Research Article

The application of indirect methods to establish and verify Argentinian paediatric thyroid function test reference intervals

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Article Info

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Keywords
thyroid function, reference ranges, paediatrics patients, indirect method, thyroid hormones

Abstract

The aim of this study was to establish and verify reference intervals for thyroid function tests using indirect methods in a paediatric hospital in Argentina. TSH and thyroid hormones were collected from a total of 19,822 patients. TSH, TT3 y FT4 were measured by Architect i4000 (Abbott) and TT4 by Immulite 2000 XPi (Siemens). Following the implementation of a series of filters to identify pathologies affecting the thyroid profile, a total of 5,871 cases remained in the database. Group A: 0-12 months (n=551). Group B: 13 months to 7 years old (n=2357). Group C: 8 to 18 years old (n=2963). Reference intervals were calculated by Hoffmann-Katayev and by CLSI28-A3. Verification was performed using the CLSI28-A3 protocol, as well as evaluated by being compared with the RCV. Reference intervals for TSH and thyroid hormones were established using Hoffmann–Katayev and CLSI28-A3 methods, showing overall agreement. CLSI28-A3 verification confirmed RIs for TSH and TT4 across all groups. For FT4 and TT3, only CLSI-derived RIs were consistently verified, while HK showed limited performance in younger groups. Comparison with published data using RCV demonstrated overall acceptable agreement, with some deviations in early ages. According to the verification procedure, suitable reference intervals calculation methods were Hoffmann–Katayev for TSH and TT4, and CLSI28-A3 for FT4 and TT3.Having reference intervals established for our patient population enhances the quality of results, improves their interpretation and safeguards patient safety. Indirect methods found to be good alternatives when a healthy population is not easily accessible.

Introduction

A test result in isolation, without accompanying information, is of limited use and fails to provide meaningful insights to the attending physician. Accurate interpretation requires the specification of units, methodology, brand of the assay and an appropriate reference interval (RI).

The concept of RI encompasses a substantial proportion of the reference population, the central 95% of the distribution, between $P_{2.5}$ and $P_{97.5}$. This leaves 5% of the reference population outside this range. There are four methods accepted by ISO 15189 norms for establishing RIs, in increasing order of strength: direct methods, indirect methods, bibliographic references, and the RI provided by the manufacturer [1,2]. All these RIs must be verified in the population to which they are to be applied.

Thyroid function is critical in several processes throughout life, especially in paediatrics. Depending on the severity and age of thyroid disease onset, early neurocognitive development and growth can be seriously affected [3]. Laboratory tests are a fundamental tool in diagnosing and monitoring thyroid disease. In addition to newborn screening, routine paediatric thyroid function screening is not clearly defined in current clinical care guidelines. The 2018 guide by the European Thyroid Association recommends screening for central hypothyroidism by testing for free thyroxine (FT4) and thyrotropin (TSH) in children with familial antecedents and/or failure to thrive, developmental delay, growth hormone (GH) deficiency, delayed or precocious puberty, or other hypothalamic–pituitary defects or lesions [3,4]. In previous research conducted by our group, the effectiveness of a full thyroid profile versus TSH plus FT4 in screening for thyroid disorders in paediatric patients was compared. The combination of TSH and FT4 has been demonstrated to be an effective method for the screening of thyroid disease in paediatric patients [5].

Therefore, TSH and thyroid hormones (THs) are the most frequently requested tests in the endocrinology laboratory of a high-complexity paediatric hospital for the evaluation and follow-up of thyroid disorders. They represent approximately 49% of all requests. TSH and free thyroxine (FT4) are particularly important, as they are essential for screening for thyroid disease in children and adolescents [5].

TSH and THs demonstrate significant variability, primarily due to age and, according to some authors, due to sex after puberty, as well as methodology and the specific brand of reagents used. Furthermore, there is a large amount of existing biological variation and inter-population differences [6]. Evident differences in methodological shows, even within the same methodology, among various reagents brands are highlighted in external quality assessment reports.

