Review Article

Reference Intervals for Blood Chemistry Parameters in the Pakistani Population: A Systematic Review of Published Literature

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Article Info

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Keywords

Reference interval, biochemical, biomarkers, clinical chemistry tests, Pakistan.

Abstract

Background

Reference intervals (RI) are a vital part of information provided with laboratory results. It is recommended that RI should be established by each laboratory following pre-laid guidelines. In this systemic review, we aim to comprehensively analyze and summarize all the published literature about establishment of RI for biochemical parameters in Pakistani population.

Methodology

We conducted a comprehensive search using Medline (PubMed interface) and PakMediNet literature, adhering to PRISMA guidelines. The search spanned from January 1984 to February 2024. All studies done for establishment of RI of biochemical parameters were included, while were nonhuman studies, case studies, preprints, no full text and articles in languages other than English were excluded. Rigorous evaluation ensured the robustness of their study analysis.

Results

Database search reveled 161 studies, 30 were analyzed as per inclusion criteria. The accumulated sample size of the studies comprised 108,563 individuals. Most of the studies were carried out on adults in Punjab and Sindh provinces. A wide variation was noted among the RIs established and units used in each study. Gaps were identified regarding description of healthy population, patient preparation sample handing and quality control

Conclusion

In this review, critical gaps in data, methodology and reporting were identified. To enhance future studies, researchers should clearly define healthy populations, incorporate rigorous sample handling and quality control, and collaborate across centers.

Introduction

Modern day clinicians rely heavily on laboratory tests, for disease diagnostics and monitoring. Research from Germany and the USA suggest that approximately 60–70% of clinical decisions are influenced by laboratory test results. Furthermore, 80% of diagnostic and disease management guidelines also incorporate laboratory testing in clinical guidelines [1,2]. Reference intervals (RIs) are a vital part of information provided by laboratories along with patient test results in order to provide a context and allow clinicians to make well-informed clinical decisions. RI usually represents a range of values seen in 95% or more of healthy population for that particular parameter [3]. The process of RI establishment involves selection of appropriate reference individuals, a reference sample group, then reference values are obtained and finally reference distribution, reference limits and RIs are calculated through various statistical methods [4]. The quality of RI is as important as the quality of laboratory result itself. However, the validity of RI is not universal. A large number of factors like reference population, sampling strategies, sample size, genetics, ethnicity and diet can affect the accuracy/ transferability of RI. The International Federation of Clinical Chemistry (IFCC) has recommended that each laboratory should follow defined procedures to produce its own reference values [5,6,7] International Organization for Standardization (ISO) 15189 standard for clinical laboratory accreditation states that each laboratory should periodically re-evaluate its own RIs [8].
Pakistani population exhibits wide genetic, demographic, dietary and environmental diversity [9]. Additionally, inclusion of both genders and various age groups is also essential to ensure that RI is truly representative of entire population. However, despite the critical importance of region-specific RI for blood chemistry parameters there remains a notable gap in literature regarding comprehensive and up to date RI for blood chemistry parameters in Pakistan. To the best of our knowledge, this systematic review represents the first comprehensive compilation and analysis of RIs for various blood chemistry parameters in the Pakistani population. Despite the pivotal role of accurate RIs in clinical decision-making and patient management, there exists a significant gap in the literature regarding region-specific reference values for Pakistan. In order to address this deficiency, we present this systematic review to critically evaluate the existing body of literature to compile and analyze reference intervals for various blood chemistry parameters in the Pakistani population. This review aims to provide valuable insights for laboratory professionals and researchers by identifying current research gaps, guide towards areas for further investigation and contribute to evidence-based healthcare practices in Pakistan.

