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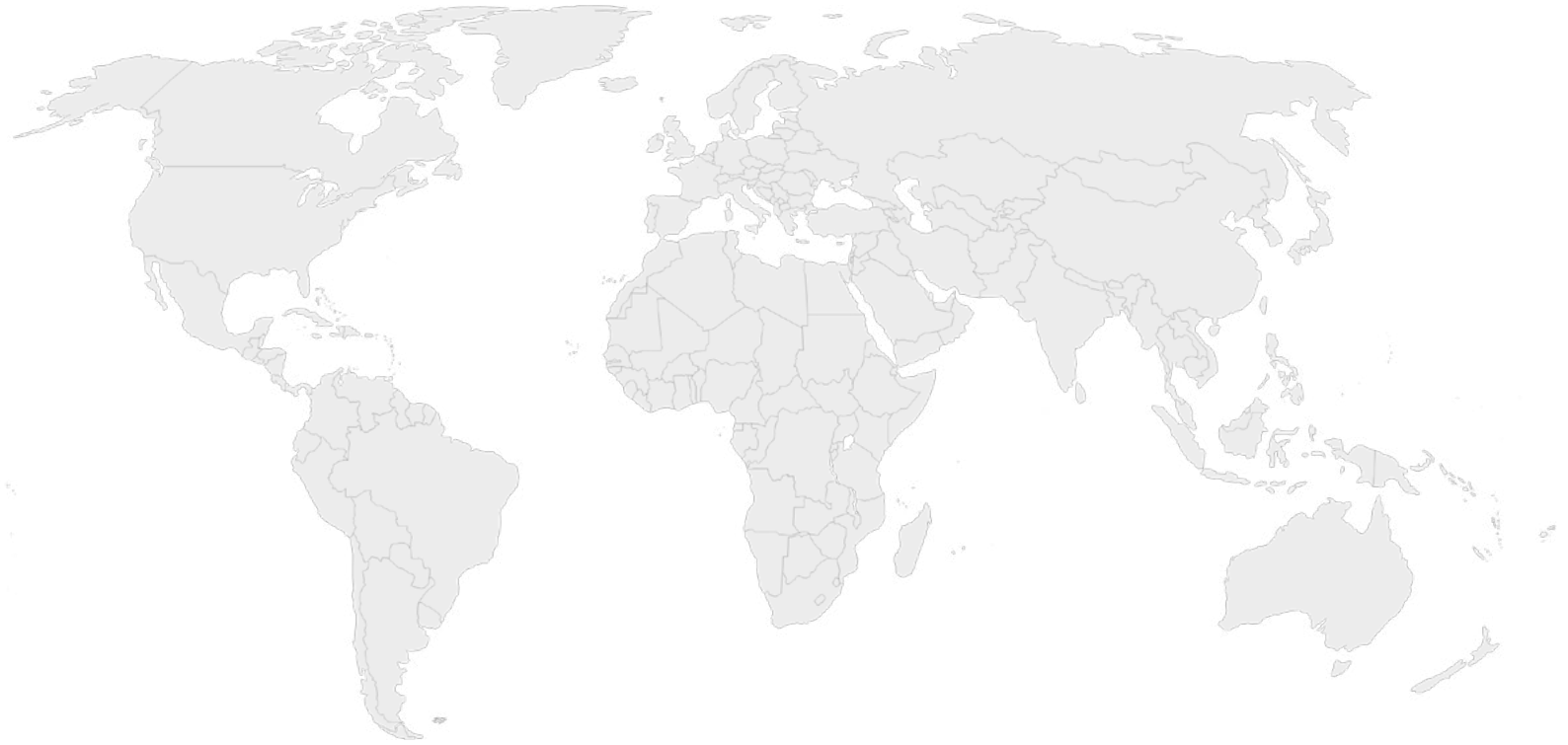

In this Issue

Letter to the Editor

Navigating Laboratory Medicine's Transformation: Embracing Doughnut Economics for Sustainable Business Redesign

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Keywords

Doughnut Economics, Sustainability, Transformation, Emerging Technologies, Innovation, Economy

Abstract

To the Editor,

In the rapidly evolving field of laboratory medicine, the integration of emerging technologies such as automation, omics, mobile health, and artificial intelligence heralds a transformative era in clinical decision-making and patient outcomes [1,2]. However, the full potential of these advancements can only be realized with a parallel evolution in management and business models within the laboratory sector. This necessitates a paradigm shift towards a sustainable and equitable economic framework that addresses both the global ecological crises and social disparities, which are more and more pronounced due to the current global inflation. The move to more sustainable laboratory practices is mandatory and has been initiated [3,4]. In such a reflection, the "Doughnut Economics" model, conceptualized by Kate Raworth, offers a compelling solution [5]. It proposes a balanced approach between meeting human needs—ensuring everyone has access to life's essentials—and operating within our planet's ecological boundaries to maintain its life-support systems. The application of the doughnut economy model in healthcare has great potential [6]. To our knowledge, a discussion around the adoption of Doughnut Economics in laboratory medicine has not yet really happens and emphasizes the importance of a holistic redesign of business practices. This includes the thoughtful integration of innovative technologies to improve clinical outcomes while ensuring equity and minimizing bias in healthcare delivery. Moreover, it advocates for resource optimization strategies that align with ecological boundaries, promoting sustainable practices to minimize environmental impact. The model also calls for community-centric engagement, fostering collaborations that prioritize community well-being and inclusivity in healthcare solutions. Furthermore, it underscores the need for educational and ethical imperatives, embedding ethical considerations and sustainability in the training of healthcare professionals regarding technology applications. The adoption of Doughnut Economics in laboratory medicine presents a transformative opportunity to redefine our science's approach to sustainability, equity, and innovation. By applying this model, clinical laboratories

can navigate the complex landscape of technological advancements, environmental challenges, and societal needs, ensuring a future where healthcare contributes positively to both human and planetary health. Based on the general concepts around doughnut economy and according to ongoing initiative in healthcare [6-8]. We identified five major applications of Doughnut Economics to laboratory medicine (Figure 1), exploring their potential impacts and the perspectives needed to implement them effectively.

Sustainable Laboratory Practices

Traditional laboratory operations often involve significant resource consumption, including energy, water, and materials, contributing to environmental degradation. Adopting sustainable laboratory practices, such as energy-efficient equipment, water-saving technologies, and waste reduction measures, aligns with the ecological ceiling principle of Doughnut Economics. It not only mitigates the environmental impact but also leads to cost savings and operational efficiencies. In this perspective, clinical laboratories must adopt a long-term view, recognizing that initial investments in sustainability yield long-term benefits. This requires a shift in mindset from short-term cost considerations to long-term value creation for both the laboratory and the environment.

Equity in Healthcare Access

Doughnut Economics emphasizes ensuring no one falls below the social foundation, advocating for universal access to essential services, including healthcare. In laboratory medicine, this translates to making diagnostic services accessible and affordable to all segments of the population, addressing disparities in healthcare access. According to this perspective, clinical laboratories should foster partnerships with public health agencies, non-profits, and communities to expand access to underserved populations. Innovations in mobile health and telemedicine can bridge geographical barriers, but they must be deployed in ways that ensure equity and inclusivity.

Integration of Ethical Artificial Intelligence (AI) and Data Analytics

The potential of AI and machine learning in enhancing diagnostic accuracy and patient outcomes is immense. However, ethical considerations around bias, privacy, and accountability must be addressed. Laboratories adopting AI should ensure these technologies are used in ways that are transparent, equitable, and with a focus on enhancing patient care, reflecting the distributive and regenerative dynamics central to Doughnut. Considering this perspective, developing ethical guidelines and frameworks for the use of AI in laboratory medicine is essential. Engaging with ethicists, patients, and the wider community in these discussions ensures that the deployment of AI technologies aligns with societal values and needs.

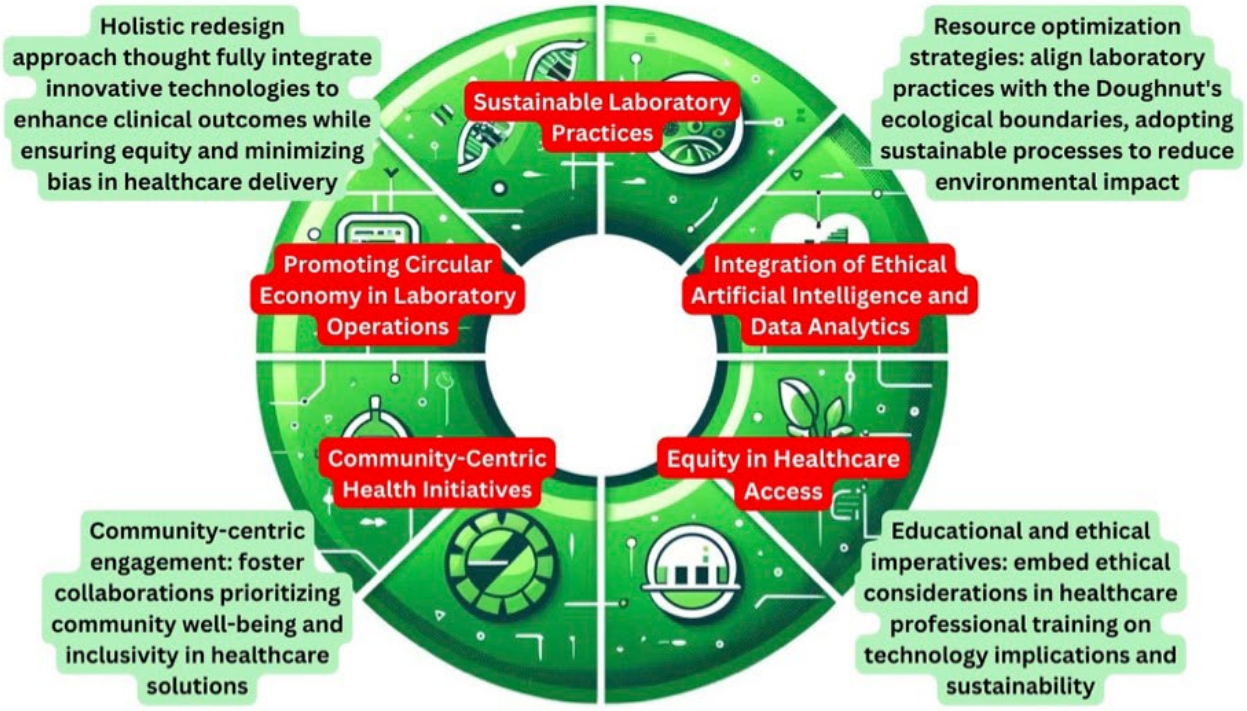
Community-Centric Health Initiatives

Beyond providing diagnostic services, clinical laboratories can play a pivotal role in community health by participating in public health campaigns, disease prevention programs, and health education initiatives. This approach aligns with the Doughnut Economics model by fostering a healthcare system that prioritizes the well-being of the community and the environment. For this perspective, clinical laboratories need to view themselves as integral members of the healthcare ecosystem, with a responsibility that extends beyond the lab's walls. This involves active collaboration with public health organizations, educational institutions, and community groups to address broader health determinants.

Promoting Circular Economy in Laboratory Operations

The circular economy model, closely related to Doughnut Economics, focuses on minimizing waste and making the most of resources. In laboratory medicine, this could involve recycling programs for lab materials, reprocessing of single-use devices where safe and feasible, and adopting green procurement policies. Clinical laboratories must explore innovative ways to reduce their environmental footprint through waste management and resource optimization strategies. This requires collaboration with suppliers, regulators, and waste management services to create a more sustainable laboratory supply chain.

Figure 1: Application of the Doughnut Economy Model to Laboratory Medicine



This figure illustrates the adaptation of the doughnut economic model to laboratory medicine, highlighting sustainable practices within the ecological and social thresholds. The inner ring ('Social Foundation') represents essential healthcare and social equity goals that laboratory practices must meet to ensure accessibility and fairness in medical services. The outer ring ('Ecological Ceiling') defines the environmental limits that laboratory operations should not exceed to avoid detrimental impacts on the planet. Between these rings, the diagram maps specific strategies for sustainable and equitable laboratory practices, such as integration of ethical AI, promoting circular economy, and community-centric health initiatives, aligning with both global sustainability goals and healthcare equity. The figure was partially built by the use of CANVA.

In conclusion, the application of Doughnut Economics to laboratory medicine could offer a roadmap for transforming our science into one that is sustainable, equitable, and innovative. It challenges laboratories to rethink their operations, technologies, and their role in society, aligning their practices with the needs of both people and the planet. Implementing these changes will require a shift in perspective, embracing long-term sustainability and equity as core values. By doing so, laboratories can contribute to a healthier future for all, demonstrating the power of combining scientific innovation with ethical and environmental responsibility.

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Review Article

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

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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

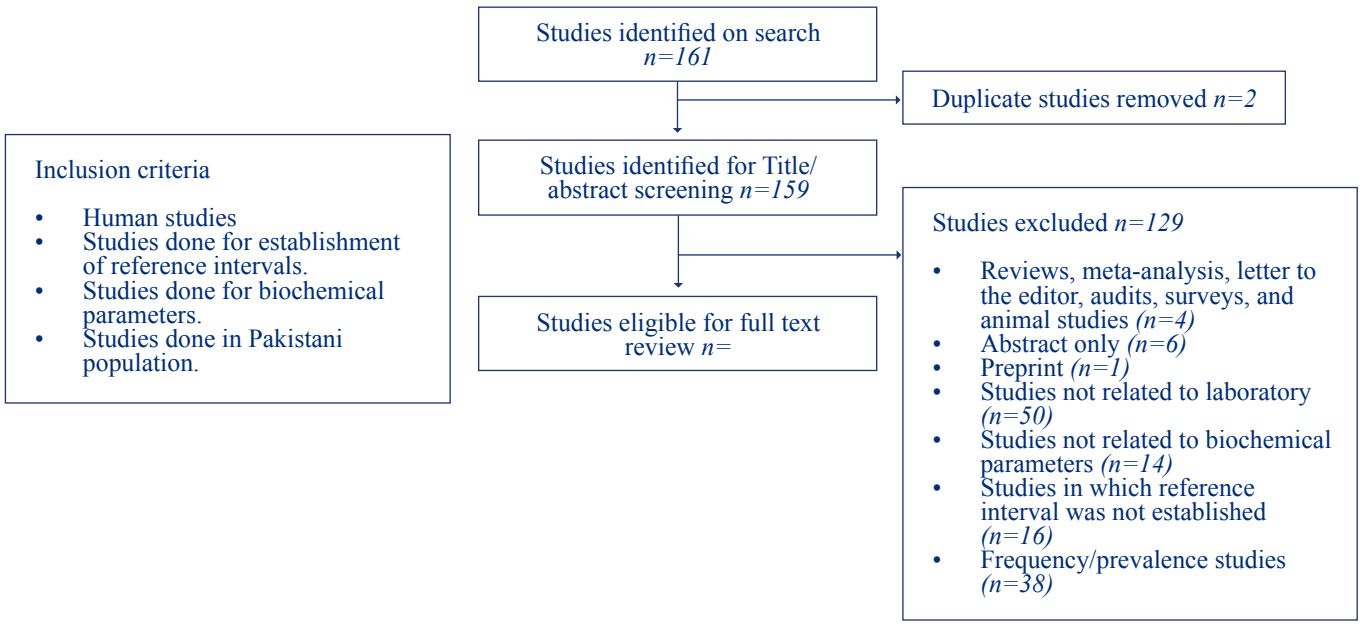

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Table 1: Listing of 30 manuscripts of RIs in Pakistan including details on author, year(s) of study, type of study, sample size and study population.

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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Review Article

Uric acid status in subclinical hypothyroidism

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Abstract

Overt hypothyroidism is associated with high levels of serum uric acid (UA) however, the association between UA and thyroid function in patients with subclinical thyroid dysfunction remains unclear. Subclinical hypothyroidism (SCH) is a common endocrine disorder characterized by normal thyroxine (T4) and triiodothyronine (T3), and elevated thyroid stimulating hormone (TSH) levels, usually without clinical manifestations. Therefore, we carried out a study of patients with subclinical thyroid dysfunction to assess the relationship between thyroid function and UA. This lead us to review the literature to find to what extent subclinical hypothyroidism is associated with uric acid. This study adopts the method of retrospective analysis to collect general information and laboratory results aimed at assessing the correlation between uric acid and thyroid hormone levels. We searched 3 databases using different keywords. Literature search was done for articles published in the last ten years, between 2013-2023. All relevant studies were screened. A total of eighteen articles were finalized for the review. Some studies supported T3 supplementation resulting in SCH correction. Our study indicates that it is important to screen for serum uric acid levels routinely in patients with subclinical hypothyroidism.

Introduction

Thyroid gland is one of the largest endocrine gland in the body, it secretes thyroxine (T4) and triiodothyronine (T3). Hypothyroidism is a progressive disorder that presents with diverse degrees of thyroid failure and metabolic consequences. Subclinical hypothyroidism (SCH) is a common endocrine disorder characterized by normal thyroxine (T4) and triiodothyronine (T3), and elevated thyroid stimulating hormone (TSH) levels, usually without clinical manifestations. SCH implies an absence of symptoms; however, it is perhaps better thought of as mild hypothyroidism. Moreover, mild hypothyroidism can progress to overt hypothyroidism. Physiological interactions exist between thyroid hormones and uric acid synthesis and excretion. Minor degrees of hypothyroidism can lead to adverse effects in various tissues, although clinically the patients may be euthyroid. Subclinical hypothyroidism is an early mild form of hypothyroidism, a condition in which the body doesn't produce enough thyroid hormones. Subclinical hypothyroidism is associated with an increased risk of metabolic disorders and cardiovascular events. Although

overt hypothyroidism shows increased levels of uric acid (UA), there is gap in knowledge about the association between uric acid (UA) and subclinical hypothyroidism [1,2]. This study was conducted to determine whether subclinical thyroid dysfunction has deleterious effects on renal function.

Review Methods

We searched three databases (Google scholar, PubMed Central and PubMed) from 2013 to 2023, i.e., last 10 years, for the article published on relationship between hypothyroidism and uric acid levels. The first stage involved screening titles and abstracts to identify and exclude irrelevant articles. All full-text studies that were potentially relevant were then read carefully in relation to the inclusion criteria. Observational and review/systematic review articles except the experiment studies on animal were selected. In total, eighteen articles met the inclusion criteria and were reviewed in the present study. Keywords used were: "Uric acid and hypothyroidism", "uric acid and subclinical hypothyroidism" in three databases separately. Table 1 shows the database searched, keywords used, date of search and the number of relevant publications identified. Reference lists of the articles were checked to identify more studies. Ethical approval was not required.