Specific populations exhibit distinct thyroid homeostasis. For instance, obese children have been found to have significantly higher TSH values than non-obese children [7-9]. One study demonstrated that substantial weight loss led to a significant decrease in TSH levels without the need for thyroid-specific treatment [8].

Consequently, it is essential for each laboratory to determine its own RIs, according to the population to be applied. Meanwhile, the establishment of an RI using direct methods is particularly problematic. The establishment of laboratory-specific direct RIs requires significant investment in reagents and specialized human resources, which is a limiting factor for several laboratories. Direct methods involve selecting a representative number of reference subjects, typically a minimum of 120 for each age and sex group, depending on the specific analyte requirement. Additionally, the availability of suitable reference individuals and the quantity of blood sample required are both significant considerations, particularly in a tertiary care paediatric hospital

In order to achieve the required number of individuals for each age/sex partition, a healthy volunteer program involving an interdisciplinary team is necessary. The establishment of RIs by direct methods for several analytes, including TSH and THs, was achieved through the CALIPER programme by Bailey et al [10]. They found a high variance in THs reference interval at birth, which significantly decreases by the first year of life and then remains constant. Absolute concentrations decline with age, except for total thyroxine (TT4), which decreases during childhood and surges between 14 and 18 years. Significant sex differences were also found in the adolescence group for TT4 and triiodothyronine (TT3). Regarding TSH, they demonstrate high variance at birth, this variance decreases gradually as the analyte levels increase with increasing postnatal age. These findings served as reference to constitute partitioning groups, in that study [10].

An alternative to direct methods is to use indirect ones, with a large data pool base of results. It has been proposed by some authors that the analysis of thousands of results, including those from pathological subjects, is statistically more robust than that of only 120 assuming healthiness subjects [11].

Indirect methods constitute a variant of data mining whereby a substantial database undergoes rigorous filtration, resulting in considerably lower costs when compared with direct methods. Hoffmann indirect method, which was developed in 1963, is the most widely applied [12,13]. Hoffmann assumed that the data would follow a Gaussian or near-Gaussian distribution, but this is a significant limitation in the field of endocrinology. The majority of hormones do not have a normal distribution.

In 2010, Katayev put forward a computerized modification to the Hoffmann method [11]. The present modification is an attempt to reduce the subjectivity associated with RI calculations and enhance reproducibility by establishing a logical progression. After a thorough and meticulous database filtration procedure, outliers are eliminated. Following this, the data is analysed using a cumulative frequency chart.

The methodology entails the iterative removal of the distal portions of the graph until a satisfactory linear approximation is attained. The RI is determined through the extrapolation of the 2.5th and 97.5th percentiles of the cumulative frequency plot on

the concentration axis.
The calculated RI must undergo verification in order to confirm its validity and reliability. Katayev proposes the implementation of reference change values (RCV) as a statistical significance indicator, in comparison with RI as previously documented in the bibliography [11].
The Clinical and Laboratory Standards Institute (CLSI) has published a recommendation for a RI verification algorithm in the CLSI28-A3 guide [14]. This algorithm involves the recruitment of 20 subjects selected from the target population, who are required to be free of any underlying conditions that could affect the analyte. In instances where no more than two patients fall outside the RI, the RI is considered confirmed and suitable for clinical application. In the case of three or four results falling outside a second verification step, identical to the first, this step is repeated. When the number of individuals exceeding the RI limits is five or more, the RI is not appropriate to the target population [15].

Objective

The aim of this study was to establish and verify reference intervals for thyroid function tests using indirect methods in a paediatric tertiary care hospital in Argentina.

Material and methods

Assay Methods

TSH, TT3 and FT4 were performed using chemiluminescent microparticle immunoassay (CMIA) by Architect i4000 (Abbott Ireland Diagnostics Division, Longford, Ireland), while TT4 was tested using chemiluminescent immunoassay by IMMULITE© 2000XPi (Siemens Healthineers, Llanberis, UK). The serum samples collected in gel tubes were processed on the same day as their collection.