Data Retrieval

The team of investigators performed a systematic literature review based on Medline (PubMed interface) and PakMediNet (https://www.pakmedinet.com/) literature on reference intervals from January 1984 until February 2024. PakMediNet is Pakistan's largest medical database containing research articles published in Pakistani medical journals. The strategy adopted was in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [10]. A manual search was performed based on references from other articles.
9 Keywords and medical subject heading (MeSH) terms searched included "reference interval" OR "reference intervals" OR "reference range" OR "reference ranges" OR "reference value" OR "reference values" "normal value" OR "normal values"), Pakistan[Affiliation]) without language restrictions. Nonhuman and biological model studies were not included. Moreover, two separate investigators (NA and SA) reviewed the titles and abstracts of all articles identified for inclusion in the final analysis; alongside, the references of the scrutinized articles and the PubMed-related article feature were also explored for any additional publications of potential interest. The inclusion criteria were structured upon the following conditions: (1) study methodology establishment of reference interval (2) studies done on Pakistani population (3) studies done for biochemical parameters; (4) study design: cross sectional. Manuscripts comprising preprints, abstracts only, case studies, case series, reviews, meta-analysis, letters to the editor, surveys, commentary, perspectives, opinion papers, hypothesis, viewpoints, animal studies, basic sciences/nonclinical studies, studies done outside Pakistan, article full text in language other than English, were omitted. Furthermore, the full-text versions of abstracts included in the final study analysis underwent additional evaluation by two independent chemical pathologists. This rigorous approach ensured the robustness of our study analysis. The two reviewers autonomously compiled the data using Excel enlisting the region of study publication, number of study participants, type of study participants, time of recruitment, biochemical parameters studied, method used for RI calculation and RI values. Inter-rater reliability and agreement were assessed using kappa statistics.

Results

The reviewed literature spanned from 1984 to 2024, with a focus on establishing reference intervals for blood chemistry parameters within different subsets of the Pakistani population. The databases searched revealed 161 studies. Moreover, 2 duplicate studies were excluded. Based on the stringent inclusion criteria as depicted in Figure 1, 30 articles were included in the final analysis based on autonomous evaluation by two investigators with an excellent agreement of κ statistic=0.90. The accumulated sample size of the studies comprised 108,563 individuals. Reference intervals were established through cross sectional studies done across various age groups ranging from neonates to adults, covering multiple ethnicities and physiological conditions like various trimesters of pregnancy. The majority of research was concentrated in the provinces of Punjab and Sindh as shown in table 1. Non-probability consecutive technique was the most common sampling approach. The majority of the researchers failed to properly define the standard for healthy population, did not conduct screening tests for study population and relied only on history of comorbid and other clinical conditions. Only a

few studies explained the patient preparation, sample collection, handling, processing and quality control measures employed during the study. RI was established for 29 biochemical including total bilirubin, albumin, alkaline phosphatase (ALP), and alanine aminotransferase (ALT), thyroid profile (FT3, FT4 and TSH) during different trimesters of pregnancy, as well as comprehensive sets of parameters such as fasting plasma glucose (FPG), serum cholesterol, triglycerides, urea, creatinine, uric acid, total protein and electrolytes. Trace metals like copper and zinc were also studied. RI for novel biomarkers like Soluble FMS-like tyrosine kinase-1 (sFIt-1), Chitinase-3-like protein 1 (CHI3L1), Protein induced by vitamin K absence II (PIVKA-II) and Placental growth factor (PGF) were also established. Most of the researchers used direct methods (2.5th-97.5th percentile, 5th-95th percentile) while a few opted for indirect approaches like data mining, KOSMIC algorithm etc. Biochemical parameters were analyzed using various methods of analysis with photometric assays, electrochemiluminescence assays and chemiluminesence assays being the most common as presented in Table 1.