Table 1: Electronic search result

Database	Keyword	Date of search	Number of articles
Pubmed Central	Uric acid and hypothyroidism	15/11/2023	2717
Pubmed Central	Uric acid and subclinical hypothyroidism	15/11/2023	804
Pubmed	Uric acid and hypothyroidism	15/11/2023	121
Pubmed	Uric acid and subclinical hypothyroidism	15/11/2023	33
Google scholar	Uric acid and hypothyroidism	15/11/2023	18
Google scholar	Uric acid and subclinical hypothyroidism	15/11/2023	9

Inclusion criteria:

We included full text articles published in English language from all geographical locations between 2013 and 2023, which were related to the topic. Articles had at least one measurement of TSH and Uric acid by any method; however, this rule was not applied for the review articles.

Exclusion criteria:

Incomplete data and /or not meeting the inclusion criteria, and animal studies were the main reasons for exclusion.

Results

We identified 121 articles which were related to the topic by database searches, out of which 99 articles were excluded based on title and/or abstract not relevant to our study. Out of the remaining 22 full-text articles, we excluded four articles after further reading. Finally, we approved eighteen articles for the study. Figure 1 shows how the research articles were finalized.

Discussion

The articles reviewed are depicted in Table 2. Table 2 shows the various study articles reviewed and their results. Table 3 demonstrates typical laboratory data of some studies.

Figure 1: Process of article selection.

Table 2: shows the various study articles reviewed and their results

Source	Sample Size	Age Group	Study Type	Result	Conclusion
Lu et al [3]	15,955 euthyroid	≥ 18 years	Cross-sectional study	Subjects with reduced sensitivity to thyroid hormones had increased levels of UA in both genders ($p<0.001$).	Association between increased UA levels and impaired sensitivity to thyroid hormones.
Xie et al [4]	4,460 euthyroid	Adults	Cross-sectional study	A significant rise in serum UA with an increase in FT3/ FT4, TFQ/FT4, TFQ/FT3, TSHI, TT4R1 and TT3RI.	Association of Higher levels of serum UA with decreased sensitivity to Thyroid hormones.
Ittermann et al [5]	7,933 pooled data	20 – 93 years	Cross-sectional study	UA levels were 294 µmol/L in Hypothyroidism and 292 µmol/L in euthyroids.	Hypothyroidism might be associated with a reduced kidney function. Thyroid function might be more tightly related to the eGFR (OR: -3.35 (95%CI: -5.19 - -1.51) $p<0.05$) than to albuminuria (OR: 1.35 (95%CI: 0.93 - 1.97)) in the general population
Song et al [6]	26,342 hypothyroid patients	Adults	Observational study	Autoimmune hypothyroidism has a causal effect on gout, IVW results show (OR= 1.13, 95%CI: 1.03–1.21, PFDR= 0.0336); Autoimmune hyperthyroidism has a causal effect on gout, IVW results show (OR= 1.07, 95%CI: 1.01–1.12, PFDR= 0.0314).	Hypothyroidism and hyperthyroidism of autoimmune origin have increased risk of gout.
Huang et al [7]	6,587	Adults	Retrospective cohort study	Mean of UA 350 µmol/L, range of UA 274- 425 µmol/L had a significant ($p=0.028$) , non-linear ($p=0.516$) association with the development of thyroid nodules.	Uric acid is an independent non-linear risk factor for the formation of thyroid nodules.
Yang et al [8]	19,013	Adults (47.5 ± 14.5 years)	Cross-sectional study	The risk of developing Hyperuricemia in mild hypothyroidism (adjusted ORs (95%CI) of 1.370 (1.006-1.866) in males and 1.256 (0.858-1.838) in females).	Males with high TSH levels had significant risk of hyperuricemia.

Zhou et al [9]	443	Adults	Cross-sectional study	A positive correlation (p=0.005) between the severity of disease and UA in active patients is seen.	UA can be a Laboratory indicator for thyroid-Associated Ophthalmopathy (TAO)
Yang et al [10]	2831 euthyroid	Adults	Retrospective study	eGFR CKD-EPI was positively associated with FT3/FT4 (β = 23.31), and inversely correlated to PTFQI FT4 (β= -2.69) (both p <0.001). Thyroid hormone sensitivity index was negatively correlated to renal function.	Decreased sensitivity to thyroid hormone is associated with reduced renal function.
Xing et al [11]	4 databases	Adults	Systematic search	Significantly high UA levels in SCH as compared to controls. Prevalence of hyperuricemia in patients with subclinical thyroid dysfunction was higher than that of subjects with normal thyroid function, and the difference was statistically significant (I2 = 0%, p = 0.50, Z = 2.09, p = 0.04, OR : 1.16, 95% CI: 1.01–1.34.	SCH was significantly associated with hyperuricemia.
Torkian et al [1]	118	Adults (49.8 ±16.0 years)	Case-control study	UA (p<0.001) and TSH (p=0.006) are significantly high in SCH as compared to euthyroid controls. In SCH, TSH level correlated to creatinine levels but not with uric acid (r=0.302,p=0.001) and (r=0.033,p=0.772), respectively.	High UA and TSH in the SCH (P<0.05) as compared to controls. In SCH, significant correlation was found with creatinine but not with uric acid.
Jialin Li et al [13]	3,563 CKD patients	Adults	Retrospective study	Per 0.5 μIU/mL increment in TSH increased the risk of CKD stage 5 by 8% (1.08, 1.02-1.14). Per 0.3 ng/dL increase in FT4 was significantly associated with 21% reduced risk of CKD at stage5 (OR, 95%CI: 0.79, 0.69–0.89)	FT4 and TSH can be used as advanced-stage biomarkers among Chinese adults.

Sayari et al [14]	107	2–14 years	Case- control study	TSH (<0.001) but not UA (0.200) were significantly high in SCH as compared to controls. Non-significant correlation was found between TSH= 8.94±4.80 (mIU/L) and UA = 374.8 ±106.0 umol/L (r=0.043, p=0.759) in SCH.	UA in SCH children was not significantly different from control (p=0.200) and a non-significant correlation between TSH and UA was found in SCH.
See et al [15]	87,813	Adults	Retrospective, cross-sectional study	No significant correlation between TSH and serum UA (r=-0.005, p=0.164).	Both hyperthyroid and hypothyroid status were weakly associated with hyperuricemia.

UA: Uric acid, TSH:Thyroid stimulating hormone, eGFR: estimated glomerular filtration rate , FT3: free triiodothyronine, FT4 : free thyroxine, TFQ: thyroid feedback quantile-based index TSHI: thyroid stimulating hormone index, TT4R1: total thyroxine (T4) resistance index and TT3RI: total triiodothyronine (T3) resistance index. IVW: inverse variance weighting method, CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration equation PTFQI: Parametric Thyroid Feedback Quantile-based Index.

Table 3: Demonstration of typical laboratory data of some studies.

	UA	**TSH**	**eGFR**	**r/p**	**OR (95%CI)**
Torkin et al [1]	450.8 ± 123.7 µmol/L	5.4 (2.3-9.7) mIU/L	-	r = 0.033 p = 0.72	
Yang et al [8]	Males: 372.81±76.93 µmol/L Females: 272.2±61.25 µmol/L	Males- 2.18±2.48 mIU/L Females 2.52±2.25 mIU/L			Males: 1.370(1.006-1.866) Females: 1.25(0.858-1.838)
Yang et al [10]	362.05±91.74 µmol/L	1.68 (1.21) mIU/L	eGFR (CKD-EPI) <90 mL/ min/1.73m^2		Correlation of eGFR with TSH 1.29 (1.13~1.47) p= <.001
Zhang et al [17]	-	-	Estimated glomerular filtration rate was significantly depressed in both genders with mild hypothyroidism		The significantly elevated risk for hyperuricemia was observed in mild hypothyroidism male participants with an odd ratio of 1.49 (1.10–2.02), whereas no statistical risk was found in female.

Kuzell et al in 1955 first proposed the association between hypothyroidism and hyperuricemia. High levels of serum UA were associated with reduced glomerular filtration rate (GFR) and renal plasma flow in hypothyroidism patients [15,16].

Thyroid hormones can affect purine metabolism involving the de novo purines synthesis, salvage pathway and degradation. Alteration in these pathways can culminate in UA production and impair its degradation. SCH could decrease cardiac contractility, as a result the GFR can decrease by 20–30% to below normal levels, hence, changing reabsorption and secretion in the tubules, which increase the levels of UA and decrease in UA excretion, respectively [17]. Another study found an inverse association between serum TSH levels and eGFR, suggested hypothyroidism might be associated with a reduced kidney function [5]. Some studies demonstrated that hyperuricemia is associated not only with gout but also with numerous cardiometabolic diseases, such as hypertension, metabolic syndrome, diabetes, and obesity [3].

The difference, due to gender, regarding the association between SCH and hyperuricemia could be caused by the protective effect of estrogen in females [18]. A study suggested that the effect of the UA metabolism in patients with recent-onset SCH was mediated by insulin sensitivity [18]. Huang et al suggested

UA as an independent risk factor for the formation of thyroid nodules [7]. The cause-effect still cannot be determined. See et al, on the other hand, found no significant correlation between TSH and serum UA levels, with a correlation coefficient of r=-0.005 (p=0.164) [15]. Deng et al found individuals in the SCH group (337.95 ± 105.28 µmol/L) presented with lower UA ($p<0.05$) as compared to euthyroid (UA levels 352.11 ± 106.07 µmol/L) subjects [12]. Thyroid hormones play important roles in renal development and function of many transport systems along the nephron. Thus, hypothyroidism may contribute to the exacerbation of pre-existing chronic kidney disease or the occurrence of acute kidney injury in the presence of other renal insults [19].

Study limitations

We could not do the systematic review and meta-analysis of all the articles which would have strengthened our paper. Secondly, the data cannot be generalized to Indian population. Thirdly, valuable findings in the articles published in the local languages might have been missed since we included the articles published only in the English language. Furthermore, we reviewed articles published since 2013 only, this could have excluded important conclusions from articles published earlier.

Conclusion

This study indicates the profound influence of thyroid hormone on renal function. The levels of serum UA significantly increased in SCH compared to normal controls. High levels of serum UA were associated with reduced glomerular filtration rate (GFR) and renal plasma flow in hypothyroidism patients. The thyroid function should, therefore, be routinely assessed for evaluation of patients presenting with impaired renal function and vice versa. This demonstrated the negative impact of hypothyroidism on renal function. As a result, it is advised to examine the renal state both at the time of hypothyroidism diagnosis and during the follow-up period.

Conflict of interest

None

Ethical clearance

The approval from the institutional ethical committee was not required.

Source of funding

None

Data Availability

Data included within this article.

Authors' Contributions

Dr Premjeet Kaur: Designed the study, Retrieved literature, extracted data and wrote article.

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Research Article

Quality Control in RT-PCR Viral Load Assays: Evaluation of Analytical Performance for HIV, HBV, and HCV

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Keywords
Internal Quality Control; External Quality Control; RT-PCR (Reverse Transcription Polymerase Chain Reaction); HIV (Human Immunodeficiency Virus); HBV (Hepatitis B Virus); HCV (Hepatitis C Virus); Coefficient of Variation; Random Error; Systematic Error; Total Error; Westgard Rules; Levey-Jennings Chart.

Abstract

Introduction
Quality Control Management (QCM) in clinical laboratories is crucial for ensuring reliable results in analytical measurements, with biological variation being a key factor. The study focuses on assessing the analytical performance of the Reverse Transcription Polymerase Chain Reaction (RT-PCR) system for Human Immunodeficiency Virus (HIV), Hepatitis B (HBV), and Hepatitis C (HCV). Five models proposed between 1999 and 2014 offer different approaches to evaluating analytical quality, with Model 2 based on biological variation and Model 5 considering the current state of the art. The study evaluates the RT-PCR system's analytical performance through Internal Quality Control (IQC) and External Quality Control (EQC).

Materials and Methods
The Laboratório Central de Saúde Pública do Estado do Ceará (LACEN-CE) conducted daily IQC using commercial kits, and EQC was performed through proficiency testing rounds. Random error, systematic error, and total error were determined for each analyte.

Results
Analytical performance, assessed through CV and random error, met specifications, with HIV and HBV classified as "desirable" and "optimal." EQC results indicated low systematic error, contributing to total errors considered clinically insignificant.

Conclusion
The study highlights the challenge of defining analytical specifications without sufficient biological variability data. Model 5 is deemed the most suitable. The analytical performance of the RT-PCR system for HIV, HBV, and HCV at LACEN-CE demonstrated satisfactory, emphasizing the importance of continuous quality control in molecular biology methodologies.

Introduction

Quality Control

The analytical measurement of a given human biological parameter is subject to a range of variations due to laboratory and physiological causes. Among the laboratory ones, those related to the pre-analytical phase stand out, that is, posture at the time of collection, sample transport time and conditions, centrifugation time, collection method, order of tubes used, among others; Examples of the analytical phase are: methodology required for the test, particulars and maintenance of the equipment used, technical team that will carry out the test, climatic conditions, validity and batch change of reagents, among others. Biological variability, in turn, is associated with physiological factors, resulting from diet, circadian cycle, menstrual cycle, stress and emotional, diseases, psychological use of medications, sex, age, etc., causes to the patient and the analyte of interest to be assessed [1-3]. For Health Care Establishments (HCE), as well as clinical laboratories, ensuring the reliability of the results issued through Quality Control Management (QCM) is a requirement provided for by the Resolução da Diretoria Colegiada (RDC), which translates to Directors' Collegiate Resolution in English, Nº 786 of 5 May 2023. "The HCE that performs the Clinical Analysis Examination (EAC) must guarantee the reliability of the results through the QCM". The QCM consists of the routine evaluation of the analytical system according to each assay analyzed, considering the internal and external performance of the HCE [4]. The International Organization for Standardization (ISO), through standard 15189:2015, which deals with the quality and competence requirements of clinical laboratories, recommends that laboratories implement analytical procedures to verify the achievement of the desired quality in the results in addition to transporting the resulting variability the imprecision and inaccuracy of analytical methods [5]. To meet the requirements of ISO 15189:2015 and RDC No. 786, the HCE must provide a clinical result with an analytical measurement error lower than the limit allowed after sample processing in all analytical phases (pre-analytical, analytical and post-analytical). Therefore, the correspondence and clinical quality of this result must be guaranteed for medical and therapeutic management [4,5]. To monitor the accuracy of the analytical system, Internal Quality Control (IQC) is used. It is a sample, normally commercial, with an already determined analytical value, whose processing is carried out before the beginning of the laboratory routine in order to evaluate the precision, that is, the agreement between the results of the control sample among themselves, as well as the result that must be within the range recommended by the manufacturer [6]. The Coefficient of Variation (CV) measures the relative variability of the data in relation to the average. This is the statistical parameter most used to evaluate the precision of the analytical method. The CV classification can be according to the following description: CV within the recommended reference, "Minimum" CV, when the value is within 75% of the reference, "Desirable" CV, when the value is within 50% of the reference, "Optimal" CV, when the value is within 25% of the reference [7]. The standard statistical model for monitoring IQC data was proposed in 1950 by researchers Stanley Levey and E. R. Jennings, which is based on a graphical representation of the results participating in the mathematical model of the Gaussian distribution. initially in the industrial sector, until the 1980s, the use of the Levey-Jennings chart aimed to keep the results of the measurand within the range of two standard deviations. In 1981, researcher James O. Westgard proposed a series of rules for evaluating random and systematic errors according to the graphical behavior of IQC results. Levey-Jennings graphs and Westgard rules were then consolidated in the laboratory [8-10].

In addition to IQC, External Quality Control (EQC) is intended to measure the accuracy of the analytical method. To this end, the laboratory must regularly participate in proficiency testing or interlaboratory comparison programs, in order to receive samples from an institution and process them in its routine, providing similar treatment to patient samples. The result is reported and subsequently published by the evaluation group. Unlike the IQC, the EQC provides a qualitative-quantitative assessment of accuracy, as it allows the assessment of systematic errors, such as analytical method biases (bias) [6-11]. Sending precision by IQC and accuracy by EQC enables GCQ to mathematically calculate the random error caused by inaccuracy, and the systematic error resulting from inaccuracy, respectively, and, by adding them together, determine the total measurement error of the method which if you are following.

Models of Analytical Specifications

In 1999, during the first "Strategies to set Global Quality Specifications in Laboratory Medicine" in Stockholm, five hierarchical models were defined for the specification of analytical quality for clinical laboratories [12]. Leading the initiative at the Conference was a group of researchers headed by Carmen Ricos, who also published a study in the same year titled "Current databases on biologic variation: pros, cons and progress," containing data for 350 analytes. Until 2019, this group compiled information in partnership with the Westgard Q.C. website. Subsequently, the maintenance of the database and its updates would be the responsibility of the European Federation of Clinical Chemistry and Laboratory Medicine (EFML) [13]. The EFML provides in its biological variation database the records of the intraindividual Coefficient of Biological Variation (CVbi) and intergroup or interindividual Coefficient of Biological Variation (CVBg) for 2716 analytes, with data based on metadata analysis and estimates corroborated by scientific research [14]. The project began after the first conference organized by the same institution, titled "Defining analytical performance goals 15 years after the Stockholm Conference on Quality Specifications in Laboratory Medicine." The central objective of the EFML is to assess the quality of biological variation data to enable users, including clinical laboratories, to make critical analyses of their processes regarding inherent variations in specific analytes [14]. Differences between the models determined at the two conferences can be observed in Table 1. In table 1, it is possible

to observe the advancements between the years 1999 and 2014 regarding models for monitoring the performance of the analytical system [12-15].

Table 1: Models proposed at the Stockholm and Milan conferences.

