

Harnessing the Power of Big Data Analytics-Estimation of Reference Intervals for Neonatal Serum TSH in Pakistan Using RefineR Algorithm

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ABSTRACT

Objective: To estimate the population-specific reference intervals (RIs) for neonatal thyroid stimulating hormone (TSH) in Pakistani neonates, utilising the refineR algorithm.

Study Design: Observational study.

Place and Duration of the Study: Department of Pathology and Laboratory Medicine, The Aga Khan University Hospital, Karachi, Pakistan, from 17th May to 30th November 2023.

Methodology: A data mining analysis was conducted on serum TSH results of neonates (≤ 1 month) over a period of six years, following approval from the Institutional Ethical Review Committee. Two subgroups were assessed based on the age as 0 - 5 days and 6 - 30 days. The refineR algorithm was implemented using refineR package (version 1.0.0), ensuring accurate analysis and insights.

Results: A total of non-duplicate 82,299 neonatal serum TSH tests were retrieved, including 70,788 (88%) aged 0 - 5 days and 11,511 (12%) aged ranging from 6 - 30 days. The estimated RI was from 0.67 $\mu\text{IU/mL}$ (90% CI 0.641 - 0.72) to 15.0 $\mu\text{IU/mL}$ (90% CI 13.2 - 17.3) for the first age group and 0.65 $\mu\text{IU/mL}$ (90% CI 0.6 - 0.84) to 8.6 $\mu\text{IU/mL}$ (90% CI 8.05 - 9.71) for the second age group.

Conclusion: Reference intervals for neonatal serum TSH of the Pakistani population were estimated, considering the genetic differences of this demographic in comparison to the Western population. Results aligned with global literature, validating the refineR indirect approach's applicability.

Key Words: Reference intervals, Neonatal, Thyroid stimulating hormone, RefineR algorithm, Big data, Pakistan.

How to cite this article: Muneer S, Ahmed S, Agravatt AK, Siddiqui I. Harnessing the Power of Big Data Analytics-Estimation of Reference Intervals for Neonatal Serum TSH in Pakistan Using RefineR Algorithm. *J Coll Physicians Surg Pak* 2024; **34(07)**:775-779.

INTRODUCTION

Congenital hypothyroidism (CH) emerges as one of the prevailing factors contributing to cognitive impairment in newborns.¹ Timely recognition and intervention through neonatal screening followed by confirmatory testing play a pivotal role in averting irreversible cognitive impairments.²

Estimating reliable and accurate reference intervals (RIs) is fundamental for the precise interpretation of clinical laboratory results and clinical decision-making.³ It is recommended that laboratories adopt RIs specific to their population for each analyte tested. Currently, numerous laboratories in Pakistan use RIs derived from the Caucasian population, either sourced from literature or provided by manufacturers in kit package inserts which are potentially unsuitable for the Pakistani population due to intrinsic population differences.⁴

Furthermore, applying adult or paediatric reference intervals for neonates, particularly concerning thyroid stimulation hormone (TSH), is inappropriate due to the substantial modifications in thyroid physiology during the neonatal phase. A surge in serum TSH levels is observed approximately 30 minutes post-delivery, which gradually diminishes by the third to fifth day of life. This underscores the importance of estimating age-specific RIs for serum TSH levels.⁵ These age-based RIs of TSH play a pivotal role in complementing newborn screening as a confirmatory test following a positive result.

The Clinical and Laboratory Standards Institute (CLSI), along with scientific and clinical societies, endorses the implementation of direct methods in estimating RIs, which involves applying a robust strategy of carefully selecting ≥ 120 healthy reference individuals.⁶ However, this approach is associated with substantial, financial, and logistical challenges, constrained by noteworthy ethical limitations in paediatric applications.⁷

The landscape of medical research has transitioned into a data-centric era. The "Big data" characterises datasets of considerable size beyond the capability of traditional IT software and hardware tools to process, or serve within reasonable timeframes.^{8,9}

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Received: April 05, 2024; Revised: June 12, 2024;

Accepted: June 15, 2024

DOI: <https://doi.org/10.29271/jcpsp.2024.07.775>

Data mining is the process of retrieving potentially valuable insights and knowledge hidden within the vast amounts of incomplete, unclear, and unpredictable data. Access to vast medical databases empowers researchers in medical data mining, fostering expectations for rapid clinical progress.⁹