Figure 1: Flowchart of search strategy

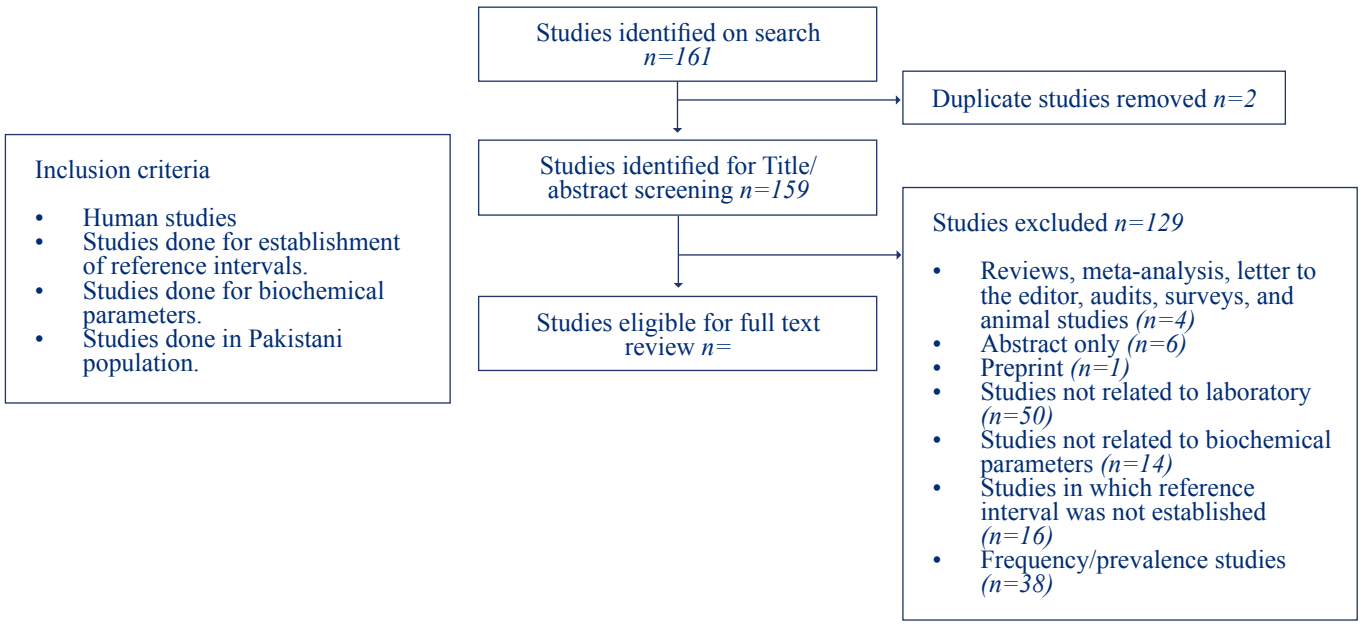

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Table 1: Listing of 30 manuscripts of RIs in Pakistan including details on author, year(s) of study, type of study, sample size and study population.

Author (Year)	Province, City	Type of Study	Sample size, n	Study population	Ages, years
(Mean/ Median)	Sindh, Karachi	Prospective cross sectional study	100	Euthyroid, Pregnant women within 12 weeks gestation	26
Husnain F et al [12] (2013)	Punjab, Lahore	Cross-sectional study	408	202 healthy and 206 liver fibrosis cases	39.6 ± 12.6
Ali SK et al [13] (2010)	Punjab, Rawalpindi	Descriptive cross-sectional study	254	Healthy Adults 18-80	41.07±15.6
Hashmi SB et al [14] (2018-2019)	Sindh, Karachi	Cross-sectional	120	Healthy children <6 yrs	29 ± 22.3months
Younas A et al [15] (2017 -2018)	Punjab, Rawalpindi	Cross-sectional study	754	Pregnant women with single intrauterine pregnancy	24.25 ± 3.97 1st trimester 25.42 ± 3.71 2nd trimester
Gilani M et al [16] (2016-2017)	Punjab, Rawalpindi	Cross sectional study	384	Pregnant women with single intrauterine pregnancy	25.3±3.7yr1sttrimester 26.54±4.65yr 2ndTrimester
Bhatti N et al [17] (2016)	KPK, Wah cantt	Cross sectional study	164	Healthy adults	_
Raza A et al [18] (2016- 2018)	Punjab and KPK	Multicentre cross sectional study	14147	<1 month	5.6±4.8 days
Bibi A et al [19] (2019)	Punjab, Rawalpindi	Cross sectional study study	120	Healthy neonates 2-6 days	_
Muneer S et al [20] (2018-2019)	Sindh, Karachi	Prospective cross sectional study	131	1-24 months	Median (IQR) age 12 months
Khan A et al [21] (2018-2019)	Baluchistan, Quetta	Cross sectional study	322	Healthy pregnant females	25.1±3.7
Ahmed S et al [22] (2013-2017)	Sindh, Karachi	_	42,711	_	_
Khan HR et al [23] (2013-2014)	Punjab, Islamabad	Cross sectional study	1000	Adults 18-60	28.4