	Comparative Models	
Model	**Stockholm 1999**	**Milan 2014**
Model 1	Evaluation of the impact of analytical performance on clinical restults in specific clinical settings.	Evaluation of the impact of analytical performance on clinical results. To develop quality specifications using results, one of the following procedures must be followed: A. A results study investigating the impact of analytical performance on the probability of clincal outcomes; C. A survey of opnions from physicians and/or specialists investigating the impact of analytical performance on medical decisions.
Model 2	Evaluation of the impact of analytical performance on overall clinical decisions: A. Data based on components of biological variation; B. Data based on the analysis of physicians' opinions.	Based on components of biological variation: The goal is to ensure that "analytical niose" does not drown out the biological signal. In the new project, it was emphasized that there are indeed significant limitations to this approach, including the relevance and validity of biological data.
Model 3	Published professional recommendations: A. From national and international sepcialized bodies; B. From local specialized groups or individuals.	
Model 4	Performance goals defined by: A. Regulatory bodies; B. From Organizers of External Quality Assessment (EQA) schemes.	
Model 5	Goals based on the current state of the art. A. As demonstrated by data from EQA schemes of Proficiency Tests; B. As found in current publications on methodology.	Based on the current state of the art: It is based on the realistic performance "as-is" in the market. If the best laboratories can only achieve a certain quality but cannot meet the quality required by models 1 and 2, then the current performance is accepted (for now).

Following the implementation in 1999, Model 2 became the most sought after by clinical laboratories due to its provision of a tangible numerical parameter for statistical analysis for HCE. However, after the publication of the Milan conference report, the limitations of the model became evident, including the lack of data for a variety of analytes measured in routine laboratory practice, as well as the validity of the then-available data [13-15]. In the following years, international standards such as ISO and the Clinical and Laboratory Standards Institute (CLSI), along with national regulations developed by regulatory bodies in various countries, based on Model 2, sought to determine desirable limits of imprecision, bias, and total error using CVBi and CVBg data [13].

Molecular Biology and Viruses

Polymerase Chain Reaction (PCR) is a molecular biology technique developed and automated since the 1980s [16,17]. The success of this method lies in its heightened sensitivity and accuracy in detecting and identifying genetic material unique to the analyte of interest through genetic material extraction, followed by amplification (composed of denaturation, annealing, and extension of genetic material), culminating in its analysis [18-20]. Subsequently, to maximize the analytical process, Reverse Transcription Polymerase Chain Reaction (RT-PCR) was developed. The main attraction of this modification was the condensation of the amplification and result analysis steps, as well as the reduction of the minimum genetic material required for the reaction, the ability of the method to process RNA template strands, and provide quantitative results according to gene expression [20-21]. Given the various permissible applications of RT-PCR, the quantification of microorganisms such as viruses and bacteria made it unique for monitoring and guiding medical interventions in the management of highly complex conditions [20]. In this context, Human Immunodeficiency Virus (HIV), Hepatitis B and C (HBV and HCV, respectively), conditions of public health interest, once assessed through rapid

and serological tests incapable of providing quantitative results regarding the viral load in the patient, are currently evaluated by molecular biology methods, which have advantages in terms of specificity, sensitivity, and better monitoring of therapies employed for the treatment of such infections.

In the pursuit of continuous improvement, Clinical Laboratories employ new technologies and methods, such as molecular biology, to provide healthcare professionals with more sensitive clinical data to assist in therapeutic decision-making. This effort aims to minimize costs associated with unnecessary or inappropriate therapies, expedite the diagnostic process, and enhance the capacity for short and long-term therapeutic monitoring. To ensure that such methods are under analytical control, i.e., their results are reliable and under stabilized random and systematic errors, is of utmost importance for issuing clinically meaningful reports. QCM, therefore, plays a fundamental role in ensuring this success. The objective of this study was to evaluate the analytical performance of the RT-PCR system used for the assay of determining the viral load of HIV, HBV, and HCV. Thus, random error was calculated according to IQC, systematic error according to EQC, and total error based on the two previous ones.

Materials and Methods

The Laboratório Central de Saúde Pública do Estado do Ceará (LACEN-CE) is the Reference Laboratory for the State of Ceará, with the responsibility of conducting Laboratory Surveillance through analyses of interest to Health Surveillance, acting, among other functions, in monitoring the epidemiological situation in the State of Ceará. It has more than 11 sectors dedicated to monitoring various health issues, such as bacterial and mycobacterial diseases, parasitic diseases, arboviruses, mycoses, and viral diseases, totaling 787,861 assays during the year 2023. To achieve this, it relies on a wide variety of technologies for monitoring the respective analytes. In this context, the Laboratory for HIV and Viral Hepatitis Viral Load (BHH) operates at the forefront of monitoring viral diseases using the molecular biology method, RT-PCR, detecting and quantifying the viral load of previously diagnosed patients undergoing pharmacotherapeutic monitoring. Routine procedures involve processing an internal Roche quality control kit containing three IQC levels for each condition for every 21 samples, with low, high and negative levels [22]. The quantitative data from the low and high levels are manually entered into the Google Sheets© software, which automatically transforms this data into logarithmic values of base 10. They are then evaluated using Levey-Jennings graphs, initially following the knowledge principles of manufacturer. After 100 observations, obtaining mean and standard deviation values, the results are evaluated according to pre-established Westgard rules. The determination of random error, considering 95% reliability, was made from the coefficient of variation mathematically obtained, as demonstrated by equation 1, using data collected during the months of October, November, and December 2023. Only results for low and high levels, within 2 standard deviations, the limit recommended by the manufacturer, were considered valid [23].

Equation 1:

$$Coefficient\ of\ Variation\ (\%) = \left(\frac{Standard\ Deviation}{Mean}\right) * 100$$

To obtain the random error, equation 2 was applied. The evaluation of the random error results was done according to the classification of “minimum,” “desirable,” and “optimal.” The specification criterion used was the maximum limit recommended in the package insert for each analyte.

Equation 2:

$$Random\ error\ (95\%\ confidence) = CV * 1.65$$

Trimestrally, the laboratory participates in external quality control rounds, during which it receives samples with unknown presence and viral load results. The determination of the systematic error of the method was obtained with the results from the two rounds of the year 2023, covering the months of August, September, October, November, and December of the year 2023. For this purpose, equation 3 was employed:

$$Systematic\ error\ (\%) = \left(\frac{Laboratory\ result - Round\ Mean}{Round\ Mean}\right) * 100$$

The total error for each assay was calculated by summing the systematic error and random error. It was classified according to the total error guidelines recommended by the Ministério da Saúde (MS) which translates to Ministry of Health, in English. [24]. As specification criteria for the IQC, reference values from the manufacturer’s instructions were used, and then the corresponding CV and random error limits for analytical performance were mathematically calculated.

Results

During the last quarter of 2023, 7,119, 231, and 336 samples of HIV, HCV, HBV, respectively, from different healthcare units in the State of Ceará, were processed, totaling 7,686 assays during the period. This represents 22.4% of the analyses conducted by the sector throughout the year. Table 2 shows the quantity of controls processed during this interval. In table 2, it is possible to observe the number of samples processed during the year 2023, in the last quarter of 2023, and the number of controls processed for the same period.

Table 2: Historical series of analyses and IQC processed in 2023.

	Samples		Control Kits
Virus	**Number of Samples in 2023**	**Number of Samples in the Last Quarter**	**Number of Control Kits in the Last Quarter**
HIV	32699	7119	339
HBV	980	336	16
HCV	606	231	11

The results of the IQC were categorized based on the data from the IQC kit manufacturer, meeting the terms "minimum," "desirable," and "optimal," as shown in Table 2. The analytical performance, by month, can be seen in Table 3. Considering the cumulative results for the quarter, it is observed that HIV maintained a "desirable" result, HBV an "optimal" result, and HCV maintained a "desirable" result in two months. All evaluated analytes remained within specifications for both CV and random error. In table 3, the manufacturer's data regarding the analytical performance of the IQC is presented. The CV and Random Error Specification were calculated based on the Mean and SD values.

Table 3: Parameters of the manufacturer's IQC kit.

	Manufacturer's IQC Parameters					
	HIV		HBV		HCV	
Control level of analytes	**HIV Low**	**HIV High**	**HBV Low**	**HBV High**	**HVC Low**	**HVC High**
Mean (Log)	2.57	5.31	2.17	6.30	2.15	6.24
SD (Log)	0.32	0.32	0.32	0.32	0.32	0.32
Reference CV (%)	9.38	9.38	10.08	10.08	10.16	10.16
Minimun (%)	7.04	7.04	7.62	7.62	7.56	7.56
Desirable (%)	4.69	4,69	5.08	5.08	5.04	5.04
Optimal (%)	2.35	2.35	2.54	2.54	2.52	2.52
Random Error Specification (95% CI%)	15.48	15.48	16.63	16.63	16.76	16.76
Minumum (%)	11.62	11.62	12.57	12.57	12.47	12.47
Desirable (%)	7.74	7.74	8.38	8.38	8.32	8.32
Optimal (%)	3.88	3.88	4.19	4.19	4.16	4.16

In table 4, you can observe the results of Coefficient of Variation (CV) and random error for each analyte in each observed month, along with their averages. The classification values are based on the parameters from Table 3.

Table 4: IQC Performance Results

Analyte	CV (%)	Random Error (%)	Classification
October			
HIV	2.74	4.52	Desirable
HBV	1.41	2.32	Optimal
HCV	2.99		Desirable
November			
HIV	2.60	4.30	Desirable
HBV	2.48	4.09	Optimal
HCV	2.32	3.83	Optimal
December			
HIV	2.68	4.43	Desirable
HBV	1.86	3.07	Optimal
HCV	0.80	1.32	Optimal
Quarterly Comulative Average			
HIV	2.68	4.42	Desirable
HBV	1.86	3.16	Optimal
HCV	2.04	3.36	Optimal

EQC was assessed based on the results of the last two rounds of the year 2023. The average percentage of systematic error was calculated for each condition from the obtained results, as shown in Table 3.

In table 5, it is possible to evaluate the results of the EQC according to the rounds in August and November, as well as the total systematic error per analyte during the assessed period. The systematic error was calculated in absolute value.

Table 5: EQC Results.

	External Quality Control			
Round	**Group Round Mean**	**LACEN-CE Mean**	**System Error (%)**	**Average Systematic Error (%)**
HIV				
August	4.16	4.00	3.89	3.37
November	2.79	2.66	2.85	
HBV				
August	3.34	3.32	0.51	0.65
November	2.54	2.52	0.79	
HCV				
August	5.82	5.73	1.58	1.75
November	4.48	4.39	1.92	

The total error was determined by summing the average systematic and random errors, as shown in Table 6.

Table 6: The table presents the sum of IQC and EQC

	Total Error		
Analyte	**Average Random Error (%)**	**Average Systematic Error (%)**	**Total Error (%)**
HIV	4.42	3.37	7.79
HBV	3.16	0.65	3.81
HCV	3.36	1.75	5.11

In Table 7, the simulation involved calculating the deviation of the result from the total error value, derived from the average value in the leaflet, for each control level. This was done to assess whether the variation between the average result and the result considering the error is clinically significant, based on the recommendation of the MS, which determines a variation of up to 0.5 Log as a limit [24].

Table 7: Mathematical simulations of the clinical impact of total error on system-released results.

		Total Error	
Control level of Analyte	**LACEN-CE EQC Result (Log)**	**Maximum Limit considering Total Error of table 6 (Log)**	**Variation between LACEN-CE Result and Maximum Value (Log)**
HIV-high	4.00	4.31	0.31
HIV-low	2.66	2.87	0.21
HBV-high	3.32	3.45	0.13
HBV-low	2.52	2.62	0.10
HCV-high	5.73	6.02	0.29
HCV-low	4.39	4.61	0.22

Conclusion

Biological variation describes the observed variation in the concentration or activity of different constituents in an individual, reflecting regulation by homeostatic processes in the body [25]. The use of IQC provides daily elements that allow the operator to identify errors or atypical behaviors in the analytical performance of the system. In the event of IQC errors, the analytical routine should be postponed until the cause of the error is identified, and appropriate actions are taken to correct the analytical performance and initiate the routine [6]. In seeking literature specifications for Intra- and Inter-Individual Biological Variation (CVBi and CVBg) related to the analytes of interest, no information was found in the reference databases, EFLM and Westgard Q.C., regarding HIV, HBV, and HCV or even other analytes measured by RT-PCR techniques. The absence of information, a limitation foreseen since the Stockholm Conference in 1999, leads the clinical laboratory to determine specifications that align with the models presented in the conferences of either Stockholm or Milan [12-16]. The LACEN-CE employs the use of commercial Roche© kits for internal control, which have specifications that should guide the analytical performance of the system at the national and international market levels. From these specifications, presented in Table 2, mathematically determined values of CV and Acceptable Random Error were established to assess the analytical performance of the system. Although the analysis and treatment pattern follows the Model 2, which relates to biological variability, the analytical specification that best suits the procedure adopted in this study is Model 5, which expresses the "state of the art" available for a particular analyte. This term refers to "a methodological procedure that aims to develop a mapping of scientific productions" whose result is the "descriptive inventory of academic and scientific production on the topic investigated" [26]. Given the fact that methodologies involving biological variation have been widely disseminated since the first decade of 2000, after the Stockholm conference, the limitation of content involving the biological variability of analytes such as HIV, HCV and HBV in infected individuals is understandable. The MS, through the Unidade de Assistência e Laboratório da Coordenação Nacional de DST e Aids, Assistance and Laboratory Unit of the National STD and AIDS Coordination, in english, recommended, in a technical note from 1999, as a significant analytical variation for the viral load assay for HIV the value of 0.5 Log10 between measurements of the same patient, that is, a precision parameter [24]. The use of data from the KIT manufacturer, on the other hand, supports the specification of the selected model 5, due

to the fact that it has mastery of the production technique and monitoring of the quality of control KITs and the performance of the equipment itself. Thus, the "optimal" results for HBV and HCV, and "Desirable" for HIV are corroborated by mathematical projections based on the manufacturer's data; and the automation of the equipment, which allows for the minimization of variation in the analytical phase, arising from the operator, instrumentation analytical and related interferences. The EQC can also be evaluated according to the state of the art, just like the IQC, based on the results available in each round by the proficiency test advisory group. However, unlike the IQC, there are a range of factors that disadvantage the model with regard to quantitative assessment, including the number of participants in each round, which directly interfere with the predictive value of random or systematic error.Thus, it was not possible to establish a single model for the critical assessment of systematic error. It was observed, however, that the results obtained during the rounds, for the three analytes evaluated, presented values slightly below the group average, suggesting trends. There is no clear specification regarding the total allowable error for testing HIV, HCV and HBV. This supports the limitation of model 2 parameters, in addition to the scarcity of information that can adapt to the requirements of model 1, making it therefore necessary to resort to model 5, with more restrictions in the evaluation process. To monitor the significance of the total error, reflecting systematic and random error, mathematical simulations were carried out that considered the LACEN result in each round of the proficiency test and the variation in the result, resulting from the calculated total error. This value was compared with the significant clinical variation proposed by the MS. From the data obtained in Table 7, it is possible to verify that the total error measured in the study, when applied to the results obtained, did not demonstrate variations, which in the clinical environment would be considered significant. This corroborates the stability of the analytical process, recommended by current legislation. It is important to note that during the monitored period, there were no rule violation infractions or operational problems. The present study made it possible to determine random, systematic and total error. In order to be able to indicate the analytical performance of the RT-PCR methodology for HIV and viral hepatitis at LACEN-CE. The limitation of biological variability data and clinical studies that provide parameters for clinical laboratories and especially for public health laboratories to use as specifications, demonstrates the relevance of the current initiative. The achievement of "optimal" and "desirable" performance for the monitored analytes indicates the quality with which the processes are evaluated and monitored by LACEN-CE, as well as confirmation that the total error values do not imply clinically significant results. With access to molecular biology technologies by clinical laboratories, it becomes increasingly necessary to pay attention to the data and elements available to manage methodologies and ensure the quality of results released by laboratories.

Disclosure Statement

I, Gabriel Thé Araújo Gomes, the primary author of this manuscript, certify that there are no conflicts of interest or impediments that could influence the results or interpretations presented in this work. I declare that: I have no financial affiliations with organizations or entities that may have a direct or indirect interest in the content of this manuscript. I have not received funding or financial support from any entity that could influence the conduct or presentation of this work. I have no financial interests, patents, or stock holdings in companies related to the topic addressed in this manuscript. This disclosure statement is provided in good faith and reflects complete transparency regarding potential conflicts of interest.

Declaration of Helsinki

The present work did not use information, samples or biological material from patients treated by the Laboratório Central de Saúde Pública do Estado do Ceará. All scientific research was carried out using commercial internal quality control kits.

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Research Article

Diagnostic Utility of Pan-Immune-Inflammation Value (PIV) in Predicting Insulin Resistance: Results from the National Health and Nutrition Examination Survey (NHANES) 2017–2020

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Article Info

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Keywords

PIV, pan-immune-inflammation value, insulin resistance, inflammation, diabetes, metabolic syndrome.

Abstract

Background

Insulin resistance (IR), a hallmark feature of diabetes and metabolic syndrome, is characterized by chronic low-grade inflammation. Pan-immune-inflammation value (PIV), an emerging immune cell count-based inflammatory index, is the global quantifier of systemic inflammation. This study analyses the levels of PIV and its association with various markers of IR.

Materials and Methods

This retrospective, cross-sectional study was done using the Center for Disease Control-National Health and Nutritional Examination Survey (CDC-NHANES) pre-pandemic data from 2017–2020. Data from 4620 survey participants was included after screening. Homeostasis model assessments of insulin resistance (HOMA-IR) and beta-cell function (HOMA-B), triglyceride glucose (TyG) index, visceral adiposity index (VAI), and lipid accumulation product (LAP) were used as markers of IR. Multiple logistic regression and trend analysis were done to determine the associations, and receiver operator characteristic curve (ROC) analysis was done to estimate the diagnostic utility of PIV to predict IR.

Results

PIV levels were significantly higher in obesity, diabetes, and metabolic syndrome. HOMA-IR, HOMA-B, LAP, VAI, and TyG levels were found to be higher in those with higher PIV (i.e., quartiles 4 and 3). Regression and trend analysis showed that the odds ratio for IR increased with PIV. However, ROC indicated that the diagnostic utility of PIV to predict IR is low compared to the other surrogate markers.

Conclusions

PIV levels differed significantly based on glycemic status, BMI, and metabolic syndrome status. PIV showed a significant positive association with IR. However, the ability of PIV to predict IR is not optimal compared to other surrogate markers.