Database

The data for TSH and THs were collected from 19,822 patients of J.P. Garrahan Paediatric Hospital between January 2020 and December 2021. In cases where multiple values were available for the same analyte in the same patient, only the oldest result was selected for inclusion in the analysis.
In order to avoid the inclusion of patients affected by non-thyroidal illness syndrome, all inpatients were excluded from the database. Exclusion criteria included prior diagnosis of thyroid disease (thyroiditis, thyroid cancer, hypothyroidism, or hyperthyroidism) and previous thyroidectomy. The general laboratory data available was subjected to evaluation in order to identify patients with possible abnormal thyroid profiles. This was achieved by filtering out patients with abnormal liver function tests and/or a pathological hematology panel, as well as those with positive anti-thyroid antibodies results, using as positive the manufacturer-reported cutoff values of anti-thyroid peroxidase antibodies >35 IU/ml and/or anti-thyroglobulin antibodies >40 IU/ml [16, 17]. The reference intervals applied for filtering in this study, for both the haematology panel (haematocrit and haemoglobin) [18, 19] and the liver function panel [20] were those previously established and verified for routine clinical use at our institution. Following the curation of the initial 19,822 patients' data, a total of 5,871 remained in the database for the purposes of further analysis.

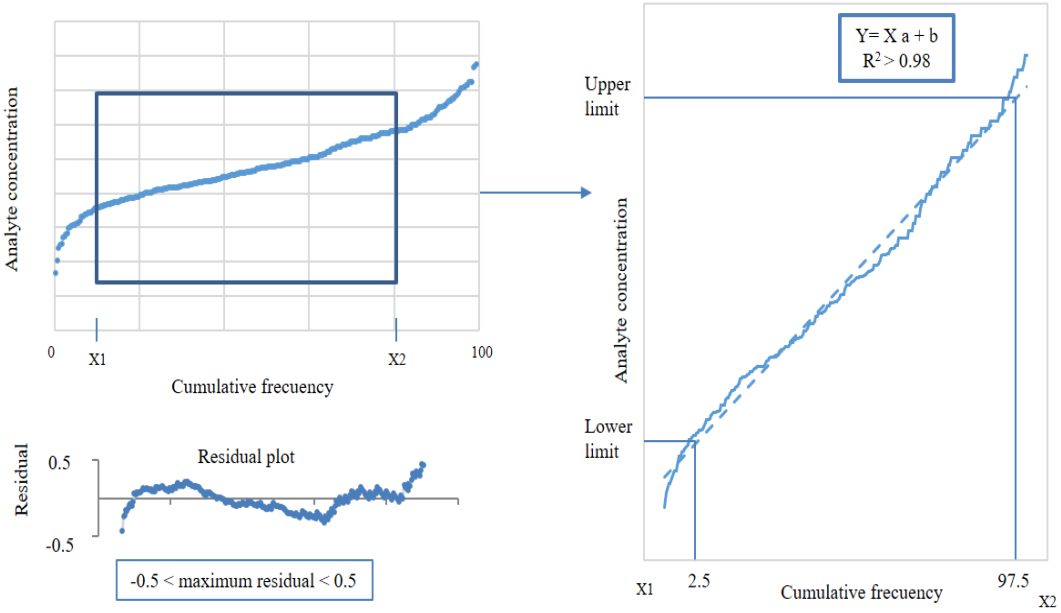
Partitions group

A total of 5871 patients were divided into three groups for the purposes of the study. Group A comprised infants aged 12 months and under (n = 551, female: 43.4%). Group B included children aged 13 months to 7 years old, inclusive (n =2357, female: 49.9%). Group C contained children from 8 to18 years old (n = 2963, female: 56.9%).

