was not investigated in this study (13, 14).

The reference values for sex were 24.44 ng/mL and 16.82 ng/mL for males and females, respectively. In the study by Gonzales et al. (2013), 10 799 RN samples were analyzed, and they found that 17 OHP levels in females were statistically lower than males, so it is important for the estimation of reference values because of possible false negatives in females (15).

The type of feeding does not affect the reference values of 17 OHP, there is little research in this regard, however, our values were for maternal feeding 21.15 ng/mL, 10.57 ng/mL and 9.96 ng/mL for artificial and parenteral feeding.

Regarding sampling, the reference value for newborns screened before 24 hours is 25.83 ng/mL, from 24 to 72 hours is 16.36 ng/mL, from 3 to 7 days is 25.11 ng/mL and after 7 days is 35.65 ng/mL. In a study by Hayashi et al, they analyzed 271,810 newborns, indicating that they had a greater number of false positives when the sample was taken between 24 and 72 hours of life, in comparison to samples taken after 72 hours, in several countries the sample is taken between the 3rd and 7th day of life, in order not to harm the diagnostic precision (16).

Regarding the type of delivery, the reference value in cesarean delivery was 15.85 ng/mL and 30.62 ng/mL in normal or vaginal delivery. In the study by Gonzales et al. (2013), 17 OHP concentrations were statistically different in those born vaginally and those born by cesarean delivery, with a slight increase in values in those born by cesarean delivery (15). The type of delivery is important to take into account since stress can occur during pregnancy, affecting the hypothalamic-pituitary-adrenal axis, increasing the levels of 17 OHP, which occurs in normal delivery. Some cesarean deliveries occur after labor or when complications occur, transiently increasing 17 OHP values (14,15,17).

Immunoreactive trypsinogen is elevated in patients with suspected Cystic Fibrosis, due to the obstruction of pancreatic canaliculi, which are responsible for metabolizing the IRT component. The reference values for this marker are chosen based on the 99.5th percentile due to its proximity to the current reference values, and it is 63.54 ng/mL (Table 4). It is worth mentioning that significant differences in TRI values have been found between healthy and carriers, with a tendency to a higher TRI concentration in carrier NBs, which was not the objective of this study (18, 19).

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The reference values for IRT in terms of sex are 67.4 ng/mL for males and 58.76 ng/mL for females. Several studies have found elevated TRI values in women compared to men, but without statistical significance (20).

With reference to weight, those who weighed less than 2.2 pounds, their reference value is 21.81 ng/mL, from 2.3 to 4.2 ng/mL 47.8 ng/mL, those from 4.3 to 5.2 lb 52.54 ng/mL, those from 5.3 to 6.2 lb 68.11 ng/mL and those over 6.3 lb, 58.44 ng/mL. Preterm NBs had a reference value ranging from 12.90 to 55.13 ng/mL, those from 37 to 38 SMG 67.49 ng/mL and from 39 SMG onwards 59.91 ng/mL. Preterm and extreme preterm NBs have significantly high values of IRT, which was not evident in this study (21).

Breastfed NBs had a baseline value of 62.96 ng/mL, formula-fed NBs had 48.85 ng/mL and parenterally fed NBs had 23.46 ng/mL. It has been established that formula-fed or parenterally fed NBs have elevated IRT values since they are related to having some inherent pathology; however, in this study there was no evidence of an increase (20).

In terms of sample collection, those less than 24 hours were 68.94 ng/mL, from 24 to 72 hours 58.34 ng/mL, from 3 to 7 days 48.91 ng/mL and greater than 7 days 68.30 ng/mL. IRT levels remain constant until day 20 of life, then it drops significantly, which was not evident in this study since samples taken after 7 days are similar to samples taken before 24 hours (20).

Normal deliveries had a reference value of 67.03 ng/mL and cesarean deliveries had a reference value of 58.41 ng/mL. As for normal deliveries, stress and perinatal asphyxia can occur, these situations elevate IRT values, which was evidenced in this study (22).

Another of the diseases that make up the basic NT panel is Galactosemia, in which total galactose is quantified; the reference value was chosen based on the 99.9th percentile due to its proximity to the current reference value and was 8.78 mg/dL (Table 5) (23) (Table 5).

The reference values for sex were 7.16 mg/dL for males and 9.40 mg/dL for females. In reference to weight and weeks of gestation, for preterm NBs with a weight less than 3.2 lb was 3.50 mg/dL, from 3.3 to 6.2 lb was 7.47 mg/dL, from 6.3 to 7.2 lb was 9.46 mg/dL and greater than 7.3 lb was 6.76 mg/dL. In relation to weeks of gestation, for those with less than 32 SMG was 2.50 mg/dL, from 33 to 36 SMG 4.60 mg/dL, from 37 to 40 SMG 8.92 mg/dL and greater than 40 SMG 5.56 mg/dL. The reference value for normal deliveries was 8.52 mg/dL and 8.10 for cesarean delivery.

Regarding the type of feeding, those fed by breastfeeding have 8.97 mg/dL, 6.14 mg/dL those fed artificially and 6.59 mg/dL those fed parenterally. Regarding the time of sampling, those taken before 72 hours have a reference value of 6.73 mg/dL and after 3 days 9.43 mg/dL. In the study by Marrero-Gonzalez et al. suggest a slight increase in total galactose values with respect to the age of the NB, and found a decrease in NBs more than 168 hours old. In the case of patients with classic Galactosemia, it is not affected by the time of sampling if galactose-1-phosphate activity is measured since it is detectable in cord blood before milk intake. In this study, total galactose was quantified, which requires lactose or galactose intake, in order to detect the different conditions that occur in hypergalactosemia (24).

