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ORIGINAL ARTICLE

Reference Intervals of Serum TSH from Mixed Distributions Using Truncation Points and the Kolmogorov-Smirnov Distance

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SUMMARY

Background: **Serum TSH reference intervals (RIs) are methodology, population, and age specific. However, the ethical and practical challenges restrict the establishment of pediatric RIs using conventional approaches and advocates the use of indirect data mining-based algorithms. This study was carried out to estimate the reference interval of neonatal serum TSH in Pakistani population using an indirect approach.**
Methods: **A data mining of serum TSH results of neonates (≤ 1 month of age) from 2013 - 2018 was done. Two subgroups on the basis of age from birth to 5 days and 6 - 30 days were assessed. The German study group's pre-validated indirect algorithm 'KOSMIC' was utilized for the statistical analysis.**
Results: **A total of non-duplicate 82,299 neonatal serum TSH tests were retrieved over a period of 6 years, including 88% (n = 70,788) aged 0 - 5 days and 12% (n = 11,511) ranging from 6 days to 1 month. The estimated RIs for the first age partition was 0.7 (90% CI 0.6 - 0.8) to 15.5 (90% CI 12.9 - 16.2) and for the second group 0.7 (90% CI 0.5 - 0.9) to 7.8 (90% CI 6.1 - 9.9) µIU/mL.**
Conclusions: **This study revealed age related trends in serum TSH. The study advocates the need for population specific RIs owing to the significant variations noted on comparison with previously published literature. Precise RIs become vital particularly when serum TSH is undertaken as a confirmatory test for presumptive positive results on newborn screening for congenital hypothyroidism.**
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INTRODUCTION

In most clinical scenarios, decision making regarding the diagnosis and therapeutics to be followed rely on biochemical test results, supplemented with precise reference intervals (RIs). The RIs in turn are dependent on broad ranging variables from the type of analyzers and reagents utilized, methodology adopted, the population concerned, and the vitality of the quality control protocol of the laboratory [1]. Moreover, adequate representation of the age dependency of laboratory analytes and partitioning of RIs is of utmost importance. This be-

comes more important in the neonatal age group, as physiological advances following birth can influence biochemical framework [2]. With regard to good laboratory practices, self-establishment of RIs by laboratories has become a key element in laboratory medicine [3,4]. Congenital Hypothyroidism (CH) is a commonly occurring cause of avertible intellectual deficit in newborns with an overall incidence of 1 in 3,000 newborn infants [5,6]. Timely screening and prompt confirmation for CH has been proven to be effective [7,8]. Literature from Pakistan on the prevalence of CH is scarce; however, in a study, Raza et al. reported that CH was found in three babies of 1,337 screened [9]. Another study also reported a similar incidence of one case per 1,000 newborns which is substantially higher compared to Caucasian data [10]. Vigorous alterations in thyroid physiology occur in the neonatal period. There is a rapid surge of thyrotropin-releasing hormone and TSH, beginning at 30 minutes following delivery, followed by a declining level within RIs by the third to fifth day of age. [11] Moreover, age based RIs for serum TSH are necessary to complement newborn screening as a confirmatory test following a positive result.

Most of the clinical laboratory regulatory/accreditation bodies, as well as scientific and clinical societies recommend that RIs should be derived from a predefined group of healthy subjects from a specific population relevant to the analyte of interest [12]. Moreover, a minimum of 120 individuals after prior screening are required to establish RIs using validated statistical analysis as recommended by the clinical laboratory standards institute (CLSI) [13]. However, as these conventional approaches are often hindered by ethical and practical limitations, data mining of laboratory systems with an indirect methodology is being increasingly utilized.

In Pakistan, population specific RIs for most biochemical analytes including serum neonatal TSH have not been widely established. The RI in-use by most laboratories in the country are mainly obtained from published studies, textbooks or clinical guidelines centered across European or American population; hence, in most instances they are misleading in the local scenario. Therefore, this study was undertaken to estimate age specific neonatal RIs for serum TSH and assess them against published literature to evaluate the probability of population-based differences.