Indirect methods analysis

RIs were calculated in each group applying two methods. Firstly, the Hoffmann-Katayev adapted method (HK) was employed [11]. This method involved graphing cumulative frequency versus test value without transformation. Outliers were identified using Tukey's method and were excluded [21]. Then, the extremes were trimmed to improve the linear regression within an acceptable margin of error. The following rules were applied: A $r^2 \geq 0.98$ was considered adequate and the maximum absolute residual do not exceed 0.5. Upon fulfillment of both criteria, the trimming process was halted (Figure 1). After that, the RIs were obtained by extrapolating the values from the linear regression of the 2.5th and 97.5th percentiles.
In the second method, RIs were calculated using percentiles according to the procedure recommended for non-parametric analytes by the Clinical and Laboratory Standards Institute (CLSI) (CLSI28-A3) [14].

Figure 1: Calculation scheme for RIs using the Hoffmann-Katayev method.



RI verification

A total of 20 independent serum samples from children corresponding to each age group with presumably normal thyroid function, who had undergone pre-surgical laboratory testing, were used to verify the calculated RI. A specialist was assigned to review the subjects' clinical records with a view to excluding any pathologies or medications that might have an impact on thyroid function. The presence of outliers was detected and subsequently excluded by Tukey method [21]. The processing of the data was conducted in accordance with the guidelines stipulated in CLSI EP28-A3c [14].

Assessment of clinical relevance (comparison test with RCV)

In addition, as a comparative exploration, the calculated RIs were compared to those previously published by Bailey et al. as part of the CALIPER project. (CALG) [10] and by Bokulić et al. (CROG) [22] both were obtained by direct method. The percentage of difference was calculated for each comparison and then compared to the RCV defined as: RCV = $\sqrt{2}$ x Z x $\sqrt{(CVa^2 + CVi^2)}$. Where Z is the probability selected by significance is 1.96 for p=0.05. CVa is the inter-assay coefficient of variation and CVi within–subject biological variation from the biological variation table. For this purpose, the RCV was used as the maximum accepted variation when comparing the lower and upper limits of the RI of each group and calculation methodology.

In instances where the comparison age groups do not correspond to those that have been established in this study, a comparative analysis was conducted with all RIs that include age groups G1, G2, and G3.

Results

Obtained TSH and THs RIs are shown in Table 1. The percentage of values trimmed to obtain the RIs using the HK method was as follows: TSH: 14.6%, 15.2%, and 10.3%; FT4: 8.4%, 10.0%, and 7.7%; TT3: 5.7%, 12.5%, and 8.9%; TT4: 18.0%, 14.3%, and 9.4%, for Groups A, B, and C, respectively. Both methods yielded similar RIs, lower and upper limits showed a difference of less than 20%, except for TSH at upper limit in group A and B, where significant differences were observed, although the RCV was exceeded only in group A (Table 1). Calculated upper limits by HK were lower than those obtained by CLSI28-A3, the opposite occurred with lower limits (Table 1).

Table 1: Reference interval calculated by indirect methods.

	group	Hoffmann-Katayev		CLSI28A3 (90% confidence interval)		Relative difference % Hoffmann-Katayev vs CLSI28A3	
		Lower limit	Upper limit	Lower limit	Upper limit	Lower limit	Upper limit
TSH (µUI/ml)							
0 - 12 m	A	0.68	4.48	0.56 (0.51-0.70)	8.26 (7.50-8.65)	19.4	-59.3
13 m - 7 y	B	0.85	4.84	0.80 (0.75-0.87)	6.15 (5.93-6.34)	6.1	-23.8
8 - 18 y	C	0.76	4.27	0.72 (0.69-0.79)	5.17 (5.09-5.26)	5.4	-19.1
FT4 (pmol/l)							
0 - 12 m	A	10.81	15.83	10.42 (10.04-10.68)	17.25 (16.60-17.50)	3.7	-8.6
13 m - 7 y	B	11.33	15.06	10.04 (9.78-10.30)	17.25 (16.99-17.37)	12.0	-13.5
8 - 18 y	C	10.55	14.29	9.91 (9.90-10.04)	15.96 (15.83-16.09)	6.3	-11.1
TT3 (nmol/l)							
0 - 12 m	A	1.84	3.56	1.70 (1.60-1.75)	4.07 (3.92-4.16)	7.8	-13.3
13 m - 7 y	B	1.64	3.12	1.52 (1.49-1.55)	3.59 (3.52-3.61)	7.8	-14.2
8 - 18 y	C	1.41	2.73	1.17 (1.14-1.27)	3.04 (2.98-3.07)	19.0	-10.6
TT4 (nmol/l)							
0 - 12 m	A	84.94	162.16	78.51 (74.65-82.37)	191.76 (184.04-196.91)	7.9	-16.7
13 m - 7 y	B	83.66	146.72	72.07 (70.40-73.10)	158.30 (154.44-160.88)	14.9	-7.6
8 - 18 y	C	77.22	132.56	65.64 (64.86-66.92)	142.86 (140.28-144.14)	16.2	-7.5