Sattar A et al [24] (2008)	Punjab, Rawalpindi	Cross sectional study	214	18-50 healthy adults	Males 35+12 Females 28+09
Ain Qurat Ul [25] (2022)	Punjab, Rawalpindi	Cross sectional study	807	Pregnant female in 1st and 2nd Trimester	1st trimester 22.37 ± 2.54 2nd trimester 27.14 ± 3.62
Ahmed S et al [25]	Sindh, Karachi	_	36766	_	_
et al [27] (2013-2021)	Sindh, Karachi	Cross sectional study	40,914	0-18	_
Rasheed A et al [28] (2023)	Punjab, Lahore	Cross-sectional study	260	130 disease free non-pregnant and 130 disease free pregnant females	_
Ahmed AR et al [29] (2023)	Punjab, Lahore	Observational study	240	Healthy Pregnant and non-pregnant females	_
Khan A et al [30] (2018 - 2019)	Baluchistan, Quetta	Cross-sectional study	388	Healthy women with singleton pregnancy	1st trimester 25.1±3.7 2nd trimester 26.7±4.5 3rd trimester 26.8±4.8
Abbas R et al [31] (2009 - 2011)	Punjab, Lahore	Cross-sectional study	852	Euthyroid adults	46
Sarfaraz L et al [32] (2014)	Punjab, Bahawalpur	Cross-sectional study	800	Healthy adults 19-60 yrs	_
Hussain W et al [33] (2012)	Punjab, Lahore	Cross-sectional study	450	Healthy adults 20-29yrs	25
Hussain W et al [34] (2012)	Punjab, Lahore	Cross-sectional study	450	Healthy adults 20-29yrs	_
Khan DA et al [35] (2007-2009)	KPK, Wah Cantt	Cross-sectional study	297	Healthy adults >18yrs	39
Husnain F [36] (2020)	Punjab, Lahore	Cross-sectional study	240	120 diagnosed cases of HCC and 120 healthy individuals	39.5 ± 13.4 years
Abbas HG et al [37] (1998-2000) Punjab, Lahore	Punjab, Lahore	Cross-sectional study	1153	Neonates	_
Khokhar AR et al [38] (1998 -2000)	Punjab,Dera Ghazi Khan	Cross-sectional analytical study	30	Normal healthy, euthyroid pregnant women in last trimester	25.77±5.10

Mumtaz A et al [39] (2019)	Punjab, Lahore	Cross-sectional study	500	Pregnant women	25.03 ± 4.06
Iqbal S et al [40] (2012)	Sindh, Karachi	Cross-sectional study	146	Adults <30 years	24

Table 2: Studies clustered according to biochemical parameters, with RI and methodology used for establishment of RI and method of analysis.

Parameters, units	**Reference interval established**	**Methodology used for establishment of RI**	**Method of analysis**	**Author**
HbA1c, mmol/mol	4.6–6.56% OR 2.69–4.81	CLSI recommendation	HPLC using Biorad D-10.	Ali SK et al [13]
HbA1c, %	20-30 yrs: 2.09-5.57 (3.83±0.87) 31-40 yrs: 2.63-5.99 (4.31±0.84) >40yrs:2.8-6.4 (4.31±0.9)	RI using the formula Mean ± 2SD	Ion-Exchange Chromatography technique on Microlab 300	Bhatti N et al [17]
HbA1C,%	1st trimester:3.8-5.2% 2nd trimester:4.1-5.4% 3rd trimester: 4.2-5.7%	5th & 95th percentile	TINIA	Khan A et al [21]
Free Ionized Calcium, mmol/l	Males 1.12 ± 0.05 Females 1.12 ± 0.04	2.5th & 97.5th percentiles were taken	ISE method	Sattar A et al [24]
Serum calcium, mg/dl	KOSMIC Algorithm > 1 yr-8.5-11.2 >1-4yr 8.6-10.6 >4-18 yrs 8.5-10.5 Hoffman's Method > 1 yr 8.3-10.6>1-4yr 8.7-10.2 >4-18 yrs 8.4-10.5 Bhattacharya Analysis > 1 yr-8.1-11.3 >1-4yr 8.5-10.6 >4-18 yrs 8.4-10.5	KOSMIC algorithm,Hoffman's method and Bhattacharya analysis	-	Ahmed S et al [27]
TPO Ab, IU/ml	2.4-23.1	Parametric robust method	Micro particle immunoassay technique (AxSyms, Abbott)	Iqbal S et al [11]
TSH,µIU / ml	Hypothyroidism group:15.47-165.21, Normal group:0.15-6.32, borderline group:9.15-17.6	Reference value advisor	DELFIA	Raza A et al [18]
TSH, µIU / ml FT4, pmol/l TT3, nmol/l	1st Trimester-TSH:0.05-2.8, FT4:.4-22.7, TT3 1.5-3.3 2nd Trimester-TSH:0.16-3.3, FT4:14.2-24.60, TT3: 1.6-3.1	2.5th & 97.5th percentiles were estimated, 2SD on each side of mean	TSH by CLIA TT3 and FT4 by competitive immunoassay	Gilani M et al [16]