Introduction

Insulin resistance (IR) is the major hallmark feature of type 2 diabetes mellitus (DM) and metabolic syndrome. IR is a complex metabolic defect leading to a decreased response toward insulin, impaired regulation of blood glucose levels, and other adverse events [1]. IR is recognized as a chronic low-grade inflammation state affecting various tissues, mainly adipose tissue, liver, and skeletal muscle [2]. Adipose tissue-derived cytokines (i.e.,) adipokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), adipokines (leptin, adiponectin, and resistin), monocyte chemoattractant protein-1 (MCP-1), and nuclear factor kappa-B (NFκB) are widely reported to promote low-grade inflammation, which could play a central role in IR [3,4].

IR can be determined to an extent by various biochemical and anthropometric indices. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and Beta-cell Function (HOMA-B) are widely used markers of IR [5]. Triglyceride glucose (TyG) index, visceral adiposity index (VAI), and lipid accumulation product (LAP) are other surrogate markers of IR [6,7]. The associations between inflammatory markers and markers of insulin resistance have been studied extensively. High-sensitivity C-reactive protein (hs-CRP), a widely used marker of systemic inflammation, showed a significant positive association with insulin resistance as measured by HOMA-IR [8], and high CRP could independently predict IR in the future [9]. Estimation of serum CRP is usually done in those with inflammation and infection. The accumulating evidence regarding the pathogenesis of the disease and advancements in diagnostic assays have led to the development of various biomarkers of inflammation, such as IL-6, IL-1 beta, and TNF-alpha.

In this regard, several blood cell count-based inflammatory biomarkers have gained importance in cancer. Pan-immune-inflammation value (PIV), a relatively new biomarker of inflammation derived using the counts of neutrophils, lymphocytes, platelets, and monocytes, was a better prognostic marker in cancer. As it encompasses all major immune cells, PIV is considered the global quantifier of the cellular compartment of systemic inflammation [10]. The PIV values predicted mortality in ST-elevation myocardial infarction (STEMI) [11], end-stage renal disease (ESRD) [12], and hepatic steatosis [13]. However, PIV levels in patients with diabetes mellitus and metabolic syndrome and their association with IR have not been addressed.

Hence, this study was done to determine the levels of PIV in those with diabetes and metabolic syndrome. The association of PIV with various markers of IR was also explored in this study.

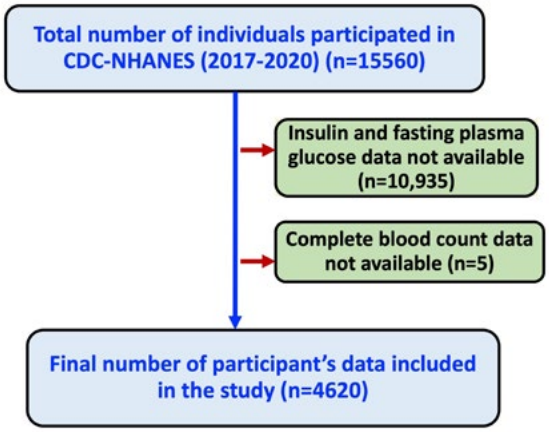
Methods

Data source

The study is done using the data obtained from the Center for Disease Control's (CDC) National Health and Nutritional Examination Survey (NHANES) pre-pandemic data from 2017–2020. The survey was approved by the National Center for Health Statistics (NCHS) Ethics Review Board (ERB) (Protocol #2018-01, Continuation of Protocol #2011-17, effective October 26, 2017). The survey was carried out in compliance with the Declaration of Helsinki. The participants were interviewed, and subsequent physical examination and laboratory investigations were done at the mobile examination center (MEC) after obtaining informed consent [14]. This completely de-identified data is available in the public domain; hence, subsequent approval from the NCHS ERB and institutional review board is exempted for this study.

Among the participants who participated in the survey (n = 15560), only those with data on complete blood count (CBC), fasting plasma insulin, and glucose were included in the study (n = 4620) (Figure 1). The methodology used for CBC, fasting plasma insulin, glucose, and lipid profile were discussed in detail [14].

Figure 1: Flow chart to describe the retrieval of data



CDC - Center for Disease Control, NHANES - National Health and Nutrition Examination Survey

Formulas used for calculating PIV, HOMA-IR, HOMA-B, LAP, TyG and VAI

PIV is calculated using the formula [10]:

$$PIV = \frac{\text{Neutrophils (1000 cells per μL)} * \text{Platelets (1000 cells per μL)} * \text{Monocytes (1000 cells per μL)}}{\text{Lymphocytes (1000 cells per μL)}}$$

Homeostatic model assessment of insulin resistance (HOMA-IR) and - beta cell function (HOMA-B) [5] is calculated by

$$HOMA\text{-}IR = \frac{\text{Fasting insulin (μU/mL)} * \text{Fasting plasma glucose (mg/dL)}}{405}$$

$$\text{HOMA-B}=\frac{20*\text{Fasting insulin }(\mu U/mL)}{\text{Fasting plasma glucose (mg/dL)}-63}$$

Visceral adiposity index (VAI) [6] is calculated by:

$$\text{VAI (men)}=\frac{\text{Waist circumference (in cm)}}{(39.68+(1.88*\text{BMI}))}*\left(\frac{\text{Triglycerides(in mg/dL)}*0.012229}{1.03}\right)*\left(\frac{1.31}{\text{HDL-C(in mg/dL)}*0.02586}\right)$$

$$\text{VAI (women)}=\frac{\text{Waist circumference (in cm)}}{(39.58+(1.88*\text{BMI}))}*\left(\frac{\text{Triglycerides(in mg/dL)}*0.012229}{0.81}\right)*\left(\frac{1.51}{\text{HDL-C(in mg/dL)}*0.02586}\right)$$

Lipid accumulation product (LAP) [6] is calculated as follows:

LAP(men)=(Waist circumference(in cm)-65)*(Triglycerides (in mg/dL)*0.012229)

LAP(women)=(Waist circumference (in cm) 58) * (Triglycerides(in mg/dL)*0.012229)

Triglyceride glucose (TyG) [7] is calculated by:

$$\text{TyG}=\text{Ln}\left(\frac{\text{Fasting plasma glucose (mg/dL)}*\text{Triglycerides (mg/dL)}}{2}\right)$$

Criteria for Metabolic Syndrome, BMI, Prediabetes, and Diabetes Mellitus.

The metabolic syndrome is diagnosed based on the American Heart Association-National Heart Lung Blood Institute (AHA-NHLBI) guidelines [15]. BMI values are used to diagnose overweight, obesity, and underweight based on CDC guidelines [16]. The participants are categorized into normoglycemia, prediabetes, and diabetes based on the American Diabetes Association 2023 guidelines [17].

Statistical analysis

All statistical analyses were performed using the R programming language, version 4.3.1. The parameters were checked for their distribution by the Shapiro-Wilk test, and appropriate statistical tests were conducted. The data across the quartiles were analyzed using the Kruskal-Wallis test with post-hoc Bonferroni correction. Receiver operator characteristic (ROC) curves were plotted for PIV and other surrogate measures of insulin resistance to predict metabolic syndrome. The ROC curve is plotted using the "pROC" R package, which calculates the sensitivity, specificity, and optimal cut-off value of Youden's index [18]. The diagnosis of metabolic syndrome is done by the R package "MetabolicSyndrome" [19].

Results

The baseline characteristics of the participants included in the study are represented in Table 1.

Table 1: Baseline characteristics of the participants.

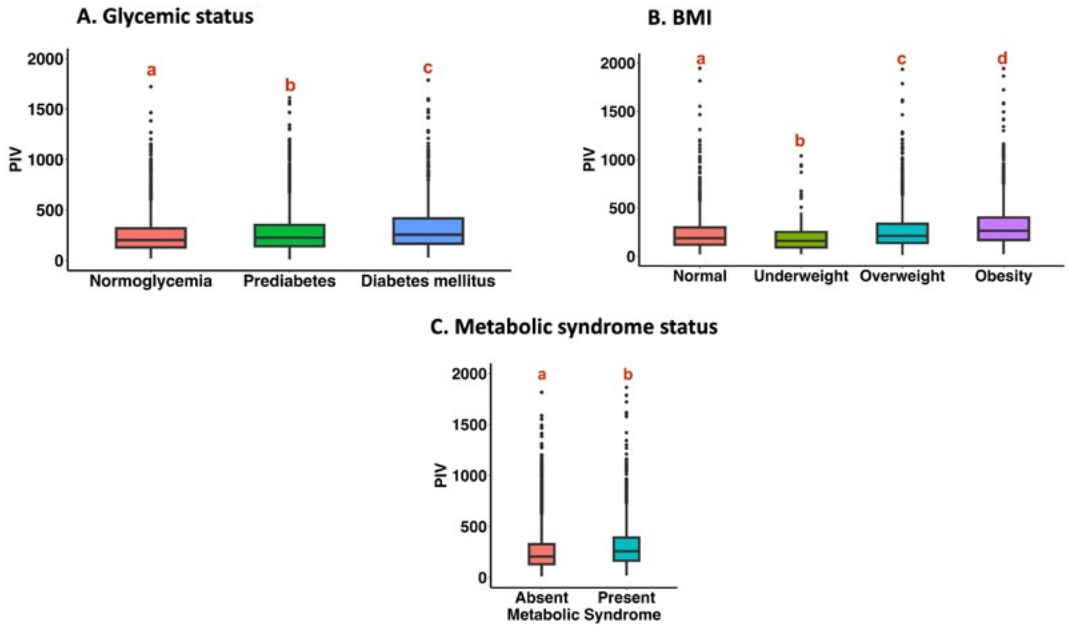
Parameter	
Number of participants	4620
Age in years	46 (27-62)
Gender (%)	
Male	2251 (49)
Female	2369 (51)
BMI status (based on CDC guidelines)	
Underweight	141 (3.1)
Normal	1276 (28.1)
Overweight	1370 (30.2)
Obesity	1753 (38.6)

Glycemic status (based on ADA criteria, 2023)	
Normoglycemia	1593 (34.5)
Prediabetes	2270 (49.1)
Diabetes mellitus	757 (16.4)
Metabolic Syndrome status (based on AHA-NHLBI criteria, 2005)	
Yes	1616 (35)
No	3004 (64)
Pan - immune inflammation index value (PIV)	221.9 (139.6-352.1)
CRP, mg/L	1.7 (0.7-4.2)
Fasting plasma glucose, mg/dL	102 (95-112)
HbA1C, %	5.5 (5.3-5.9)
Fasting plasma insulin, µU/L	10.2 (6.3-16.6)
Markers of insulin resistance	
HOMA-IR	2.6 (1.6-4.6)
HOMA-B	4.9 (3.1-8)
VAI	1.36 (0.83-2.22)
TyG	9.22 (8.85-9.76)
LAP	39.88 (20.37-69.31)

The continuous data are represented by the median (interquartile range). The categorical data (gender, BMI status, glycemic status, and metabolic syndrome status) are represented in numbers (percentages). Homeostatic model assessment of insulin resistance (HOMA-IR) and beta cell function (HOMA-B), visceral adiposity index (VAI), triglyceride-glycemic index (TyG), and lipid accumulation product (LAP) are shown as makers of insulin resistance.

PIV values differ significantly based on glycemic status, as they were higher in those with diabetes and pre- diabetes compared to those with normoglycemia (Figure 2A). The increase in PIV values paralleled BMI, as it was found to be higher in those with overweight and obesity and lower in those with underweight (Figure 2B). PIV levels were significantly higher in those with metabolic syndrome. (Figure 2C)

Figure 2: Comparison of PIV values based on Glycemic status, BMI and Metabolic Syndrome status



The pan-immune-inflammation values (PIV) were compared based on the glycemic status (A), body mass index, BMI (B), and metabolic syndrome status (C). The box and whisker plots showing dissimilar alphabets are significantly different from one another ($p<0.05$). THe Kruskal-Wallis test iwth post hoc Bonferroni correction was done (A, B), and the Mann-Whitney U test was dont (C).

The data was categorized into quartiles using PIV values, and baseline characteristics were analyzed across the quartiles (Table 2). The age of the participants was significantly higher in Q3 and Q4 (i.e., in those with higher PIV values). The gender distribution was similar across the quartiles. The glycemic status was significantly different across the quartiles, with significantly higher number of diabetics in Q3 and Q4. The metabolic syndrome status was significantly different across the quartiles, with significantly higher number of metabolic syndrome participants in Q3 and Q4 (Table 2).

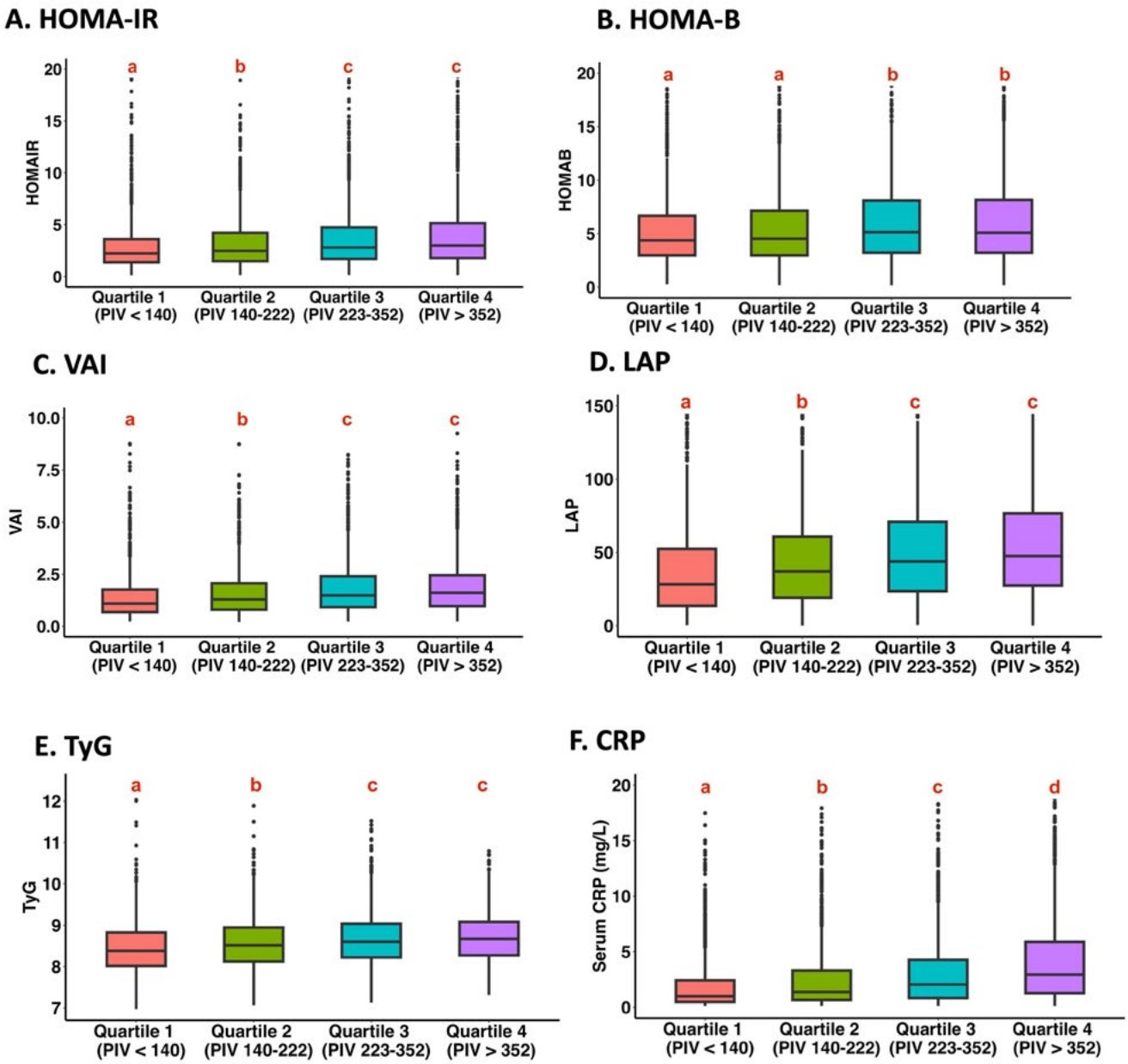
Table 1: Comparison of baseline characteristics across the PIV Quartiles

Parameter	Quartile 1, Q1 (PIV < 140)	Quartile 2, Q2 (PIV 140-222)	Quartile 3, Q3 (PIV 223-352)	Quartile 4, Q4 (PIV > 352)	P value
No of participants	1155	1155	1155	1155	-
Age in years	43[a] (24-60)	45[a] (27-60)	46[b] (28-63)	51[c] (31-67)	<0.0001
Gender (%)					
Male **Female**	556 (49) 589 (51)	589 (51) 566 (49)	548 (47) 566 (49)	548 (47) 607 (53)	0.268
Glycemic status (based on ADA criteria)					
Normoglycemia **Prediabetes** **Diabetes mellitus**	458 (40) 558 (48) 139 (12)	428 (37) 555 (48) 172 (15)	378 (33) 582 (50) 195 (17)	329 (28) 575 (50) 251 (22)	<0.0001
Metabolic Syndrome status (based on AHA-NHLBI criteria)					
Yes **No**	301 (26) 854 (74)	355 (31) 800 (69)	466 (40) 689 (60)	494 (43) 661 (57)	<0.0001

The data were categorized based on pan-immune-inflammation values (PIV) into quartiles (Q1, Q2, Q3, and Q4). The categorical data (gender, glycemic status, metabolic syndrome status) were represented as numbers (percentages) and compared across the quartiles using the Chi-Square test. Age was expressed as median and interquartile range (IQR), and the Kruskal-Wallis test with post hoc Bonferroni correction was done. Quartile with dissimilar alphabet in their superscript denote that age in that quartile were significantly different from the other.

The markers of insulin resistance and inflammation were compared across the PIV quartiles (Figure 3). There was a significant difference in the levels of these markers across the quartiles (Kruskal-Wallis test, $p < 0.0001$). The HOMA-IR, VAI, LAP, and TyG values trended upward as PIV increased (i.e., from Q1 to Q4). The values were higher in Q2, Q3, and Q4 compared to Q1. However, the values were not different between Q4 and Q3. (Figure 3A, 3C-3E). The HOMA-B values were higher in Q3 and Q4 compared to Q1 and Q2. The HOMA-B values were not different between Q1 vs. Q2 and Q3 vs. Q4 (Figure 3B). Serum CRP values were increased in parallel with the PIV values from Q1 to Q4 (Figure 3F).