Verification using the CLSI protocol showed that TSH and TT4 were approved for all groups, calculated HK, CLSI28-A3 RIs and previously published RIs. In the case of FT4, the RI calculated by CLSI28-A3 and those previously published by CALG [10] and CROG [22] were verified for all groups. However, the RI calculated by HK was not verified for any group. For TT3, the RIs calculated by CLSI28-A3 were verified for all groups. However, this was not the case for RIs calculated by HK in G1 and G2. For the G3 group, all RIs were verified. When examining the TSH values calculated by HK RIs using RCV, an acceptable percentage deviation was found compared to the published values by CALG and CROG. In the case of FT4, the RIs calculated using both methods were comparable with CROG, with the exception of G1, where the percentage difference compared to the group was greater, in the following age group 2 to 15 days.

In the instance of TT3, the RIs calculated by HK were acceptable in G2 and G3. However, when we compared the RIs obtained by CLSI28-A3 with the published values, some differences exceeded the RCV%, as is the case with FT4 in G1, where the difference was greater compared to the group from 2 to 15 days of the CROG and were unsuccessful when comparing with CALG, TSH in G1 and TT3 in G2, where there is a disagreement with the group from 6-14 of the CROG that overlaps only one year with G3 group (Table 2). All of the RIs comparisons were successful for TT4 when compared with the manufacturer's published values.

Table 2: Comparison test with RCV: differences compared with the high and low levels reported in the literature.

Group	Analite	CROG					CALG				
		ARG	Lower limit		Upper limit		ARG	Lower limit		Upper limit	
			% HK	% CLSI 28-A3	% HK	% CLSI 28-A3		% HK	% CLSI 28-A3	% HK	% CLSI 28-A3
G1 0-12m	TSH	2d-<2m	30.5	11.3	-37.4	23.2	4d-<6m	-7.1	-26.4	-6.3	53.6
		2m-<14y	-16.2	-35.3	-2.9	56.7*	6m-<14y	-2.9	-22.2	7.2	65.8*
	TT3	2d-<1y	59.5*	52.3*	-0.9	12.4	4d-<1y	35.3*	27.7*	-0.9	12.4
	FT4	2d-<15d	-9.1	-12.7	-55.3*	-47.3*	5d-<15d	-22.2	-25.8*	-89.2*	-82.2*
		15d-<1y	-4.7	-8.3	-17.8	-9.2	15d-<30d	21.1	17.4	-69.1*	-61.5*
							30d-<1y	-5.8	-9.4	-32.1*	-23.7*
G2 13m-7y	TSH	2m-<14y	6.1	0.0	4.9	28.6	6m-<14y	19.4	13.3	14.9	38.4
	TT3	1y-<6y	-2.8	-10.5	7.7	21.8	1y-<12y	-5.5	-13.2	7.1	21.3
		6y-<14y	16.2	8.4	10.9	25.0*					
	FT4	1y-<19y	8.3	-3.8	-5.0	8.6	1y-<14y	-1.1	-13.2	-15.7	-2.2
G3 8-18y	TSH	2m-<14y	8.2	2.8	-7.7	11.5	6m-<14y	8.2	2.8	2.4	21.4
		14y-<19y	47.2	42.0	17.0	35.8	14-<19y	47.2	42.0	22.4	41.0
	TT3	6-<14y	1.1	-18.0	-2.2	8.4	1-<12y	-20.5	-39.2*	-6.0	4.7
		14-<19y	7.9	-11.2	17.1	27.6*	12-<15y	-6.3	-25.3*	1.1	11.8
	FT4	1y-<19y	1.2	-5.1	-10.3	0.8	1-<19y	-8.2	-14.5	-21.0	-10.0