TSH **FT4 ng/dl**	TSH was 0.73–4.94 FT4 was 0.81–1.51	Central 95% of the population using the non-parametric approach	CLIA using ADVIA Centaur, Siemens.	Muneer S et al [20]
TSH µIU/mL **FT4 pmol/l**	TSH-1st trimester 0.6-3.3, 2nd trimester 0.6-3.8, 3rd trimester 0.6-2.7 FT4-1st trimester 9.8-10.8, 2nd trimester 10.4-20.1, 3rd trimester 11.020.9	2.5th & 97.5th percentiles	ECLIA on Cobas e601	Khan A et al [30]
FT3 pmol/L **FT4 pmol/L**	FT3 2.80 - 5.39 FT4 11.9 - 22.2	R-language (version 2.15)	RIA on Immunotech (A Beckman Coulter Company)	Abbas R et al [31]
Cord serum T4 nmol/L **TSH mIU/L**	T4 49-189 TSH 0.4-17.6	2.5th & 97.5th percentile	Ortho-clinical Diagnostics (Amersham, UK) and North Eastern Thames Regional Immunoassay (St. Bartholomew's Hospital, London, UK). T4 by competitive RIA technique.	Abbas HG et al [37]
TSH, mIU/L **FT3 pmol/L** **FT4 ng/dl**	TSH:3.3-6.0 FT3: 0.84-7.51 FT4: 0.78-5.09	5th & 95th percentile	CMIA, ARCHITECT	Khokhar AR et al [38]
Serum TSH mIU/mL **FT3 pmol/L** **FT 4 pmol/L**	TSH 0.168-4.294, 0.258-4.584 and 0.341-4.625 FT3 1.857-4.408, 1.958-4.621 and 2.025-4.821 FT4 8.815-18.006, 8.306-17.341 and 7.402-17.292	5th & 95th percentile	CLIA system on Maglumi 800	Mumtaz A et al [39]
TSH, IU/ml **TPO Ab**	3.3- 13.8	2.5th & 97.5th percentiles	CLIA on ADVIA Centaur CP Immunoassay system, Siemens.	Iqbal S et al [40]
Spot Ox:Cr ratio	Mean:0.05–0.34 Group I 0.25 (IQR: 0.06) Group II 0.19 (IQR: 0.11) Group III 0.15 (IQR: 0.04) Group IV 0.11 (IQR: 0.06) Group V 0.08 (IQR: 0.04) (pvalue <0.001)	STROCSS criteria	Urinary Oxalate by Micro lab 300 using oxalate oxidase principle by Trinity Biotech Plc	Hashmi SB et al [14]
DBS for biotinidase nmol/ml/min	3.0 to 11.0	2.5th & 97.5th percentiles were estimated	Solid phase time-resolved immunofluorescence assay, Genetic Screening Processor 2021, Perkin Elmer	Bibi A et al [19]
Uric acid umol/l	1st trimester 95.8-260.14 2nd trimester 96-268	3rd & 97th percentile was used	Uricase enzymatic method on Siemens's ADVIA 1800	Ain Qurat Ul [25]
ALP	_	2.5th& 97.5th percentiles, Data mining method (indirect method)	Photometric method, Seimens ADVIA 1800.	Ahmed S et al [22]