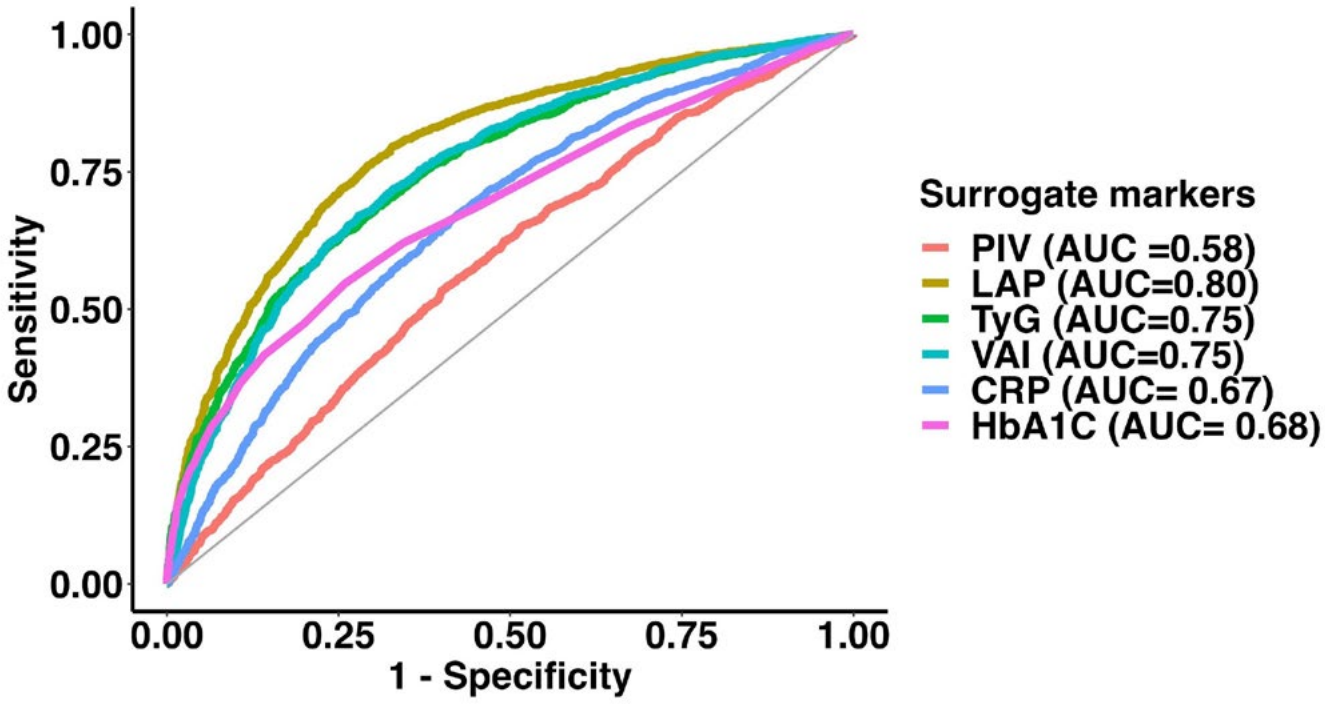
Figure 3: Comparison of surrogate markers of insulin resistance and inflammation across the PIV quartiles



The data were categorized based on pan-immune-inflammation values (PIV) into quartiles (Q1, Q2, Q3 and Q4). The markers of insulin resistance and inflammation were compared across the quartiles using the Kruskal- Wallis test and Mann Whitney U test with post hoc Bonferroni correction to do pairwaise comparisons. The box and whisker plots showing dissimial alphabets are significantly different from one another ($p<0.05$). Homeostatic model assessment of insulin resistance, HOMA-IR (A) and beta cell function, HOMA-B (B), visceral adiposity index, VAI (C), lipd accumulation product, LAP (D), triglyceride-glycemic index, and TyG (E) were shown as markers of insulin resistance. C-reactive protein, CRP (F), was shown as a marker of inflammation.

ROC curves were plotted for PIV and other surrogate measures to predict insulin resistance. HOMA-IR was used to categorize the participants into insulin-resistant (cut-off > 2.73) and non-insulin-resistant (cut-off < 2.73) [20]. LAP performed better with an AUC of 0.80 among the surrogate markers, followed by VAI (AUC =0.75), TyG (0.75), CRP (0.67), and HbA1C (0.68). The AUC of PIV is 0.58, suggesting it is not useful as a marker to predict insulin resistance (Figure 4).

Figure 4: ROC of PIV and other surrogate markers to predict insulin resistance



Receiver operator characteristic curve, ROC was constructed to predict the diagnostic utility of pan-immune-inflammation value, PIV and other surrogate markers to predict insuling resistance. Homeostatic model assessment of insulin resistance, HOMA-IR was used to cateogrize insulin resistance i.e. thode with HOMIR cutoff < 2.73 were non-insulin resistant and those with > 2.73 were insulin resistant. Predictive ability of Visceral adiposity index (VAI), lipid accumulation product (LAP), triglyceride-glycemic indic (TyG), C-reactive protein (CRP), HbAIC were also studied.

Multiple logistic regression was carried out with four models to analyze the association between PIV and IR. The effect of the model can be interpreted as an increase in PIV leading to a corresponding increase in IR. In model 1 (i.e., the unadjusted model), the incidence of IR increased by 0.08% with one unit increase in the variance of PIV, and the OR (95% CI) were 1.0008 (1, 1.001). The results of models 2 (age and gender adjusted), 3 (age, gender, and BMI adjusted), and 4 (age, gender, BMI, diabetes, and prediabetes adjusted) were similar, indicating that the strategy used for adjustment was sufficient. Collectively, PIV was independently positively associated with the occurrence of IR. However, the association was weak, as suggested by the OR (Table 3). Further, to ensure the stability of the results across various ranges of PIV, the trend test was carried out across PIV quartiles. The PIV was transformed into a categorical variable by grouping it into four levels as quartiles. Q1 was taken as the reference; the incidence of VAI and IR represented a monotonically increasing trend in all models (all P for trend < 0.001) (Table 3). This aligns with the finding that the HOMA-IR values trended upward as PIV increased (i.e., from Q1 to Q4) (Figure 3). The OR was higher as the PIV value increased (i.e., in Q2-Q4) in all models, suggesting the significant positive association of elevated PIV values with IR (Table 3).

Table 3: Multiple logistic regression model to determine the association between PIV and insulin resistance.

Viariable	n (%)	Model 1		Model 2		Model 3		Model 4	
		OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
PIV	4620	1.0008 (1-1.001)	<0.0001	1.0008 (1 – 1.001)	<0.0001	1.0004 (1.0001 – 1.0007)	<0.0001	1.0003 (1 -1.0006)	0.014
PIV Quartiles									
Quartile 1	1155	1 *(Ref)*	<0.0001	1 *(Ref)*	<0.0001	1 *(Ref)*		1 *(Ref)*	
Quartile 2	1155	1.34 (1.13-1.59)	<0.0001	1.33 (1.13-1.58)	<0.0001	1.12 (0.93-1.35)	0.233	1.14 (0.93-1.4)	0.186
Quartile 3	1155	1.82 (1.55-2.15)	<0.0001	1.8 (1.52-2.12)	<0.0001	1.31 (1.08-1.51)	0.004	1.66 (1.36-1.11)	0.002
Quartile 4	1155	2.01 (1.78-2.49)	<0.0001	2.05 (1.74-2.42)	<0.0001	1.38 (1.14-1.67)	<0.001	1.68 (1.37-1.11)	0.002
P for trend	4620	1.76 (0.57-1.98)	<0.0001	1.73 (1.54-1.95)	<0.0001	1.29 (1.12-1.47)	<0.001	1.28 (1.11-1.48)	<0.001

Model 1 – unadjusted, model 2 – adjusted for age and gender, model 3 - adjusted for age, gender and BMI, model 4 - adjusted for age, gender, body mass index, diabetes, and pre-diabetes. Homeostatic model assessment of insulin resistance, HOMA-IR was used to categorize insulin resistance (HOMA-IR cut-off < 2.73 – non-insulin-resistant and > 2.73 – insulin-resistant).

Discussion

Insulin resistance seen in diabetes and metabolic syndrome is regarded as a chronic inflammatory state [3], predisposing to impaired glucose tolerance, dyslipidemia, and hypertension [21]. PIV was significantly higher in those with diabetes, prediabetes, and metabolic syndrome. The studies have reported that increased levels of PIV seen in hypertensive [22] and NSTEMI patients [11] are associated with all-cause mortality. Hence, further prospective studies are required to study whether elevated levels of PIV seen in those with diabetes and metabolic syndrome are associated with all-cause mortality.

The interaction between insulin resistance, low-grade inflammation, and obesity has been well-elucidated in previous studies. In this study, it was found that PIV increased with BMI, and its levels were found to be significantly higher in those with overweight and obesity. Serum CRP increased in parallel with the PIV values in this study. This finding was expected, as PIV is considered a marker for inflammation. A previous study has shown that serum CRP levels are positively correlated with PIV values in patients with carcinoma [23].

In this study, there was a significant difference in the levels of surrogate markers of IR when the data was analyzed based on quartiles of PIV. The HOMA-IR, CRP, and lipid-based surrogate markers of IR (VAI, LAP, and TyG) trended upwards as PIV increased, i.e., from Q1 to Q4, suggesting the relationship between insulin resistance and inflammation (Figure 2). There are studies that have shown a positive association between elevated CRP and HOMA-IR [24,25].

ROC curve analysis showed that LAP, VAI, and TyG performed as better markers to predict insulin resistance. It has been reported in a previous study that lipid-based surrogate markers of IR can aid in identifying insulin resistance in prediabetes and diabetes [26]. However, PIV and CRP levels lacked predictive utility as markers of insulin resistance (Figure 4). The multiple logistic regression analysis of the unadjusted and adjusted models showed a weak positive association between IR and PIV. There was an increasing trend in the odds ratio for the association of PIV and IR as the PIV increased from Q1 to Q4 (Table 3).

Various complete blood count (CBC)-derived inflammatory indices such as neutrophil-lymphocyte ratio (NLR) [27], platelet-lymphocyte ratio (PLR) [28], monocyte-lymphocyte ratio (MLR) [29], and systemic immune-inflammation index (SII) [30] have been used in estimating chronic low-grade inflammation in insulin resistance. The role of PIV has been well-elucidated in cancer as a biomarker to determine prognosis and survival outcomes [10]. This study addresses the utility and association of PIV with insulin resistance in U.S. adults.

Limitation

The retrospective cross-sectional study design of this study

allows us to determine the associations. Hence, large-scale prospective studies can confirm the predictive role of PIV in insulin resistance.

Conclusion

PIV levels differed significantly based on BMI, glycemic status, and metabolic syndrome. The proportion of participants with diabetes and metabolic syndrome was higher in those with higher PIV values (i.e., quartiles 3 and 4). The participants with higher PIV values had increased levels of HOMA-IR, VAI, TyG, and LAP compared to those with lower PIV values, suggesting an association of PIV with insulin resistance. Multiple logistic regression and a trend analysis showed that the odds ratio for insulin resistance increases as the PIV value increases. However, the ROC analysis revealed a poor AUC, indicating a low diagnostic utility of PIV as a marker of insulin resistance. Hence, large- scale longitudinal studies are needed to ascertain the role of PIV as a marker for IR.

Author contributions

Ramasamy J: conceptualized and designed the study, acquired the data, analyzed data, interpreted results, and wrote the manuscript; Murugiah V: interpretation of data, drafting the manuscript; Balasubramaniam G and Dhanapalan A: analyzed data, drafting the manuscript.

Ethics Approval

The survey was approved by National Center for Health Statistics (NCHS) Ethics Review Board (ERB) (Protocol #2018-01, Continuation of Protocol #2011-17, effective through October 26, 2017). The survey was carried out in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

Disclosures

Conflict of interests

The author does not have any conflict of interest to disclose in this study.

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Data availability

This data is in the public domain and is available online.

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Research Article

From Practice to Proficiency: Evaluation of a Novel Workplace-Based Assessment (WBA) in a Postgraduate Chemical Pathology Residency Program in Pakistan

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Keywords
WBA, Chemical Pathology, Formative assessment, Trainee development, Residency, Virtual Learning Environment

Abstract

Introduction
A workplace-based assessment (WBA) model was implemented in the postgraduate (PG) residency program of Chemical Pathology at the Department of Pathology & Laboratory Medicine, Aga Khan University (AKU). PGs were assessed using direct observation of practical skills (DOPS), evaluation of clinical events (ECE) and case-based discussion (CBD) on a virtual learning environment (VLE) platform.

Objectives
To evaluate WBA frequency, case mix, feedback, and satisfaction levels of faculty and PGs of Chemical Pathology at AKU.

Methods
Data from January 2019 to June 2023 was assessed. Tool utilization and case mix frequencies were calculated. PG and faculty satisfaction levels, as well as feedback and discussion time, were averaged. A thematic analysis was conducted on descriptive comments.

Results
Out of 911 WBAs attempted, 79.1% (n=730) were CBDs, 10.8% (n=98) were DOPS, and 9.1% (n=83) were ECEs, showing a well-distributed case mix. Average satisfaction levels for CBD, ECE, and DOPS among both PGs and faculty were 8.38, 8.48, and 8.59, and 8.20, 8.36, and 8.46, respectively. Faculty feedback averaged 8.40, 8.65, and 7.85 minutes for CBD, ECE, and DOPS, respectively. Discussion times averaged 9.37, 9.52, and 13.36 minutes for CBD, ECE, and DOPS, respectively. Suggestions for development were noted in 20.82% (n=225) of CBDs, 21.69% (n=18) of ECEs, and 16.32% (n=16) of DOPS. Positives were documented in 40% (n=292) of CBDs, 28.92% (n=24) of ECEs, and 7.14% (n=7) of DOPS.

Conclusion
This study evaluated a web-based WBA model in chemical pathology training, suggesting its applicability in diverse pathology specialties and regional training programs.

Introduction

Workplace-based assessment (WBA), initially labelled as a formative or 'assessment-for-learning' was introduced in postgraduate medical education (PGME) as part of a global shift towards competency-based medical education. Its primary aim is to steer postgraduate (PG) trainees towards specific learning objectives [1]. Formative assessment does not simply assign grades to trainee performance at specific points in the curriculum. Instead, it is curated to seamlessly integrate into the instructional process with the overarching goal of fostering and enhancing learning [1]. One significant advantage of WBAs over other evaluation methods, such as multiple-choice questions or objective structured clinical examinations (OSCEs), is their ability to facilitate real-time assessments. With this approach, the supervisor directly observes the trainee in a specific aspect of clinical practice, thereby increasing the validity of WBAs compared to other assessment methods [2]. However, evidence suggests that the greatest impact of WBA lies in providing observation-based feedback making it a powerful tool for positively impacting learning behaviours by encouraging proactive pursuit of feedback [3-5]. Despite these appeals, concerns regarding the practical feasibility of WBA persist as the implementation of WBA is seen as an additional demand on the trainer and trainee in busy clinical settings [1, 4]. To complete post graduate medical training in chemical pathology, guidelines of the College of Physicians and Surgeons of Pakistan (CPSP) mandate the completion of a 4-year training program. Responsibilities of PG trainees in Chemical Pathology mainly include examining preanalytical, analytical and post analytical aspects before validating any biochemical result, investigating interferences on test results, conducting clinical audits for quality and process improvements, and collaborating with consultants in performing procedures, provocative tests and assessing new tests for potential introduction in service to name a few [6-8]. Concerns about resident involvement and postgraduate medical education (PGME) in Chemical Pathology have been brought up by developments in laboratory medicine. Multiple surveys of pathology residents have shown that Chemical Pathology's supporting learning environments are inadequate. Additionally, after finishing their residency program, many graduates have stated feeling inadequate to practice clinical chemistry on their own [9, 10]. These issues are further augmented by the concern that PG trainees in Chemical Pathology are seldom observed and given feedback, whereas feedback is central to learning and lies "at the heart of medical education [3]." To address this, a web-based WBA model was implemented in January 2019 in a postgraduate residency program of chemical pathology at Aga Khan University (AKU) using a virtual learning environment (VLE) platform, Moodle, to assess the knowledge, skills, professionalism, and critical thinking of PG trainees while providing comprehensive feedback. Validated WBA tools were chosen using Norcini AMEE guide and modified according to the needs of chemical pathology training [8]. The objective was to facilitate ongoing evaluation and learning for trainees in high-volume laboratory environments by seamlessly integrating assessment into their daily work activities. This approach ensures that trainees receive timely feedback on their performance, allowing them to identify areas for improvement and further development. By embedding assessment within the context of their work, WBA enables trainees to apply theoretical knowledge to practical situations, fostering a deeper understanding of key concepts and enhancing their skills. Moreover, the continuous nature of WBA promotes a culture of self-reflection and improvement, empowering trainees to take ownership of their learning journey. Overall, WBA serves as a powerful intervention in medical education, offering a holistic approach to assessment that aligns closely with real-world clinical practice. A grading scale ranging from 1 to 6 was employed, where ratings of 1 to 2 indicated performance below expectations, 3 denoted borderline performance, 4 signified meeting expectations, and ratings of 5 to 6 indicated performance above expectations. In instances where ratings fell within the range of 1 to 3, focused faculty feedback was provided, necessitating re-training within the respective domain, followed by reassessment. Furthermore, a predetermined minimum number of WBAs was established for each residency year. These assessments were required to be completed and documented within the VLE platform, constituting an integral component of the promotion criteria. It is widely acknowledged that the success of innovations in medical training programmes hinges on promptly identifying opportunities and pitfalls [11]. Thus, the goal of our present study is to evaluate the data recorded on VLE since the introduction of WBA five years ago and assess its efficacy and long-term feasibility. We intend to analyze the frequency of WBA tools usage, the regularity of feedback provided to the PGs, the distribution of specialties among participating PGs, the variety of cases and procedures discussed, the average time taken for feedback and discussion, satisfaction levels of both PGs and assessors, and examine qualitative feedback to identify potential gaps and areas for improvement.