*RCV: TSH:55.5%, FT4:21.4% and TT3:23.5%. ARG: Age range of the comparison group.HK: Hoffmann Katayev method. *The difference exceeds the RCV.*
CALG: Bailey et al. [10] and CROG: Bokulić et al. [22].

In accordance with the findings derived from the verification process, Table 3 presents the most suitable TSH and THs RIs for the paediatric population under study.

Table 3: Reference intervals for TSH and thyroid hormones selected on the basis of the verification results to be applied.

	Lower limit	Upper limit	Source
TSH (µUI/ml)			
0 - 12 m	0.68	4.48	Hoffmann-Katayev
13 m - 7 y	0.85	4.84	
8 - 18 y	0.76	4.27	
FT4 (pmol/l)			
0 - 12 m	10.42	17.25	CLSI28-A3
13 m - 7 y	10.04	17.25	
8 - 18 y	9.91	15.96	
TT3 (nmol/l)			
0 - 12 m	1.70	4.07	CLSI28-A3
13 m - 7 y	1.52	3.59	
8 - 18 y	1.17	3.04	
TT4 (nmol/l)			
0 - 12 m	84.94	162.16	Hoffmann-Katayev
13 m - 7 y	83.66	146.72	
8 - 18 y	77.22	132.56	

Discussion

Obtaining specific RIs for the target population with the available equipment represents a constant challenge for paediatric endocrinology laboratories. In this study, RIs were obtained using the indirect method with two alternatives, Hoffmann and CLSI. An accessible alternative was found, as it does not require a large investment in human or economic resources.

A large database comprising 19,822 patients was analysed, and following a rigorous filtration process, this was reduced to 5,871. The large number of patients, presumed to be free of thyroid disease, lends strength to the RIs obtained. In addition, the processing of data over a two-year period mitigates seasonal variations, changes in reagent batches and sampling materials, consequently reducing pre-analytical and analytical variations.

The intervals obtained were verified to determine whether they could be used for our population. To this end, the CLSI-recommended method was used, which yielded favourable results for all RIs obtained for TSH and TT4. For TT3 and FT4, the RIs calculated by CLSI28-A3 were verified, but not those calculated by HK, as they were found to be very narrow. This outcome can be attributed to the calculation procedure, which was developed with the objective of minimizing error.

Furthermore, in order to evaluate the RIs obtained, the RCV was applied as a parameter to test the clinical relevance of the RIs obtained with respect to those previously published in the literature, calculated by the direct method, determining the percentage difference. With regard to TSH, the results obtained were favourable, with the exception of the upper limit in G1. In this case, the publications utilized for comparative analysis implemented a distinct categorization of age ranges, including patients up to 14 years of age within the same category as those below 1 year of age, and their upper limits were considerably lower than our RI calculated by CLSI28-A3; the one calculated by HK was verified. In addition, TSH exhibits greater variability, with a RCV of 55.5%, which is reflected in the presence of significant differences at the upper limit of the reference range. Numerous publications have documented the phenomenon of TSH variability, even when the same measuring method or analytical platform is analysed.

Analysing the FT4 data, it was found that the comparison test with RCV was successful in comparison to CROG, with the exception of infants younger than 15 days, as this group was not separated during the process of this study. In TT3, the comparison test with RCV demonstrated that the RIs calculated by HK exhibited less discrepancy with those previously published than those calculated by CLSI28-A3.

The principal aim of this study is summarized in Table 3, which details the RIs that were found to be appropriate for the study population. These RIs were based on the results obtained using the CLSI recommended verification method and the comparison test with RCV.