Serum creatinine mg/dl	Males <2: 0.15-0.39, 2-<5: 0.15-0.80, 9-<12: 0.27-0.92 5-<9: 0.16-0.69, 12-<15:0.29-1.06, 15-<17:0.40-1.26 Females <2: 0.12-0.73, 2-<5: 0.15-0.74, 5-<9: 0.16-0.68, 5-<9: 0.16-0.68, 9-<12: 0.26-0.78, 15-<17:0.34-0.93	Data mining of the laboratory information system German Society of Clinical Chemistry and Laboratory Medicine's Working Group on Guide Limits were used.	Siemens's ADVIA 1800	Ahmed S et al [26]
Total Bilirubin µmol/l, Albumin g/l, ALP U/L, ALT U/L,	1st Trimester T.B:2.96-8.84 Alb: 31.5-45.0 ALT: 3.1-35.7 ALP:121.6-224.3 2nd Trimester T.B:2.5-7.3 Alb: 27.8-44.7 ALT: 1.4-33.1 ALP:131.5-300.4	2.5th & 97.5th percentiles were estimated by values approximately 2SD on each side of mean	Diazo (modified Jendrassik and Grof's), BCG (bromocresol green) end point; Nitro-phenyl phosphate (pNPP) Kinetic and modified IFCC (Wróblewski and LaDue) kinetic method respectively.	Younas A et al [15]
ALT & ALP U/L, Total Bilirubin, Urea, Creatinine, Uric Acid mg/dL, Total Protein &Albumin g/dL, Na, K mEq/L.	ALT: 10-68 T.B: 0.12-1.4 ALP: 51-150 Urea: 13-40 Creatinine: 0.6-1.3 Uric acid: 3.4-8.2 Total Protein: 6.1-8.3 Albumin: 3.8-5.3 Na: 136-147 K :3.1-4.8	2.5th & 97.5th percentiles	Analysis on MODULAR P 800 Serum ALT & ALP by IFCC method. T.B by Wahlefeldet method. Urea by Talke & Schuberts kinetic UV method. Creatinine by Jaffe alkaline picrate method. Total Protein by Biuret Alb by Bromocresol Green method. ISE using EASYLYTE PLUS (USA) for electrolytes	Khan HR et al [23]
FPG, Cholesterol Triglycerides Urea & Creatinine mmol/L Uric acid & Total bilirubin µmol/L, Total proteins g/l, ALT & ALP U/L,	FPG: 3.7-5.4 Cholesterol: 3.4-5.2 Triglycerides:0.7-2.2 Urea: 3.8-8.5 Creatinine:0.06-0.15 Uric acid: 209-440 Total bilirubin: 6.5-21.5 Total proteins: 55-76 ALT: 16-45 ALP: 130-280	2.5th & 97.5th percentiles	Selectra Excel	Sarfaraz L et al [32]
Placental growth factor pg/ml	Pregnant-3.8 to 12.7 Non-pregnant: 46.43-1148	2.5th & 97.5th percentiles	ECLIA on Cobas e601	Rasheed A et al [28]

Soluble FMS-like tyrosine kinase-1 (sFIt-1) pg/ml	Non-pregnant: 57.7 -118.5 Pregnant females 563.5-3288.0	2.5th & 97.5th percentiles	ECLIA analyser Cobas e601	Ahmed AR et al [29]
Chitinase-3-like protein 1 (CHI3L1) ng/ml	12.80-81.80 in healthy Cut-off for the diagnosis in hepatic fibrosis cases 102.12	–	Manual ELISA (Proprium Biotech)	Husnain F et al [12]
Serum PIVKA-II, mAU/ml	Healthy:15.55-43.03 Cut-off for the diagnosis in HCC cases: 148.810	2.5th percentile & 97.5th percentile	CMIA	Husnain F [36]
Serum Copper µmol/L	Males Mean: 18.57±6.61 RI: 4.72 to 31.7. Females Mean: 16.52±6.67 RI: 4.72 to 30.48	2.5th percentile & 97.5th percentile	FAAS (Hitachi Z2000)	Hussain W et al [33]
Serum zinc µmol/L	Overall mean 24.02±7.03 RI:11.47-36.72 Females Mean: 21.72±7.34 RI: 9.94-36.87 Males Mean: 22.33±6.42 RI: 11.93-32.4).	2.5th percentile & 97.5th percentile	Atomic absorption spectrometry (Hitachi Z2000)	Hussain W et al [34]
hS-CRP, mg/L	Adult population of Northern Pakistan 1.84 (0.37-4.81) Punjabis 1.75 (0.30-4.65) Pathans 1.93 (0.50-5.30)	2.5th percentile & 97.5th percentile	CLIA assay on Immulite 1000 (Siemens)	Khan DA et al [35]