Methods

A retrospective study was conducted at the Section of Chemical Pathology, Department of Pathology and Laboratory Medicine, AKU, from September 2023 to January 2024. The WBA tools most relevant to Chemical Pathology training including direct observation of practical skills (DOPS), evaluation of clinical events (ECE) and case-based discussion (CBD) were implemented. All PGs, including fellows, registered in the chemical pathology training program at AKU and those rotating in the section of Chemical Pathology from other Pathology, Pediatric or Medicine specialties were eligible for WBA. Assessors included faculty, senior technologists, and sectional managers of the Chemical Pathology section at AKU [8]. Data was extracted from all the responses following the completion of each WBA form on Moodle between January 2019 to September 2023. The permission of AKU's ethical review committee was sought before the initiation of this project. The cumulative number of WBAs conducted since January 2019 was calculated, including the utilization of each WBA tool on an annual basis.

Averages were computed for the feedback and discussion duration associated with each tool, categorized by year. To assess the range of topics discussed in WBAs, an analysis was performed on all cases covered in each assessment, with frequencies calculated accordingly. Furthermore, frequencies were determined for the procedures and instruments evaluated using the DOPS tool separately. Satisfaction ratings from students and faculty regarding the WBA process, recorded on a 10-point Likert scale in each WBA form, were averaged. To gain insight into the participation of PG trainees in WBAs to date, the total number of participating trainees, along with their respective specialties, was also analyzed. Each WBA form featured two sections for qualitative feedback to PG students: one for developmental suggestions and another prompting comments on particularly positive aspects of the conducted WBA. The frequency of feedback provision for each WBA tool was assessed. Additionally, a qualitative analysis was conducted on the feedback comments provided in both sections. Conceptually similar statements were grouped together and systematically organized under thematic categories.

Results

Our collected sample represents a total of 911 WBA forms out of which 80.1% (n=730) were CBDs, 10.8% (n=98) were DOPS, and 9.1% (n=83) were ECEs.

Figure 1 illustrates the case mix and percentage distribution of topics covered under all WBA tools. Table 1 represents the procedures and instruments upon which DOPS was demonstrated.

Figure 1: Percentage distribution of WBA case mix topics covered from 2019 to 2023 at the section of Chemical Pathology (n=911)

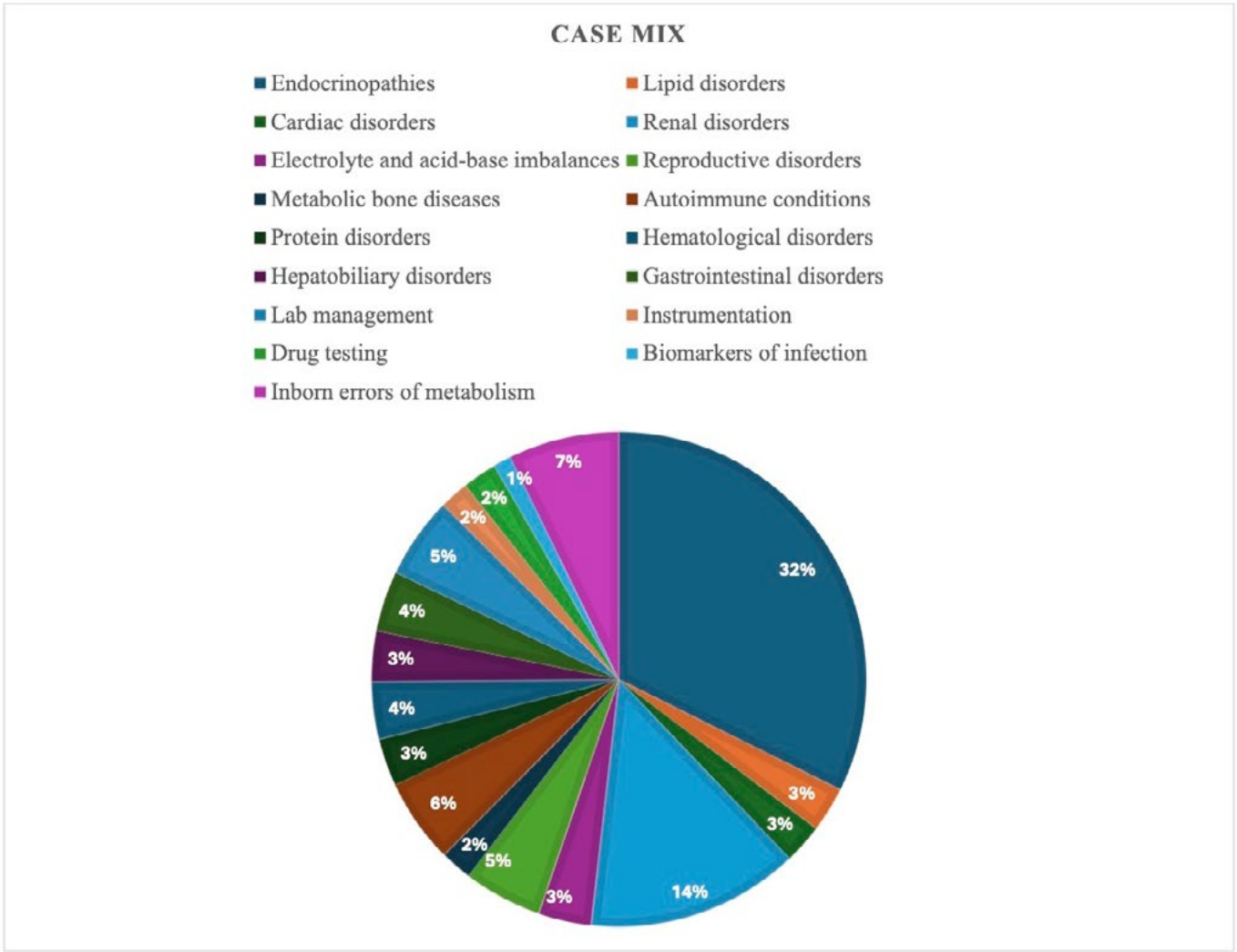

Table 1: Procedures and instruments upon which DOPS was demonstrated from 2019 to 2023 at the section of Chemical Pathology (n=98)

	Instrument/Procedure (DOPS)	**Frequency**
1.	Procedures	19
	Growth hormone insulin test	4
	Breath hydrogen test	6
	Sweat chloride test	9
2.	Lab management	11
	Complaint handling	1
	Recovery study	1
	SOPs preparation	1
	File maintenance	7
	Preparation of inventory binder	1
3.	Instrumentation	57
	Integrated clinical chemistry and immunoassay analyzer	1
	Chemiluminescence immunoassay	4
	Isoelectric focusing electrophoresis	1
	Gas chromatography mass spectrometry	1
	ABG analyzer	4
	Centrifugation	6
	Stone analyzer (FTIR)	24
	Electrophoresis	2
	Analytical balance	3
	Total lab automation	1
	Semi-automated clinical chemistry analyzer	1
	Osmometer	4
	Immunofixation electrophoresis	4
	Point of care testing	1
4.	Method validation and verification	7
	Linearity study	1
	Precision study	1
	Prolactin (PEG method)	5
5.	Lab Safety	4
	Biological and chemical spill	4

DOPS proved to be the most efficient at feedback provision with a duration of 7.85 minutes compared to CBD (8.40 minutes) and ECE (8.65 minutes). However, DOPS discussions were lengthier, averaging at 13.36 minutes compared to CBD (9.37 minutes) and ECE (9.52 minutes). Trends for these variables across the years are shown in Figures 2(a), 2(b) and 2(c) for CBD, ECE, and DOPS, respectively. The average PG satisfaction levels with CBD, ECE, and DOPS were found to be 8.38, 8.48, and 8.59 on a 10-point Likert scale, respectively. On the same scale, faculty satisfaction levels for CBD, ECE and DOPS averaged 8.20, 8.36, and 8.46, respectively. Since 2019, 47 PG trainees have taken part in WBAs. Out of these, majority were rotating trainees from histopathology (28%), followed by hematology (23%), microbiology (17%), adult endocrinology (13%), and pediatric

endocrinology (4%). The trainees of chemical pathology represented 15% of all participants. Regarding feedback comments, suggestions for development were provided in 20.82% (n=225) of CBDs, 21.69% (n=18) of ECEs, and 16.32% (n=16) of DOPS. Positive aspects of the conducted WBA were highlighted in 40% (n=292) of CBDs, 28.92% (n=24) of ECEs, and 7.14% (n=7) of DOPS. The descriptive analysis of feedback comments on the three WBA tools yielded the following themes presented in Table 2.

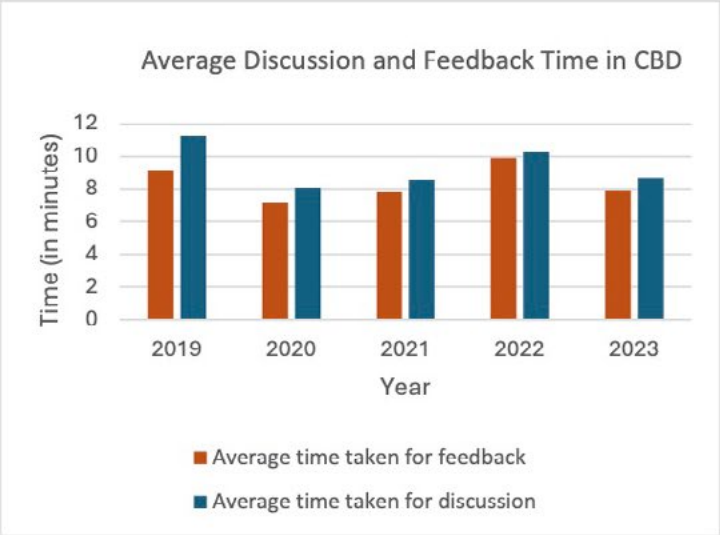

Figure 2(a). Average time taken for discussion and feedback in CBD

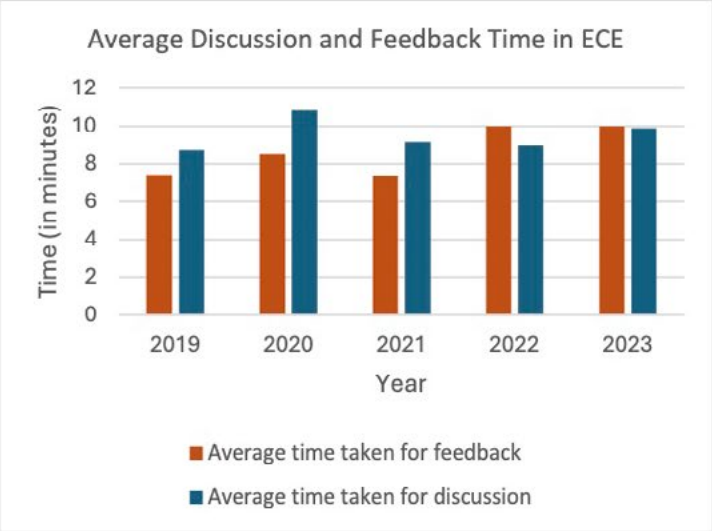

Figure 2(b). Average time taken for discussion and feedback in ECE

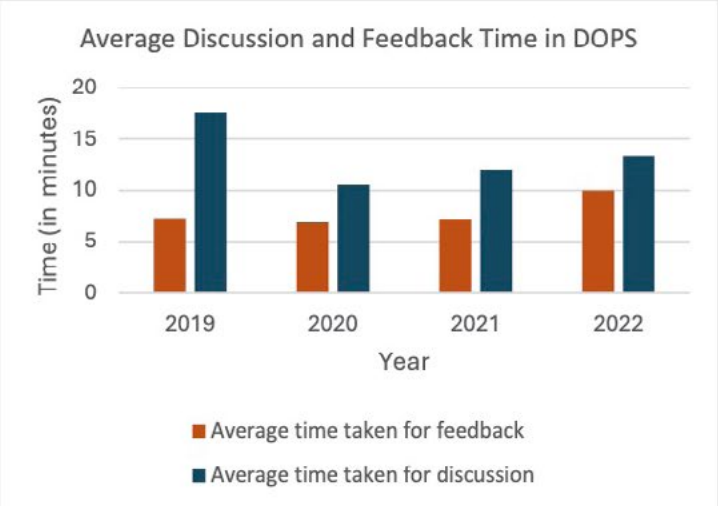

Figure 2(c). Average time taken for discussion and feedback in DOPS

Table 2: WBA tools yielded the following themes.

	ECE	DOPS	CBD
Suggestions for Development	• Research skills enhancement • Diversification of knowledge base • Quality control and methodology understanding • Improvement in technological competence	• Skill practice enhancement • Revision of SOPs • Technical and instrumental proficiency development	• Improvement in the understanding of laboratory techniques and methodologies • Further improvement in clinical and pathophysiological knowledge • Professional development and research collaboration
Positive highlights	• Communication and proactivity • Clinical competence and case management • Comprehensive theoretical understanding	• Professional conduct • Test knowledge proficiency	• Effective selection, presentation, and preparation of cases • Proactive learning opportunity and knowledge demonstration

Discussion

To our knowledge, our study represents a pioneering effort in Pakistan by introducing a WBA model within a chemical pathology training program. Although formative assessments are popular in residency programs across various medical specialties, their utilization in pathology subspecialties remains relatively scarce [12]. Given the modest size of the chemical pathology residency program, with a limited number of trainees nationally and globally, our study offers valuable insights. The growing volume of WBAs conducted over successive years adds to the validity of our study findings. Moreover, during the initial phase of our model implementation in 2019, faculty and PGs qualitative feedback on perception of WBA was taken via interviews. aimed at identifying challenges and areas for improvement [8]. This feedback was instrumental in refining the processes, ensuring enhancements were made prior to its official implementation in the residency program. Furthermore, as faculty time constrained was a leading challenge and to facilitate, few senior lab scientists and manager were also included as evaluators in the program particularly for bench skills. Our evaluation revealed that the WBA model is being successfully employed in the chemical pathology residency program at AKU. This is evident by its frequent use, feedback and discussion sessions that are convenient for both students and faculty in the clinical workplace, and a diverse case-mix. Furthermore, the high satisfaction levels expressed by both our assessors and PG trainees, alongside the substantial positive highlights documented in faculty feedback, affirm the successful integration of WBA into our chemical pathology training program and day-to-day laboratory practice. We discovered that CBD was the most frequently used WBA tool. This compares to the findings from a UK study where NHS trainee doctors and trainers preferred CBD over DOPS as they facilitate "real-learning" as opposed to DOPS which are merely "tick-box exercises [13]." In accordance with prior studies, our research also supported the value of WBAs in enabling structured narrative feedback and supporting learners with suggestions for further development [14-18]. In contrast, a relatively understudied role of WBAs is in their potential to assess entrustable professional activities (EPAs) in graduate training programs. Entrustable professional activities (EPAs) are quickly taking over as a key assessment tool in the implementation of competency-based medical education. Proposed by ten Cate in 2005, EPAs are "units of professional practice, defined as tasks or responsibilities to be entrusted to the unsupervised execution by a trainee once he or she has attained sufficient specific competence [19]. Thus, assessments based on EPA generate summative entrustment decisions, deducing an individual's ability to perform under a specified level of supervision [20]. Given the success of our current WBA model, further steps can be taken to upgrade it into an EPA-based WBA tool designed to monitor entrustment decisions, supervision levels, and the autonomy of PG trainees. A similar model implemented for fellows of pediatric critical care medicine provided compelling evidence in support of an EPA-based WBA tool to improve learning by benchmarking and monitoring entrustment levels [21]. Furthermore, we successfully demonstrated the feasibility and advantages of incorporation of a virtual learning environment platform, Moodle, as a core part of PG training. For any healthcare institute, a VLE can offer a platform where students' performance can be recorded and monitored across the years. Especially in the local context, limited literature exists for exploring the scope and potential of e-learning in medical education [22, 23]. This is particularly unfortunate because evidence suggests that Moodle has been successfully used in teaching acute medicine, urology, physiology, dental radiology, thoracic surgery, and ethics [24]. Therefore, there is an imminent need for medical institutes to consider online learning platforms to support and encourage innovations in medical education. The outcomes of the descriptive analysis revealed many aspects of the narrative feedback provided to the PG trainees. The

positive aspects of our trainees were highlighted by assessors which mainly included clinical competence, proactive approach to learning and a strong theoretical foundation. Additionally, suggestions for development were provided, serving as valuable pointers for focused learning for trainees. Our analysis across all three WBA tools demonstrated common developmental areas including the need to develop technical proficiency, cultivate a broader knowledge base and explore opportunities for professional development such as research collaborations. These findings will help the faculty in providing focused assistance and guidance to trainees for a more personalized learning experience to build their proficiency in these identified weaker areas. This also aligns with cognitive theorist's belief that emphasizing the disparity between an individual's current performance and the desired level of performance serves as an important catalyst for learning [25]. To further enhance the feedback's efficacy, assessors should consider fostering recipient reflection-in-action. This would ensure that the trainee's reflections inform the formulation of an action plan which would emphasize self-monitoring and self-regulated learning, while fostering a supportive teaching and learning environment [26, 27]. To evaluate the genuine impact of our WBA model on a graduating trainee, our way forward is to conduct a study involving graduates from our training program who were exposed to this model to assess the effectiveness of the WBA approach in enhancing their real-world practice. However, as with any novel approach, our model had its limitations and gaps. We found that the frequency of documented feedback was considerably less than the number of WBA forms attempted. This could potentially mean that there was real-time verbal feedback which was not mentioned in the WBA forms. Similar issues have emerged internationally, with many institutions now opting for WBA smartphone and tablet apps which ensure real-time completion and uploading of feedback [1]. In addition to documentation issues in feedback, we identified several other areas with incorrect documentation where assessors did not consistently complete the forms in their entirety or occasionally filled them out incorrectly. Specifically, some assessors failed to explicitly state the name and type of topics being discussed or document the agreed action with the student as required. This discovery offers the opportunity to discuss and tackle these concerns among the assessors.

Conclusion

Overall, our study contributes valuable insights to the ongoing discourse on effective assessment methodologies in competency based medical education. This research was the first of its kind to successfully evaluate the implementation of a web-based WBA model in a chemical pathology training program at a tertiary care facility. Our findings imply that there is potential for replicating this tool in other sub-specialties of pathology, as well as other post-graduate training programs both locally and regionally. Additionally, they underscore the advantages of technological integration and utilizing e-learning platforms in medical education, highlighting the numerous opportunities they can offer to medical institutions.