Limitations

Obtaining a processable database from a tertiary care institution's system proved to be challenging. The filtering of a large database, although restrictive, may still yield a set of abnormal values; however, this is something that the indirect method already takes into account in its calculation. However, certain parameters must be established to standardize the calculation, such as the method for excluding outliers or the acceptable error in the linearity of the HK curve, which determines how far to trim the tails. It is important to establish a method that ensures the RI calculation is not dependent on the operator's experience.

In order to verify the RIs using CLSI protocol, we encountered significant challenges in obtaining samples from children without any medical conditions or treatments that might affect their thyroid profile. This was due to the fact that the hospital is a tertiary care facility and the volume of paediatric samples is limited.

The sample size within the under-1-year-old group was insufficient to permit further subgroup stratification; this resulted in significant differences, particularly in FT4, when compared with previously published data from CALG and CROG, which provide reference intervals for infants under 15 days of age. Certain precautions should be taken when using the reference intervals published in this study for infants younger than 15 days. Future studies will focus on this age group in order to reach the number of subjects required to allow further subdivision, especially within the 0–12-month range, where neonatal and early infancy thyroid physiology changes rapidly.

The recruitment of 20 healthy subjects for Group A to the verification process proved to be a significant challenge. Apparently, endocrine-healthy children under 13 months of age do not require laboratory analysis. In order to achieve the necessary sample size, assistance was requested from a nearby paediatric hospital, Elizalde Hospital. The samples were also processed on the same equipment used to obtain the results for calculating the reference intervals (RIs). Despite the similarity in terms of demographics between the two patient populations, the conditions treated at Elizalde Hospital are less complex. This constitutes a limitation of the study, as identical patient populations were not used to verify the RIs for Group A, since it was not possible to obtain samples from infants under one year of age with no apparent thyroid condition.

Significant differences in THs in adolescents by biological sex. have been shown in previous studies, such as those published by Bailey et al. [10] and by Campbell et al [23]. To investigate further, a Mann-Whitney test was conducted, which revealed significant differences between males and females in the 12 to 18 age group for both TT3 and TT4. Due to the design of the database, distinguishing between the two groups to obtain the RIs is beyond the scope of this study.

Conclusions

Having reference intervals established for our patient population, under the same pre-analytical and analytical conditions as those used in our daily work, enhances the quality of results, improves their interpretation and safeguards patient safety.

Thus, indirect methods have been found to be good alternatives when a healthy population is not easily accessible. However, since these methods involve some subjective decision points, it is important to carry out a thorough verification before they are applied.

Author Contributions

Veronica E. Zaidman: Conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing - original draft, writing - review & editing, visualization and project administration.

Melina B. Pelanda: Validation, investigation, resources and writing - review & editing.

Noelia Dujovne: Investigation, resources and writing - review & editing.

Natalia Gazek: Investigation, resources and writing - review & editing.

Claudia Insua: Investigation, resources and writing - review & editing.

Rodolfo E. Recalde: Validation, investigation and writing - review & editing.

Florencia Zabaleta: Validation, investigation and writing - review & editing.

Eduardo A. Chaler: Conceptualization, methodology, formal analysis, investigation, resources, data curation, writing - review &editing, supervision and project administration.

Juan M. Lazzati: Conceptualization, methodology, investigation, resources, data curation, writing - review &editing, supervision and project administration.

Author's Disclosures

The authors declare no conflicts of interest for the conduction of the study. The corresponding author is responsible for ensuring that the descriptions are accurate and agreed upon by all authors.

Ethics Approval

This study utilised an anonymised patient database. Consequently, it did not require approval from the ethics committee. The research was conducted in accordance with the Declaration of Helsinki and national and institutional standards.

Data Availability Statement

The databases analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

IA Statement

During the preparation of this work the authors used DeepL IA in order to improve grammar and clarify language. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the published article.

Funding Statement

The authors received no financial support for the research, authorship, and/or publication of this article.

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