Finally, phenylketonuria is another disease of the basic NT panel detected through phenylalanine and is one of the most common Inborn Errors of Metabolism worldwide. Currently a reference value of 5 mg/dL is used, but worldwide more adjusted reference values are used, several NT programs use reference values higher than 2.0 mg/dL, and in Latin America values between 3.0 to 4.0 mg/dL are reported (25), so a reference value calculated with the 99.9th percentile, 2.17 mg/dL, was chosen (Table 6).

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The reference values for sex are 4.11 mg/dL for males and 2.08 mg/dL for females. No significant sex differences have been reported, what may occur is the distribution in other factors that affect the phenylalanine value. There are studies with adult population in which they report variations of phenylalanine in terms of sex, finding that in men there are elevated values of some amino acids, among them phenylalanine, however, there are no data for the neonatal population, and in this study a higher reference value was reported in the male sex (26).

In reference to weight, the reference value for preterm NBs weighing less than 3.2 lb is 1.80 mg/dL, between 3.3 to 5.2 lb 2.09 mg/dL, from 5.3 to 6.2 lb 6.90 mg/dL and greater than 6.3 lb is 2.15 mg/dL. As for weeks of gestation, preterm NBs with less than 34 SMG was 2.09 mg/dL, 35 to 36 SMG 8.26 mg/dL and greater than 37 SMG 2.10 mg/dL. In a study by Reese et al, they evaluated whether gestational age affected the values of 15 amino acids in preterm infants, among them phenylalanine, and found that between 23 and 26 weeks, elevated phenylalanine levels were present (2).

In breastfed NBs the reference value was 2.10 mg/dL, in those fed artificially 8.73 mg/dL and parenterally 1.40 mg/dL. In a study by Perko et al. found cases of NBs fed with parenteral diet in which phenylalanine levels were increased producing false positives; phenylalanine values are affected by nutrition, since dynamic interaction between carbohydrates and proteins/amino acids provided, the way it is provided (parenteral or artificial), the way it is used and the metabolized nutritional component influence values (27). Several artificial foods or milk formulas are constituted with supplements rich in essential amino acids, increasing the concentration of the amino acid in blood compared to those fed only with breastfeeding (2,26,27).

There was a difference in the type of delivery, those born by normal delivery the reference value was 2.15 mg/dL and 3.57 mg/dL for cesarean delivery.

The time of sampling, was evaluated in the same way as the other tests of the basic NT panel, the reference value for the sample taken before 24 hours was 2.06 mg/dL, between 24 and 72 hours 2.12 mg/dL, from 3 to 7 days 7.99 mg/dL and after 7 days 1.69 mg/dL. Reese et al. estimated phenylalanine values at different times of sampling, indicating that they found abnormal phenylalanine values on day 7, however, they were preterm NBs, similarly Sanchez in his doctoral thesis reported elevated phenylalanine levels in samples taken after 7 days, which does not agree with our study, since the elevated values were evidenced when the sampling was carried out between the 3rd and 7th day of life. This is possibly due to the fact that the newborns in this group had a low degree of maturity and needed special medical care (2, 26).

Table 7 shows the proposed reference values for the basic neonatal screening panel at Roosevelt Hospital. The TSH value was chosen based on the 99.9th percentile (<10 IUI/mL), close to the upper limit of the proposed range, lower than that currently used (<12.4 IUI/mL). The 17 OHP values were chosen based on the 99.5th percentile and were classified by gestational age, since perinatal factors influence these values. The IRT value was chosen by the 99.5th percentile (< 63.54 ng/mL), because of its closeness to current values (< 60.0 ng/mL). For the total galactose value, the 99.9th percentile was chosen (< 8.78 mg/dL), due to its proximity to the current reference value (< 10.0 mg/dL). For the phenylalanine value, the 99.9th percentile was chosen (< 2.17 mg/dL), since several Latin American countries use adjusted values, between 3.0 and 4.0 mg/dL (2, 13, 25).

Finally, several studies indicate the importance of each country that has a neonatal screening program to establish cut-off points for its population. Neonatal screening in Guatemala has had several difficulties, the main one being that there is no law guaranteeing nationwide screening with a national coverage of 5% (28), so it is only performed in the metropolitan area, which is a major limitation in this study.

This study opens future lines of research for the adjustment of the reference values for the different areas of the country with the purpose of reducing the cases of false positives and false negatives, reducing the anxiety of parents and supporting the timely and accurate approach to the detected endocrine-metabolic disease. Likewise, the data obtained in this study are subject to evaluation, allowing future adjustments based on the experience of the program.

CONCLUSION

The proposed reference values for the neonatal TSH, Total Galactose and Phenylalanine tests are slightly lower than the values indicated by the manufacturer; for immunoreactive Trypsinogen the proposed value is slightly higher, while for 17-hydroxyprogesterone reference values are proposed that take into account the gestational age at birth. The proposed values show the importance and need to make adjustments to the reference values of the basic neonatal panel currently available in our hospital, based on characteristics of the Guatemalan population, such as sex, birth weight, type of feeding, weeks of gestation and type of delivery, since these are factors that influence these values.

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