The trend towards extensive data mining techniques for determining RIs is becoming more pronounced.¹⁰ Diverse data mining algorithms, including refineR, Hoffmann’s method, Bhat-tacharya’s method, and KOSMIC method, are currently being used for the RI estimation.¹¹ This facilitates the derivation of RIs from existing laboratory results encompassing both healthy and diseased populations. Acknowledged for its simplicity and cost-effectiveness, the indirect method stands out as a potentially viable approach for estimating RIs adjusted for variables such as age, gender, and season, making it particularly relevant and feasible in low-income countries such as Pakistan, where efficiency and cost-effectiveness are critical considerations.¹²

The refineR stands out as the most recent addition to the latest generation of algorithms devised for computing indirect RIs.¹³

The objective of this study was to estimate population-specific RIs for neonatal TSH in Pakistani neonates using the refineR algorithm.

METHODOLOGY

This observational study was conducted at the Section of Clinical Chemistry, Department of Pathology and Laboratory Medicine, The Aga Khan University Hospital, Karachi, Pakistan, from 17th May to 30th November 2023, following approval from the Institutional Ethical Review Committee (ERC no: 2023- 8678-25064).

The study involved extracting neonatal serum thyroid stimulating hormone (nTSH) data spanning six years from the Aga Khan University Hospital's laboratory information management systems (LIMS) database. The dataset includes samples from hospitalised subjects, outpatient clinics, outside referrals, and outreach laboratories across Pakistan. All samples, regardless of clinical indication, were incorporated into the analysis. In cases where multiple test request forms existed for the same individual, only the results from the initial sample were considered for the final analysis. To explore age-related dynamics, the dataset was further divided into two groups: 0 – 5 days and 6 – 30 days.

Serum TSH levels were evaluated using chemiluminescence immunoassay (CLIA) on the ADVIA® Centaur™ Siemens platform. The ADVIA Centaur TSH-Ultra assay (third generation) had an assay range from the limit of quantitation 0.008 µIU/mL to 150 µIU/mL. The laboratory adheres to high standards for both internal and external quality assurance and is accredited by the College of American Pathologists (CAP).

The refineR algorithm was implemented using the refineR package (version 1.0.0) and its associated functions, getRI and resRI, to estimate the RIs of TSH. The refineR algorithm employs a three-step inverse modelling strategy. Initially, the algorithm identifies the parameter search region and principal peak. In the subsequent optimisation phase, a multi-level grid search is used

to estimate the optimal model parameters including λ (power parameter), σ , μ , and the 'P' scaling factor. This optimisation aims to estimate the optimal model characterising the underlying data. Ultimately, refined indices (RIs) can be derived from the optimal model at the minimal cost.

RESULTS

For each age group, Table I displays the RIs, and Figure 1 (A and B) illustrates the distribution of test results for both groups. Furthermore, a comparative analysis is presented in Table II and Table III to highlight the differences between the direct and indirect approaches. The refineR indirect approach was compared with the conventional direct approaches employed by Roche and the Caliper study in estimating RIs. Additionally, a comparison was drawn with the indirect approach for estimating RIs using KOSMIC and MedCalc by Ahmed *et al.*^{14,15}

Table I: Reference intervals for the two age groups.

	0-5 days	6-30 days
Number of subjects	70,788	11,511
2.5% percentile	0.67 µIU/ml	0.65 µIU/ml
97.5% percentile	15.0 µIU/ml	8.6 µIU/ml
Lambda	0.0637	0.236
Mu	1.28	1.19
Sigma	0.854	0.821