MATERIALS AND METHODS

Study design, setting

After exemption by the institutional ethical review committee (AKU-ERC# 2020-5626-14494), all the serum TSH results with date of collection from neonates aged up to 1 month for both genders over a 6-year period from 2013 to 2018 were extracted from Aga Khan University's (Karachi, Pakistan) clinical laboratory information management systems database. To study the age dynamics, the dataset was further stratified into 0 - 5 days and 6 - 30 days. The diversified dataset comprised of both diseased and normal cases. Samples obtained during clinical care from hospitalized subjects as well as those subjected to routine screening and outside referrals performed regardless of the clinical indication were included. In case of multiple test request forms for the same individual, only the first sample's results were included in the final analysis.

Serum TSH was analyzed by Chemiluminescence immunoassay (CLIA) on ADVIA® Centaur™, Siemens platform. The laboratory performs to the best of standards for both internal and external quality assurance and is accredited by the College of American Pathologists (CAP). The assay range for the ADVIA Centaur TSH-Ultra assay (third generation) is from the limit of quantitation i.e., 0.008 to 150 μIU/mL.

Statistical analysis

A previously validated and proposed tool by Zierk J et al., the Next-Generation Pediatric Reference Intervals, Kolmogorov-Smirnov based reference intervals (KOSMIC), was utilized for the data analysis [14]. The built-in algorithm functions upon diminishing the variations due to the estimated parametrical distribution and truncation, using the Kolmogorov-Smirnov-distance followed by a Box-Cox-transformation. A statistical program which is implemented within a software package [https://kosmic.diz.uk-erlangen.de/] is available to calculate the Box-Cox transformation parameter lambda (λ), the truncation interval, and the parameters of the Gaussian distribution Mu (μ) and sigma (σ). This procedure is based on the notion that the proportion of physiological samples in the input dataset can be modelled using parametric distribution, subsequently a truncation interval T exists within the dataset in which the proportion of abnormal test results is negligible. Moreover, to project the distribution of non-pathological test results, the lower and upper truncation limits, i.e., T1 and T2, were determined using a "Brute Force" approach.

Fine grained details of the statistical analysis utilized are available from Zierk J et al. [14]. To estimate confidence intervals, bootstrapping of the input dataset was undertaken. Subsequently, the lower and upper reference intervals with their 90% confidence interval (CI) were derived for the two age groups.

In addition, a comparison of our derived serum neonatal TSH with the values recommended by the Mayo clinical laboratory and Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) was also done [15, 16].

RESULTS

A total of non-duplicate 82,299 neonatal TSH tests were retrieved spanning a six-year time frame. A large proportion (88%; n = 70,788) were aged ≤ 5 days of life and 12% (n = 11,511) belong to the age group ranging from 6 days to 1 month. A nearly identical distribution

Table 1. Calculation of RIs for the two age groups.

	0 - 5 days	6 - 30 days
N:	70,788	11,511
2.5% Percentile:	0.682834	0.695444
50% Percentile:	3.41007	2.73867
97.5% Percentile:	15.452	7.83943
λ:	0.04	0.22
μ:	1.25733	1.12785
σ:	0.834677	0.753547
T1:	1.8	1.3
T2:	6.2	4
Decimals:	1	1
T1 min:	0.05	0.05
T1 max:	0.3	0.3
T2 min:	0.7	0.7
T2 max:	0.95	0.95
SD:	0.8	0.8
Tolerance:	1e-07	1e-07

Table 2. Comparison of lower and upper RIs for serum TSH (μIU/mL) with published literature.

Age group	Our study		Mayo Clinical Lab (15)		CALIPER (16)	
	LRI	URI	LRI	URI	LRI	URI
0 days to 5 days	0.7 (90% CI 0.6 - 0.8)	15.5 (90% CI 12.9 - 16.2)	0.7	15.2	3.2	19.0
6 days to 30 days	0.7 (90% CI 0.5 - 0.9)	7.8 (90% CI 6.1 - 9.9)	0.7	11.0	1.7	9.1

LRI: lower reference interval, URI: upper reference interval.

of in-house (n = 42,968) and outside referrals (n = 39,331) was noted, the majority being males (n = 43,275). Owing to the distribution of results as depicted in the histogram shown in Figure 1, first the cumulative density is computed. Using an optimization process ("Brute Force search" optimization), the lower and upper truncation limits (T1 and T2 and the parameters μ and σ), were derived as depicted in Table 1 from which the distribution of non-pathological test results can be projected.