TSH-thyroid stimulating hormone, FT3-free triiodothyronine, FT4-free thyroxine, TPO Ab-Anti Thyroid peroxidase, TINIA-Turbidimetric Immunoinhibition, ISE-Ion selective electrode, DELFIA- Dissociation Enhanced Lanthanide Fluorescent Immunoassay, CMIA- Chemiluminesence micro particle assay, FAAS-Flame atomic absorption spectrometry, ECLIA- Electrochemiluminescence, RIA- radioimmunoassay Ox:Cr-oxalate:creatinine, DBS-dried blood spot, PIVKA-II- Protein induced by vitamin K absence II, ALP-alkaline phosphatase, ALT-alanine transaminase, T.B- total bilirubin, hS-CRP -high-sensitivity C - reactive protein, FPG-fasting plasma glucose, CLSI- Clinical and Laboratory Standards Institute

Discussion

RIs provided with all laboratory reports serve as a benchmark for interpretation and understanding of laboratory results. Population/region specific RIs are necessary to account for differences occurring due to ethnicity, age and geographic location. In Pakistan, most laboratories use RIs provided by the manufacturers, which are usually established in a foreign population. There is severe dearth of published data on establishment of RI in local population. In this review, we gather and summarize all the published studies done for establishment of RI for various biochemical parameters in Pakistani population. Clinical and Laboratory Standards Institute (CLSI) in collaboration with the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has laid out minimum requirements for reliability and usefulness of RIs established by a laboratory. This guideline includes recommendations for appropriate population selection, inclusion exclusion criteria for "healthy" participants, population partitioning. Other factors like patient preparation, sample handling, quality control for sample analysis and equipment maintenance are also discussed.3 The studies done in Pakistan were primarily single center cross-sectional studies. While most studies enrolled more than 120 participants, as recommended in the guidelines, 2 researchers took less than 100 patients which makes these study unreliable. It was also noted that most of the studies focused on healthy adults and pregnant women while neonates and children were not adequately represented. Nine (30%) studies failed to mention the important demographic of age along with their results, this sheds a light on lack of understanding of CLSI guidelines by researchers leading to limited utility. The research was concentrated in Punjab and Sindh, with limited representation from other provinces like Khyber Pakhtunkhwa and Baluchistan. We recommend multicenter studies encompassing various regions across Pakistan to capture a broader geographic representation. When considering the criteria for selection of reference sample individuals, almost all of the research failed to provide a clear definition of 'healthy' and relied mainly on history provided by the participants. This ambiguity makes it difficult to determine if

the reference interval truly reflects a healthy population. Many studies also did not mention detailed inclusion, exclusion and partitioning criteria or screening tests. A gap was identified regarding preanalytical considerations (sample collection techniques, storage conditions, and processing times) and patient preparation protocols (fasting instructions, medication restrictions, and activity limitations). While some studies mention QC measures, details are often sparse. According to CLSI guidelines, these details must be clearly mentioned.3 Lack of such information hinders the generalizability, transferability and reliability of RIs. While comparing studies done for identical biochemical parameters it was noticed that RI varied considerably. The same parameters are measured in different units across studies (e.g., HbA1c: %, mmol/mol).13,17,21 Eight researchers established RI for TSH, however variation was noticed in the units, assay used for TSH analysis and method used for establishment of RI was not consistent. Establish consensus on the preferred units for each parameter. Varieties of methodologies are employed to establish RIs (e.g., CLSI recommendation, parametric robust method, and data mining) and Different assays are used for analysis (e.g., HPLC, Ion-Exchange Chromatography, Chemiluminescence immunoassay). This lack of standardization can lead to discrepancies. It was noted that limited studies were available for hormones and important tumor makers. This systemic review represents the first of its kind with extensive completion and analysis of RIs established in Pakistani population. Its findings can be very useful for laboratory professionals and physicians for appropriate decision-making. As several gaps have been identified in currently available literature, this review can serve as a guide for future researchers to plan and execute further studies for establishment of RI specific to Pakistani context.

Conclusion

Our review of reference interval studies in Pakistan revealed concerning gaps in data, methodology and reporting. These shortcomings can lead to inaccurate reference ranges, limited applicability of findings, and reduced confidence in the data. To improve the standard of future studies, researchers should clearly define healthy populations; incorporate sample handling, inclusion/exclusion criteria, and relevant quality control will ultimately be benefiting both clinicians and patients. Further multicenter studies must be carried out by collaboration among researchers and healthcare professionals. By addressing these gaps and adhering to guidelines, more robust and comprehensive database of RIs can be created, leading to better patient care in Pakistani population.

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Ethical approval: Study was done in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki.

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