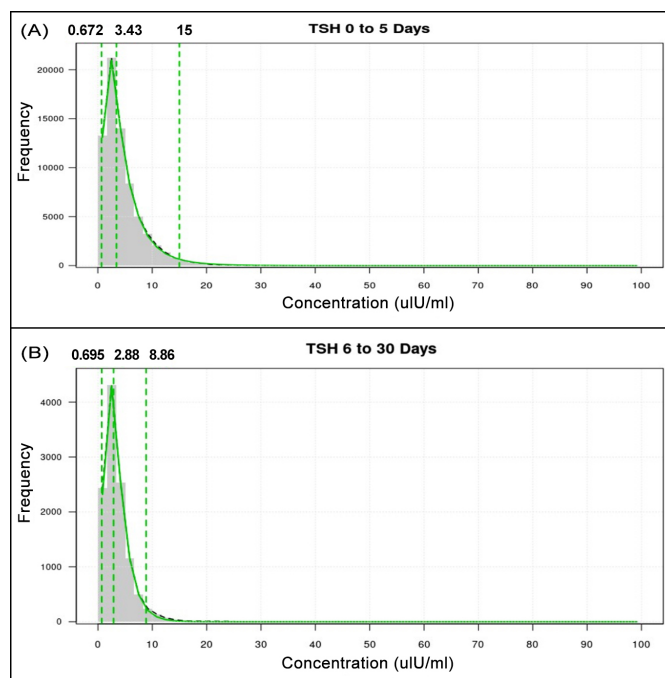


Figure 1: (A) Distribution of test results for age group 0 - 5 days (B) 6 - 30 days.

DISCUSSION

Neonatal screening (NBS) initiatives play a vital role in preventive healthcare, serving as an extensive system to reduce morbidity and mortality and enhance the health outcomes of newborns. The screening for CH in every newborn is crucial due to its favourable benefit-to-risk ratio, especially when cost-effective treatments are available. Failure to identify and treat CH within the first weeks of birth can lead to cognitive impairment.¹⁶

Table II: A comparison between the reference intervals for serum TSH levels ($\mu\text{IU/mL}$) estimated through the refineR indirect approach and the RIs derived from the direct approach in the foreign literature.

	This study using refineR		Roche RIs		Caliper RIs Roche Cobas	Caliper RIs Ortho Vitros 5600	Caliper RIs Expert consensus
Age group	0-5 days	6-30 days	0- \leq 6 days	>6 days - \leq 3 months	0- $<$ 1 month	0- $<$ 1week	1 week - $<$ 19 years
Sample Size	70,788	11,511	103	119	50	51	600
RI ($\mu\text{IU/ml}$)	0.67-15	0.65-8.6	0.7-15.2	0.7-11.0	1.23-27.2	0.71-31.5	0.86-5.7
							3.2-19.0
							5- $<$ 31 days
							N/A
							N/A
							1.7-9.1

Table III: A comparison of reference intervals for serum TSH levels ($\mu\text{IU/mL}$) estimated through various indirect approaches in the Pakistani population.

Age group	This study using refineR		Ahmed <i>et al.</i> using KOSMIC		Ahmed <i>et al.</i> using MedCalc	
	LRI $\mu\text{IU/ml}$	URI $\mu\text{IU/ml}$	LRI $\mu\text{IU/ml}$	URI $\mu\text{IU/ml}$	LRI $\mu\text{IU/ml}$	URI $\mu\text{IU/ml}$
0 days to 5 days	0.67 (90% CI 0.6-0.7)	15.0 (90% CI 13.2-17.3)	0.7 (90% CI 0.6-0.8)	15.5 (90% CI 12.9-16.2)	0.6 (90% CI 0.58-0.67)	14.9 (90% CI 14.5-15.6)
6 days to 30 days	0.65 (90% CI 0.6-0.84)	8.6 (90% CI 8.1-9.7)	0.7 (90% CI 0.5-0.9)	7.8 (90% CI 6.1-9.9)	0.46 (90% CI 0.35-0.62)	9.1 (90% CI 8.5-10.6)

LRI: Lower reference interval, URI: Upper reference interval.

On a global scale, screening for CH is integrated into newborn care through diverse programmes. However, in Pakistan, there is a noticeable absence of a NBS initiative at the state level, which is a common scenario in many developing nations. Despite the elevated prevalence of CH in Pakistan compared to global rates, most hospitals resort to screening neonates displaying signs and symptoms of CH based on serum TSH levels, as the absence of an NBS programme hinders testing by dried blood spot (DBS) samples in clinical laboratories. The Aga Khan University marked a milestone by becoming the first in the country to introduce NBS for CH using dried blood spot samples analysed *via* spectrofluorometric methods for TSH. Nevertheless, all the presumptive positive screens necessitate confirmation through serum sample analysis on a CLIA platform.^{16,17} Given the reliance on serum TSH levels for the confirmation of CH, the choice of the cut-off holds paramount importance for clinical decision-making. Presently, numerous laboratories in Pakistan rely on RIs derived from global literature, guidelines, or manufacturer-provided kit inserts. At times, even the manufacturer's kit insert lacks information specific to the neonates, compelling the laboratories to depend on textbook recommendations.¹² Therefore, there is a pressing need for age-specific neonatal TSH RIs to complement the laboratory's CH NBS regimen. This study represents a significant contribution, disseminating neonatal RIs for serum TSH through an unconventional indirect approach, based on the available data.