The respective RIs with their 90% CI for each age group are depicted in Table 2 alongside a comparison with the Mayo Clinical Laboratories USA and CALIPER study to illustrate the differences between the indirect and direct conventional approaches.

DISCUSSION

A pivotal element of preventive health programs is newborn screening (NBS) [17]. Among the various disorders screened at birth globally, CH is almost exclusively part of all such programs [17]. However, like most underdeveloped countries there is no national NBS program at the state level, where the utmost focus remains on infectious diseases [18]. Our center became the first in the country to offer NBS for CH using dried blood spot samples via spectrofluorometric analysis for TSH. However, all presumptive positive screens require confirmation via serum sample analysis on a CLIA platform. For clinical laboratories, provision of population specific RIs scrutinized by covariates, most importantly age, is a daunting yet essential task to aid clinicians. Moreover, there is a high need in our scenario of age

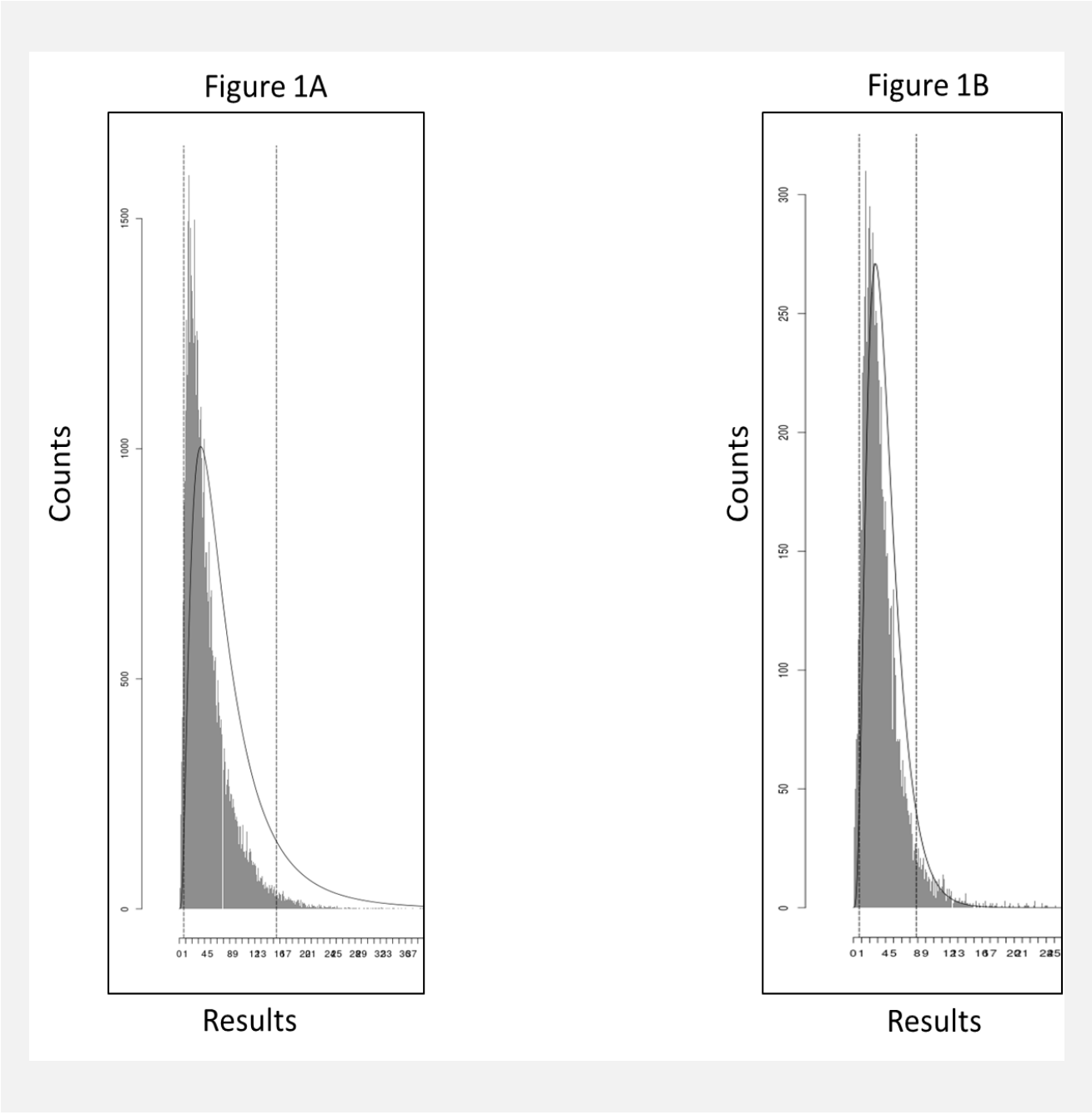

Figure 1. Estimated distribution of physiological test results for age group 0 - 5 days (1A) and 6 - 30 days (1B).

specific neonatal TSH RIs to serve the laboratory's CH NBS regimen.

On the other hand, owing to the ethical, practical, and financial constraints of establishing RIs in this tender neonatal age, most clinical laboratories in Pakistan usually implement cutoffs provided by in-vitro diagnostic companies, books or previously published literature [19]. In most instances, the populations studied are inherently not the same as those served [19]. In our case, even the manufacturer's kit insert lacks information for neonates and the laboratory had to rely on textbook recommendations. According to the available data from Pakistan, this is the first study to disseminate neonatal RIs for serum TSH using an unconventional indirect approach.

Results of the comparisons of the RI obtained in this study with the Mayo lab showed almost similar intervals for the $\leq$ 5 days age group, while the upper limits varied significantly for the second group. Conversely, the CALIPER derived values for both the lower and up-