Author disclosure statement

The authors declare that they have no conflicts of interest to disclose.

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None.

Ethical considerations

This study was conducted in accordance with the Declaration of Helsinki. The permission of AKU's ethical review committee was sought before the initiation of this project.

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Research Article

Paraprotein interferences: Insights from a short study involving multiple platforms and multiple measurands

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Keywords

Multiple myeloma, paraprotein interferences, erroneous results, wet chemistries, Bland Altman plots, regression analysis.

Abstract

Background

Though paraproteinaemic interferences is a well-known phenomenon in clinical chemistry, a large-scale evaluation study involving multiple paraproteinaemic specimens on multiple platforms including multiple measurands with an aim to provide a predictive analysis, is singularly lacking. The present study aims to fill this gap in research.

Material and Methods

This cross-sectional non-interventional observational study involved thirteen paraproteinaemic subjects, determined their gamma globulin characterization and measured their total bilirubin, direct bilirubin, HDL-cholesterol, calcium, inorganic phosphate, iron and unsaturated iron binding capacity (UIBC) levels on a dry chemistry platform (Vitros 350) as the established method and two wet chemistry platforms (AU5800 and Cobas 6000) as the evaluation methods. Data thus generated was analyzed for any significant variation and tested if such variation increased with decreasing albumin/ globulin ratio.

Results

Significant variation between dry chemistry and wet chemistry measurements were obtained for direct bilirubin, HDL and iron on AU5800 with p-values of 0.0009, <0.0001 and 0.0466 respectively. Similarly, discrepant results were obtained on Cobas 6000 for direct bilirubin and iron, with p-values of <0.0001 and 0.0002 respectively. Additionally, UIBC measurements on AU5800 varied significantly with increasing amounts of paraprotein present in the specimen (p-value = 0.0207).

Conclusion

This study emphasizes on predictive analyses to show that paraprotein interferences are fairly common on wet chemistry platforms. Evolving algorithms for monitoring of reaction curves can minimize release of erroneous results due to such interferences.

Introduction

Since the inception of quantitative estimation of measurands in clinical chemistry, analytical interferences have been a bane towards achieving accuracy in tests results. Accordingly, considerable effort has been directed in finding ways to mitigate this shortcoming. In particular, awareness against haemolysis, icterus and lipaemia have been optimized such that most modern auto-analyzers have in-built checks against such interferants. Considerable research is ongoing even against drug interferences [1] and interferences in immunoassays [2]. However, a more insidious and stealthier interferant is often overlooked – that is due to paraproteinaemia. Multiple myeloma, Waldenström's macroglobulinaemia, plasmacytoma, amyloidosis and monoclonal gammopathy of undetermined significance (MGUS) account for majority of the paraproteinaemic cases. Prevalence of these conditions are still unclear, however, the prevalence of MGUS, the most common cause of paraproteinaemia, is approximately 3 – 4 % of individuals > 50 years and 6 – 8 % of individuals > 80 years old. About 70 % of paraproteins are IgG, 15 – 20 % IgM and 10 – 15 % IgA [3].

Among original equipment manufacturers (OEMs), dry chemistry platforms like Vitros (by Ortho Clinical Diagnostics, recently renamed as Quidel Ortho) have made pioneering contributions in ameliorating paraprotein interferences [4], by incorporating a physical barrier (Spreading Layer) in their reaction slides, which filter out the potentially interfering proteins. Such measures have proven to protect against spurious results of inorganic phosphate, bilirubin, electrolytes, lipids etc., as evidenced from peer-reviewed literature [5,6]. Even though there are several instances of research involving the effect of paraproteins on individual measurands, to the best of the author's knowledge, there is a gap in research regarding large-scale comparison involving several measurands and involving two or more platforms to examine the effects of paraprotein interference. The present work-up, is thus an effort to fill up this gap, where thirteen individuals characterized as being tested positive for various forms of monoclonal gammopathies are subjected to testing of seven clinical chemistry parameters viz. Total & Direct Bilirubin, HDL-Cholesterol, Calcium, Inorganic Phosphate, Iron and Unsaturated Iron Binding Capacity (UIBC), in a Vitros 350 dry chemistry platform as the established method and two wet chemistry platforms – AU5800 (Beckman-Coulter) and Cobas 6000 (Roche) – as the evaluation methods.

Materials and Methods

This cross-sectional non-interventional observational study was undertaken at Drs. Tribedi & Roy Diagnostic Laboratory, 93 Park Street, Kolkata, India, which is a private undertaking tertiary-level referral laboratory, from January 2023 to June 2023. Informed consent from the study subjects and ethical clearance from the Drs. Tribedi & Roy Diagnostic Laboratory Ethical Committee was duly undertaken for this study, in accordance with all relevant national guidelines. Study subjects were chosen based on their decreased albumin-globulin ratio as revealed as an incidental finding during routine tests. Furthermore, subjects with known prior liver or kidney disorders, with known dyslipidaemias or anaemia and those under treatment with iron supplements, Vitamin D supplements, bisphosphonate therapy or under treatment for any sort of monoclonal gammopathies were excluded from this subset of decreased A/G ratio population. Venous blood samples were collected observing standard aseptic procedures and transferred to standard gel separator tubes [7, 8]. All samples were tested on the same day of collection.

Table 1: Study population

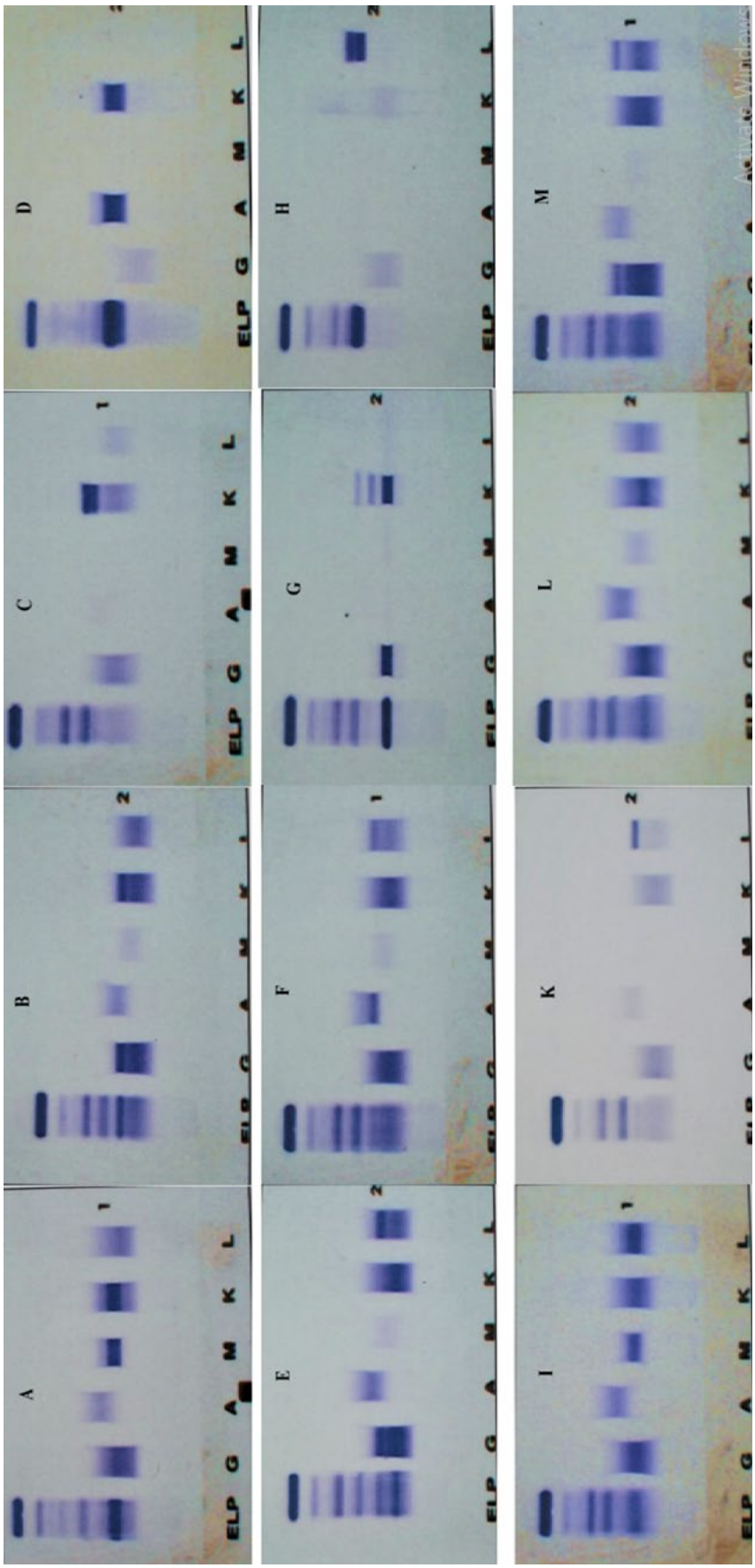
Study Subjects	Demographics	Total Protein, g/dL	Albumin g/dL	Globulin g/dL	A/G Ratio	IFE Characterization
Subject A	Male, 63 yrs, Bengali	13.9	3.1	10.8	0.29	Monoclonal gammopathy of IgM (κ – Light Chain).
Subject B	Male, 52 yrs, North Indian	11.1	2.5	8.6	0.29	Monoclonal gammopathy of IgG (κ – Light Chain).
Subject C	Female, 77 yrs, Bengali	13.9	3.2	10.7	0.30	Monoclonal gammopathy of κ – Light Chain (Two bands seen).
Subject D	Male, 49 yrs. Bengali	9.9	2.8	7.1	0.39	Monoclonal gammopathy of IgA (κ – Light Chain).
Subject E	Male, 65 yrs, Bengali	11.1	3.4	7.7	0.44	Biclonal gammopathy of monoclonal IgG (κ – Light Chain) and monoclonal IgG (λ – Light Chain).
Subject F	Male, 71 yrs, North Indian	14.4	3	11.4	0.26	Monoclonal gammopathy of IgA (λ – Light Chain).

Subject G	Female, 62 yrs, Mongoloid	10.6	3.3	7.3	0.45	Monoclonal gammopathy of IgG (κ – Light Chain) with two additional bands of κ – Light Chain.
Subject H	Male, 78 yrs, Bengali	14.4	1.3	13.1	0.10	Monoclonal gammopathy of λ – Light Chain (Two bands seen).
Subject I	Female, 72 yrs, Bengali	10.4	3.6	6.8	0.53	Monoclonal gammopathy of IgM (λ – Light Chain).
Subject J	Male, 52 yrs, North Indian	9.5	2.8	6.7	0.42	IFE not done.
Subject K	Male, 59 yrs, Bengali	10.3	3	7.3	0.41	Monoclonal gammopathy of λ – Light Chain.
Subject L	Male, 61 yrs, Bengali	11.4	2.8	8.6	0.33	Monoclonal gammopathy of IgA (κ – Light Chain) with a faint monoclonal λ – Light Chain band.
Subject M	Male, 66 yrs, South Indian	12.9	2.3	10.6	0.22	Monoclonal gammopathy of IgG (λ – Light Chain).
Range	49 – 78 yrs.	9.5 – 14.4	1.3 – 3.6	6.7 – 13.1	0.10 – 0.53	
Mean	63.6 yrs.	11.8	2.8	9	0.34	
Median	63 yrs.	11.1	3	8.6	0.32	

IFE: Immuno-Fixation Electrophoresis, IgA: Immunoglobulin A, IgG: Immunoglobulin G, IgM: Immunoglobulin M.

Thirteen subjects so chosen (Table 1) were subjected to blood tests for Total Protein and Albumin in a Vitros 350 analyzer, which acted as the established platform, for Total & Direct Bilirubin, HDL-Cholesterol, Calcium, Inorganic Phosphate, Iron and Unsaturated Iron Binding Capacity (UIBC) in the Vitros 350, an AU5800 and a Cobas 6000, of which the latter two acted as the evaluation platforms, and for gamma globulin characterization by ImmunoFixation Electrophoresis (IFE) in a Sebia Hydragel conventional electrophoresis platform (Figure 1). One of the subjects declined to undergo the last test. The method specifications of the tests performed have been elaborated in Table 2.

Figure 1: Figure depicts gamma globulin characterization of the subjects.



Subject A: Monoclonal gammopathy of IgM (κ –Light Chain). Subject B: Monoclonal gammopathy of IgG (κ – Light Chain). Subject C: Monoclonal gammopathy of κ – Light Chain (Two bands seen). Subject D: Monoclonal gammopathy of IgA (κ – Light Chain). Subject E: Biclonal gammopathy of monoclonal IgG (κ – Light Chain) and monoclonal IgG (λ – Light Chain). Subject F: Monoclonal gammopathy of IgA (λ – Light Chain). Subject G: Monoclonal gammopathy of IgG (κ – Light Chain) with two additional bands of κ – Light Chain. Subject H: Monoclonal gammopathy of λ – Light Chain (Two bands seen). Subject I: Monoclonal gammopathy of IgM (λ – Light Chain). Subject K: Monoclonal gammopathy of λ – Light Chain. Subject L: Monoclonal gammopathy of IgA (κ – Light Chain) with a faint monoclonal λ – Light Chain band. Subject M: Monoclonal gammopathy of IgG (λ – Light Chain).

Table 2: Assay Characteristics

Measurand	Ortho Clinical Diagnostics		Beckman-Coulter		Roche		Sebia	
	Methodology	**Traceability**	**Methodology**	**Traceability**	**Methodology**	**Traceability**	**Methodology**	**Traceability**
Total Bilirubin	Diazonium salt; reflectance photometry	Jendrassik-Grof; NIST SRM 916	DPD, with Caffeine, Surfactant; conventional photometry	NIST SRM 916a	DPD, with Detergents, stabilizers; conventional photometry	NIST SRM 916; Doumas method	NA	NA
Direct Bilirubin	Polycationic mordant; reflectance photometry	HPLC; NIST SRM 916	DPD,in Acid Medium; conventional photometry	Beckman-Coulter Master Calibrator	Diazo-sulphanilic acid; conventional photometry	NIST SRM 916; Doumas method	NA	NA
HDL-Cholesterol	Phosphotungstic acid-MgCl2 pptn/ cholesterol oxidase; reflectance photometry	CRMLN designated method; NIST SRM 911	Enzymatic Immuno-Inhibition; conventional photometry	US CDC Reference Method	Enzymatic Chemical-Inhibition; conventional photometry	US CDC Reference Ultra centrifugation Method	NA	NA
Calcium	Arsenazo III; reflectance photometry	Flame AAS; NIST SRM 915	Arsenazo III; conventional photometry	NIST SRM 909b L1	NM-BAPTA; conventional photometry	NIST SRM 956c L2	NA	NA
Phosphate	Heteropolymolybdenum blue complex; reflectance photometry	Phosphomolybdate/ phenylenediamine method; NIST SRM 200	UV Molybdate; conventional photometry	Beckman-Coulter Master Calibrator	UV Molybdate; conventional photometry	NERL primary reference material	NA	NA
Iron	Chromazurol B dye; reflectance photometry	Ferene dye; NIST SRM 937	TPTZ (Tripyridyl Triazine); conventional photometry	Beckman-Coulter Master Calibrator	Ferrozine; conventional photometry	NIST SRM 937	NA	NA
UIBC	Iron citrate/ chromazurol B; reflectance photometry	Ferene dye; NIST SRM 937	Nitroso-PSAP; conventional photometry	NIST SRM 937	Ferrozine; conventional photometry	Weighted purified iron SRM	NA	NA
Total Protein	Biuret; reflectance photometry	Biuret; NIST SRM 927	NA	NA	NA	NA	NA	NA
Albumin	Bromocresol green; reflectance photometry	Bromocresol green; NIST SRM 927	NA	NA	NA	NA	NA	NA
Monoclonal Proteins	NA	NA	NA	NA	NA	NA	Agarose gel electrophoresis/ immunofixation; densitometric scanning	NA

The reaction curves of all the results on the two wet chemistry platforms were analyzed; many of the results produced irregular or broken curves (for bilirubins and phosphate) or curves with high extinction coefficients (for iron). Eventually, all the results, both with normal and abnormal reaction curves, were included for data analysis. The data thus generated was analyzed with the help of Microsoft Excel and Statistical Package for Social Sciences (SPSS) software. Goodness-of-fit of comparison data with normal distribution was tested by constructing Quantile-Quantile (QQ) Plot [9], significant differences in comparison data were highlighted by constructing Bland-Altman plots [10] and finally differences in comparison were established by the Passing-Bablok regression analysis model, which assumes no special requirements regarding the distribution of samples and the measurement errors [11].