Clinical laboratory regulatory entities recommend estimating RIs using direct methods, involving the careful selection of ≥ 120 healthy individuals.⁶ However, this method presents ethical constraints when applied to neonates. It is also challenging to ascertain the true health status of the neonates. Checking the maternal health status is also essential. RIs estimated by this study have been compared with RIs derived from the direct approach in foreign literature in Table II. Roche estimated RIs on the elecsys system in two groups: 0- ≤ 6 days and >6 days- ≤ 3 months, with sample sizes of 103 and 119, respectively.¹⁸ They enrolled subjects solely based on the historical information related to prior thyroid disorders, family history, and hospitalisation. The RIs

derived from this study were similar to the first age group, but the second age group differed from this study.

Caliper developed RIs for TSH in neonates using the Roche Cobas and Ortho Vitros 5600 systems, categorising them into age groups 0- < 1 month and 0- < 1 week with sample sizes of 50 and 51, respectively.¹⁹ These samples were predominantly collected from outpatient clinics. Notably, the estimated RIs by Caliper differ from this study. However, RIs proposed by Caliper's expert consensus, although not entirely identical, exhibit a certain degree of comparability with RIs estimated by this study.

In addressing the obstacles and constraints associated with the direct approach, this study adopts an alternative method for estimating RIs. This involves extracting data from the existing pool of laboratory results, using the laboratory information system. The Aga Khan University Hospital's clinical laboratory, which serves more than 130 cities across Pakistan through over 300 sample collection units, handles a substantial volume of samples. The prevailing scarcity of expertise and understanding in data science within laboratories, particularly in developing nations such as Pakistan, often leads to underutilisation of the extensive data at hand.

An evaluation of RIs for serum TSH levels estimated using diverse indirect approaches such as refineR, KOSMIC, and medCalc in the Pakistani population reveals that the results from all three methods are nearly comparable.^{14,15} Clinical guidelines propose a serum TSH level of 20 mIU/L as the confirmatory cut-off for CH. If an NBS result for CH is abnormal and the confirmatory serum TSH exceeds 20 mIU/L, treatment initiation is recommended. Conversely, if serum TSH is above the age-specific RI but falls below 20 mIU/L, close monitoring without immediate treatment may be appropriate. Recommendations advise commencing the treatment if TSH of >10 mIU/L persists beyond four weeks of age.^{20,21} The RIs derived from refineR, KOSMIC, and MedCalc, employing diverse indirect approaches, closely correspond to the recommended cut-off in the guidelines. Consequently, the utilisation of these RIs in the Pakistani population ensures that genuine cases of CH are not missed.

Agaravatt *et al.* validated RIs for thyroid hormones in adult patients using Hoffman, KOSMIC, and refineR methods, comparing them with reference ranges provided in kit literature or standard textbooks.²² A similar study by Ma *et al.* employed five indirect algorithms to validate RIs for thyroid hormones in older individuals.²³ Both studies advocate for the adoption of indirect approaches, particularly in resource-constrained nations. The efficacy of these indirect methods, however, hinges on various attributes of the input dataset. Notably, larger sample sizes, around 5000 data points, with a pathological proportion of approximately 30%, have been shown to yield better results.²⁴ Ammer *et al.* proposed that when the proportion of pathological samples exceeds 20%, refineR produces superior results compared to KOSMIC.¹³ In this study, the sample size was 82,299, sufficiently large for producing reliable results. Moreover, since the laboratory receives samples from across the country for neonatal screening of congenital hypothyroidism (CH), as newborn screening (NBS) programmes with dried blood spots (DBS) are not present, the pathological proportion is not expected to be excessively high.