per limits differed significantly from our estimated data. While assessing the differences between our study and direct approach based RIs from other sources, differences are found to be more prominent in the upper limits, which are deemed as essential cutoffs for CH diagnosis. These findings essentially supplement the fact that RIs established in one population should not be applied to other populations as they ultimately can lead to misdiagnosis and irreversible neurological deficit.

Furthermore, a noteworthy strength of our study was the use of an entirely indirect approach, purposively planned on laboratory data acquired as part of standard care. The indirect approach is convenient for the neonatal age bracket as no additional pricks for sample collections are required, reducing the risk and making it financially feasible as well [20].

Furthermore, the recently modified KOSMIC approach based on utilization of lab data with a high percentage of pathological values alongside varying overlap of normal and pathological distributions have been validated to produce reliable RIs in such perplexing big data analysis. Adeptly, these estimated RIs are thought to remain stable, even when results from high prevalence areas like endocrine specialty units in case of TSH samples are included.

Another potential advantage of the KOSMIC methodology is less time consumption as the statistical analysis is less labor intensive. A novice user with a simple computer can estimate RIs in less than 3 seconds with confidence intervals [14]. Another advantage of this statistical package is that it can be incorporated into laboratory systems via simple programmable interface [14].

The findings of this real-world big data study will serve as an interpretative guide for serum TSH results especially for CH screening programs in Pakistan. The results are also reflective of the importance of the population based RIs, as demonstrated by the variations noted with other studies. Additionally, the RI will be beneficial for most clinical care setups relying on serum TSH for newborn screening or high-risk screening of neonates with suspicion of CH in Pakistan, owing to the absence of wide scale dried blood spot based NBS programs in the country.

CONCLUSION

This study will further advocate the use of the indirect approach for RI derivatization especially in a resource constrained set up. Moreover, these RIs can also be adopted by other clinical laboratories in Pakistan on a broader scale, by appropriate transference protocol to facilitate reporting of neonatal TSH.

Declaration of Interest:
None declared.

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