The results of this study will serve as an extensive reference for interpreting serum TSH results, complementing the NBS programme for the confirmation. These findings will have significant implications for various clinical settings that depend on serum TSH levels for newborn screening.

CONCLUSION

The findings of this study indicate that refineR surpasses the direct method when dealing with a reference population of fewer than 120 individuals, making it particularly valuable in estimating age-related reference intervals for the paediatric population. While direct methods are considered more accurate, indirect approaches can still provide valuable insights, especially when resources are limited or when direct methods are impractical due to logistical constraints or ethical considerations.

ETHICAL APPROVAL:

Ethical approval from the Institutional Ethical Review Committee (ERC no: 2023-8678-25064) was obtained before commencing the research work.

PATIENTS' CONSENT:

Not applicable (exemption granted by the institutional ethical review committee).

COMPETING INTEREST:

The research was conducted without any financial support, and the authors affirm that there is no conflict of interest.

AUTHORS' CONTRIBUTION:

SM: Designing, acquiring, analysing data, and drafting the manuscript.

SA: Conceptualising, acquiring, interpreting data, and drafting the manuscript.

AKA, IS: Conceptualising, analysing and interpreting data, and critically reviewing the manuscript.

All authors agreed to be accountable for all aspects of the work, ensuring accuracy and integrity.

REFERENCES

1. Ford G, LaFranchi SH. Screening for congenital hypothyroidism: A worldwide view of strategies. *Best Pract Res Clin Endocrinol Metab* 2014; **28(2)**:175-87. doi: 10.1016/j.beem.2013.05.008.
2. Muneer S, Siddiqui I, Majid H, Jafri L, Humayun KN, Ahmed S, *et al.* Establishing reference interval for thyroid-stimulating hormone in children below two-year ages in Pakistani population. *Ann Med Surg (Lond)* 2021; **68**:102601. doi: 10.1016/j.amsu.2021.102601.
3. Ahmed S, Zierk J, Siddiqui I, Khan AH. Indirect determination of serum creatinine reference intervals in a Pakistani pediatric population using big data analytics. *World J Clin Pediatr* 2021; **10(4)**:72-8. doi: 10.5409/wjcp.v10.i4.72.
4. Ahmed S, Zierk J, Khan AH. Establishment of reference intervals for alkaline phosphatase in pakistani children using a data mining approach. *Lab Med* 2020; **51(5)**:484-90. doi: 10.1093/labmed/lmz096.
5. Segni M. Disorders of the thyroid gland in infancy, childhood and adolescence 2017. In: Feingold KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E, *et al.*, Eds. *Endotext*. South Dartmouth (MA) 2000.
6. Institute CLS. CLSI Document C28-A3: Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline ed. 3rd, Clinical and Laboratory Standards Institute, Wayne, PA, USA; 2008.
7. Zierk J, Arzideh F, Kapsner LA, Prokosch HU, Metzler M, Rauh M. Reference interval estimation from mixed distributions using truncation points and the kolmogorov-smirnov distance (kosmic). *Sci Rep* 2020; **10(1)**:1704. doi: 10.1038/s41598-020-58749-2.
8. Ehrenstein V, Nielsen H, Pedersen AB, Johnsen SP, Pedersen L. Clinical epidemiology in the era of big data: New opportunities, familiar challenges. *Clin Epidemiol* 2017; **9**:245-50. doi: 10.2147/CLEP.S129779.
9. Wu WT, Li YJ, Feng AZ, Li L, Huang T, Xu AD, *et al.* Data mining in clinical big data: The frequently used databases, steps, and methodological models. *Mil Med Res* 2021; **8(1)**:44. doi: 10.1186/s40779-021-00338-z.
10. Ma C, Zou Y, Hou L, Yin Y, Zhao F, Hu Y, *et al.* Validation and comparison of five data mining algorithms using big data from clinical laboratories to establish reference intervals of thyroid hormones for older adults. *Clin Biochem* 2022; **107**:40-9. doi: 10.1016/j.clinbiochem.2022.05.008.
11. Zhong J, Ma C, Hou L, Yin Y, Zhao F, Hu Y, *et al.* Utilization of five data mining algorithms combined with simplified preprocessing to establish reference intervals of thyroid-related hormones for non-elderly adults. *BMC Med Res Methodol* 2023; **23(1)**:108. doi: 10.1186/s12874-023-01898-5.
12. Ahmed S, Agravatt AK, Siddiqui A, Jafri L, Siddiqui I. Indirect reference intervals of serum calcium in pakistani children using KOSMIC, bhattacharya, and hoffman approach. *Clin Lab* 2023; **69(9)**. doi: 10.7754/Clin.Lab.2023.230305.

13. Ammer T, Schutzenmeister A, Prokosch HU, Rauh M, Rank CM, Zierk J. RefineR: A novel algorithm for reference interval estimation from real-world data. *Sci Rep* 2021; **11(1)**:16023. doi: 10.1038/s41598-021-95301-2.
14. Ahmed S, Jafri L, Khan AH, Siddiqui I, Majid H, Ghani F. Establishment of age specific reference intervals of neonatal thyroid stimulating hormone in Pakistani population: Experience at a tertiary care center. *Ann Transl Med* 2017; **5(Suppl 2)**:AB041. doi: 10.21037/atm.2017.s041.
15. Ahmed S, Zierk J, Khan AH, Jafri L, Majid H, Ghani F, et al. Reference intervals of serum TSH from mixed distributions using truncation points and the Kolmogorov-Smirnov distance. *Clin Lab* 2022; **68(7)**. doi: 10.7754/Clin.Lab.2021.210833.
16. Majid H, Ahmed S, Siddiqui I, Humayun K, Karimi H, Khan AH. Newborn screening for congenital hypothyroidism: Improvement in short-term follow-up by audit and monitoring. *BMC Res Notes* 2020; **13(1)**:563. doi: 10.1186/s13104-020-05400-y.
17. Majid H, Jafri L, Khan N, Khan AH. Establishing a nationwide newborn screening program for congenital hypothyroidism: A systematic review. *Pak J Pathol* 2022; **33(3)**:104-8. doi:10.55629/pakjpathol.v33i3.715.
18. Cobas®. Pediatric and adult thyroid test reference intervals for elecsys and cobas e analyzers [28 May 2024]. Available from: <http://www.hormonerestoration.com/files/Thyroid%20Reference%20data%20Roche.pdf>.
19. Adeli K, Higgins V, Trajcevski K, White-Al Habeeb N. The Canadian laboratory initiative on pediatric reference intervals: A CALIPER white paper. *Crit Rev Clin Lab Sci* 2017; **54(6)**:358-413. doi: 10.1080/10408363.2017.1379945.
20. Rose SR, Wassner AJ, Wintergerst KA, Yayah-Jones NH, Hopkin RJ, Chuang J, et al. Congenital hypothyroidism: Screening and management. *Pediatrics* 2023; **151(1)**. doi: 10.1542/peds.2022-060420.
21. van Trotsenburg P, Stoupa A, Leger J, Rohrer T, Peters C, Fugazzola L, et al. Congenital hypothyroidism: A 2020-2021 Consensus Guidelines Update - An ENDO-European reference network initiative endorsed by the European Society for Pediatric Endocrinology and the European Society for Endocrinology. *Thyroid* 2021; **31(3)**:387-419. doi: 10.1089/thy.2020.0333.
22. Agaravatt A, Kansara G, Khubchandani A, Sanghani H, Patel S, Parchwani D. Verification of reference interval of thyroid hormones with manual and automated indirect approaches: Comparison of hoffman, KOSMIC and refineR methods. *Cureus* 2023; **15(5)**:e39066. doi: 10.7759/cureus.39066.
23. Ma C, Cheng X, Xue F, Li X, Yin Y, Wu J, et al. Validation of an approach using only patient big data from clinical laboratories to establish reference intervals for thyroid hormones based on data mining. *Clin Biochem* 2020; **80**:25-30. doi: 10.1016/j.clinbiochem.2020.03.012.
24. Ammer T, Schutzenmeister A, Prokosch HU, Zierk J, Rank CM, Rauh M. RIbench: A proposed benchmark for the standardized evaluation of indirect methods for reference interval estimation. *Clin Chem* 2022; **68(11)**:1410-24. doi: 10.1093/clinchem/hvac142.