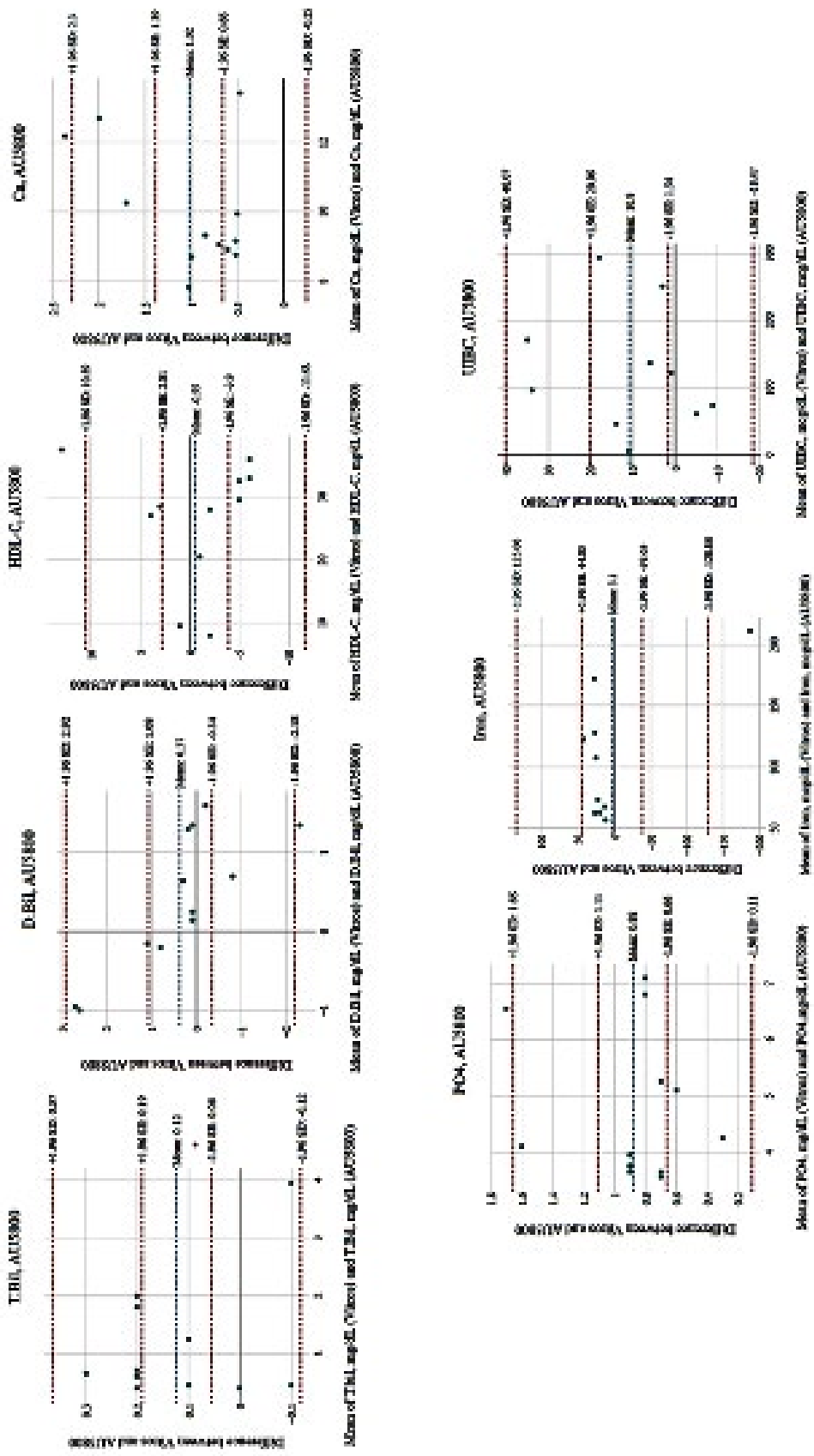
Table 3: Comparison of Test Methods

Evaluation of Variation w.r.t. Established Method								
		T.Bil	**D.Bil**	**HDL**	**Calcium**	**I. Phosphate**	**Iron**	**UIBC**
AU5800 w.r.t. Vitros 350								
Range of Variation (In Absolute Values)		0 – 40 %	7.1 – 1150 %	7.4 – 29.6 %	4.9 – 17.7 %	6.8 – 32.6 %	15.1 – 158.5 %	0.8 – 91.7 %
Regression Analysis	Intercept	-0.16	-0.86	2.26	0.634	-0.61	-90.2	-6.7
	Slope	0.04	1.22	-2.11	-0.165	-0.06	0.8	0
	p-Value	0.2745	**0.0009**	**<0.0001**	0.1364	0.5494	**0.0466**	0.585
Cobas 6000 w.r.t. Vitros 350								
Range of Variation (In Absolute Values)		0 – 228.6 %	0 – 75 %	0 – 57.1 %	4.6 – 14.5 %	0 – 20.3 %	7.7 – 23.7 %	0 – 83.3 %
Regression Analysis	Intercept	0.06	-0.22	-2.5	-0.883	-0.21	4.8	-15.8
	Slope	0.12	0.43	0.1	-0.001	-0.08	-0.3	0
	p-Value	0.3868	**<0.0001**	0.5977	0.9902	0.4022	**0.0002**	0.9242
Evaluation of Variation w.r.t Variation in A/G Ratio								
AU5800 w.r.t. A/G ratio								
Regression Analysis	Intercept	14.4	78.99	8.302	11.52	16.08	3.357	75.26
	Slope	15.3	634.8	22.03	-6.375	3.628	101.3	-172.3
	p-Value	0.6551	0.5289	0.3334	0.6603	0.8719	0.458	**0.0207**
Cobas 6000 w.r.t. A/G Ratio								
Regression Analysis	Intercept	48.8	2.085	22.83	9.025	18.87	25.72	67.92
	Slope	-11.31	92.34	-12.66	0.9345	-23.04	-26.22	-135.3
	p-Value	0.9467	0.1621	0.7785	0.9428	0.2456	0.1103	0.0653

Results

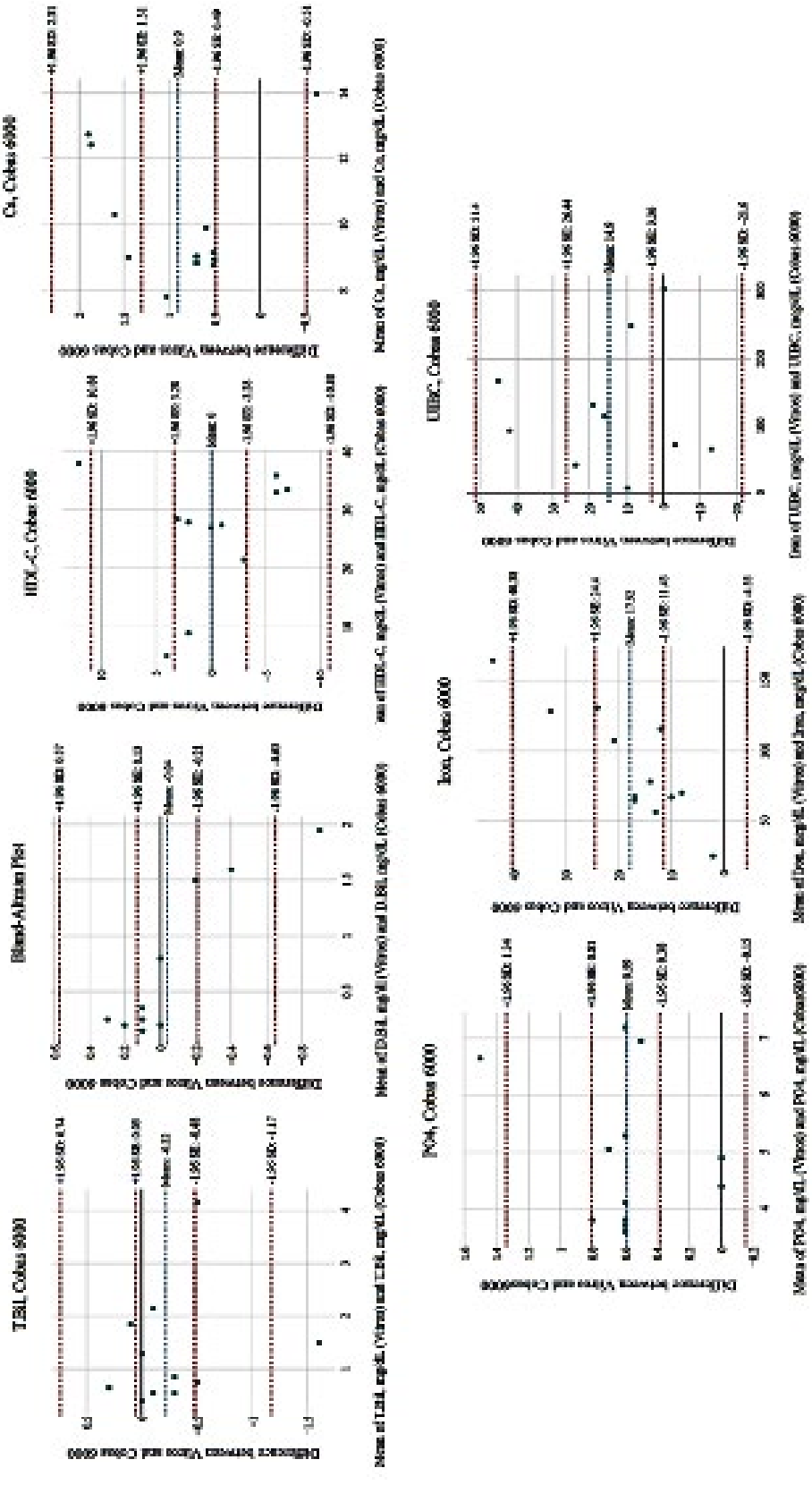
Data analysis was done with two research questions as the basis: 1) Whether the evaluation methods (wet chemistry platforms AU5800 and Cobas 6000) display a significant variation with respect to the established method (dry chemistry platform Vitros 350), and 2) Whether the evaluation methods display an increasing variation w. r. t. decreasing A/G Ratio. The findings of the analysis are summarized in Table 3, and Figures 2 and 3.

Figure 2: Figure depicts Bland-Altman plots of paired data between Vitros 350 (Established Method) and AU5800 (Evaluation Method)



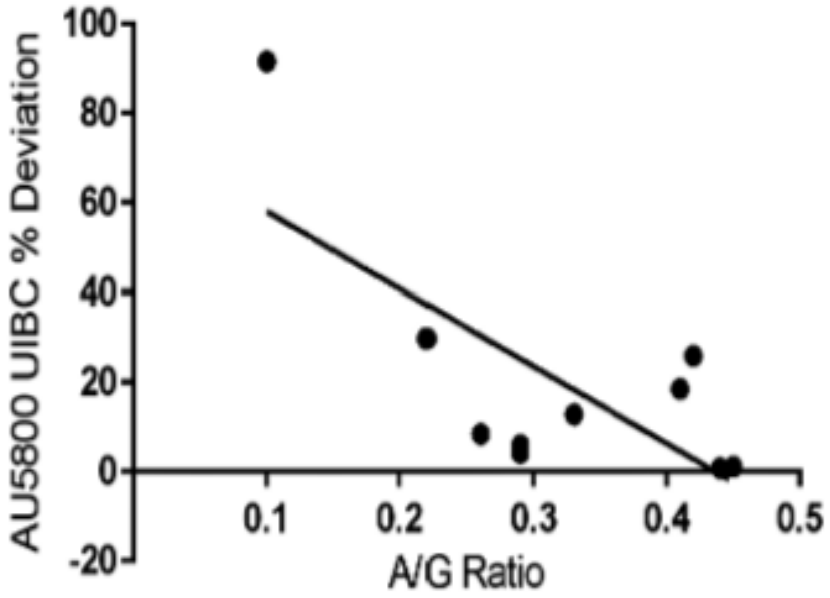
Top row (Left to right) includes Total Bilirubin, Direct Bilirubin, HDL-Cholesterol and Calcium. Bottom row (Left to right) includes Inorganic Phosphate, Iron and Unsaturated Iron Binding Capacity. Means of paired data are plotted on the x-axis and differences of the same data are plotted on the y-axis. Mean of differences is along the blue dotted line and the uppermost and lowermost red dotted lines denote the 1.96 SD limits.

Figure 3: Figure depicts Bland-Altman plots of paired data between Vitros 350 (Established Method) and Cobas 6000 (Evaluation Method).



Top row (Left to right) includes Total Bilirubin, Direct Bilirubin, HDL-Cholesterol and Calcium. Bottom row (Left to right) includes Inorganic Phosphate, Iron and Unsaturated Iron Binding Capacity. Means of paired data are plotted on the x-axis and differences of the same data are plotted on the y-axis. Mean of differences is along the blue dotted line and the uppermost and lowermost red dotted lines denote the 1.96 SD limits.

Figure 4: Figure depicts Passing-Bablok Regression Analysis of variation of Unsaturated Iron Binding Capacity measurements on AU5800 w.r.t



Vitros 350 as a function of changes in A/G ratio. The equation obtained was [UIBC] = -172.3*(A/G ratio) + 75.26, with a p-value of 0.0207. Thus, the increase in variation of UIBC measurements with decreasing A/G ratio is significant.

On the first question, a cursory look at the data reveals that the bilirubins and iron profile present the problem areas: AU5800 exhibits a variation of 7.1 – 1150% for Direct Bilirubin, 15.1 – 158.5% for Iron and 0.8 – 91.7% for UIBC; Cobas 6000 reveals a variation of 0 – 228.6% for Total Bilirubin and 0 – 83.3% for UIBC. In particular, the two wet chemistry platforms occasionally threw up bizarre results for the bilirubins, sometimes even in the negative. For example, for a Total Bilirubin of 0.7 mg/dL measured on Vitros350, the corresponding value on Cobas 6000 was 2.3 mg/dL; for a Direct Bilirubin of 0.2 mg/dL measured on Vitros350, the corresponding value on AU5800 was 2.5 mg/dL (more than Total Bilirubin of 0.5 mg/dL measured on the same AU5800!); for a Direct Bilirubin of 0.3 mg/dL measured on Vitros350, the corresponding value on AU5800 was -2.3 mg/dL; and so on. Since none of the data sets exhibited normality on the Q-Q Plots, they were subjected to transformation, mostly logarithmic, but sometimes Box-Cox or inversion, until normality was achieved. Bland-Altman Plots were constructed along two panels – on the first, variation of the test results of the respective measurands on AU5800 vis-à-vis Vitros 350 were plotted (Figure 2), while on the next, variation of test results of the same measurands on Cobas 6000 vis-à-vis Vitros 350 were plotted (Figure 3). Regression analysis at this level was done by plotting the results of the respective measurands in two sets, viz. Vitros 350 vs. AU5800, and Vitros 350 vs. Cobas 6000. Regression analysis with the assumption that both the data sets are same returned significant p-values (< 0.05) for Direct Bilirubin, HDL-Cholesterol and Iron in AU5800 and for Direct bilirubin and Iron in Cobas 6000, indicating that the measurands mentioned have significant variation vis-à-vis the Vitros 350 values.

On the second question, percent variation w. r. t. the established method was plotted against A/G ratio to determine the correlation, if any. In this level of data analysis, regression plotting was done on A/G ratio vis-à-vis percent deviation of the respective measurands on AU5800, or alternately, on Cobas 6000. None of the measurands produced any significant p-value, except UIBC measured on AU5800 (Figure 4, p-value = 0.0207). This signifies that the variation of UIBC measurement on the AU5800 platform increases with the decrease in A/G ratio.

All data generated or analysed during this study are included in this published article and its supplementary information file.

Discussion

Research regarding interference due to paraproteinaemia was already in the upswing by the 1980s, with an important communiqué emerging in 1980 about interference in thyroxine measurements [12] and two seminal papers appearing in 1986 regarding urea [13] and creatinine [14]. In fact, the last paper kickstarted such a flurry of research that almost all the major OEMs modified their Jaffé creatinine methods, and many major laboratories around the world have shifted to enzymatic creatinine assay. So much so, that it was decided against including creatinine and urea in the present study, because of the glut of information already available. Regarding information about paraprotein interferences in measurement of measurands

included in this study, a general consensus of the researchers [15-31] can be stated as 1) interferences due to paraproteins lack reproducibility across samples with similar paraproteins and across methods, and sometimes even in the same sample, and 2) interferences due to paraproteins are not proportional to the amount of paraprotein present in the sample. This implies that, even if comparison data between an established method and an evaluation method is satisfactory in one instance, such concordance may not be reproducible on repeat testing of the same sample set. Despite recognizing this fallacy, most studies till date have relied only on comparison data of actual runs. While not discarding real data altogether, the present study has put some emphasis on a regression model such that reliable predictions can be arrived at regarding general trends.

Paraprotein interference on measurement of bilirubin is a well-known phenomenon. In the present study, in view of the significant variation observed in D Bil estimation on both the wet chemistry platforms (AU5800 p-value = 0.0009, Cobas 6000 p-value ≤ 000.1, Table 3), four observations can be elucidated: 1) interference cannot be predicted based on the type of paraproteinaemia; e. g. T. Bil measured 0.7 mg/dL was falsely measured as 2.3 mg/dL on Cobas 6000 in a subject with monoclonal gammopathy of IgA (λ – Light Chain) (Subject F, Table 1); D. Bil measured 0.4 mg/dL was falsely measured as -2.3 mg/dL on AU5800 in a subject with monoclonal gammopathy of IgM (κ – Light Chain) (Subject A, Table 1); D. Bil measured 0.2 mg/dL was falsely measured as 2.5 mg/dL on AU5800 in a subject with monoclonal gammopathy of IgA (λ – Light Chain) (Subject F, Table 1); D. Bil measured 0.3 mg/dL was falsely measured as -2.3 mg/dL on AU5800 in a subject with monoclonal gammopathy of IgM (λ – Light Chain) (Subject I, Table 1). Reaction curves of all these improbable results were found to be broken or irregular. 2) interference in a sample on one platform is non-transferable to the other platform, e.g., in the above instances corresponding measurements in the other wet chemistry platform correlated well with the dry chemistry results; 3) interference is not correlated to the concentration of the measurand, in this case, bilirubin and 4) interference is not proportional to the amount of paraproteins present, as the absolute percentage variation of the wet chemistry vis-á-vis dry chemistry results does not correlate inversely with the A/G ratio (Table 3). As explained by King et al [30] and Madenci et al [31], such interferences in bilirubin measurement by diazotization are usually due to precipitation of proteins in the extremely acidic pH of the reaction mixture.

As regards to HDL-Cholesterol, the present study has found significant variation between AU5800 measurements and the established Vitros 350 values, with a p-value of <0.0001, though the range of variation in absolute percentage (7.4 - 29.6 %) was unremarkable. Variation of results of Cobas 6000 was found to be not significant. This finding seemed to be a bit perplexing because a brief review of existing literature pointed to the problem specifically against the Roche HDL-C reagent used in Cobas 6000 [16,18–20]. However, on a detailed enquiry with the OEM, it was noted that the HDL-C kit in use during 2003 – 04, when the articles were published, were of 2nd generation. The current lot of reagents is from the 4th generation, which has undergone several modifications, some of which were to address the issue of paraprotein interference. On the question as to why the performance of the AU5800 HDL-C platform faltered is difficult to explain, especially when the reaction curves were found to be normal, but the method being based on immuno-inhibition of non-HDL fraction of lipoproteins may serve as an indicator for further research.

Pseudohypercalcaemia is an oft-repeated reporting in the field of research on paraprotein interference [21,22]. In comparison with the o-cresolphthalein complexone (OCPC) method, which uses an alkaline medium, or the NM-BAPTA method, which also uses a neutral to alkaline medium, the Arsenazo III method, which uses an acidic medium, seems to be the more common culprit. But the present study, which examines the Arsenazo III method on AU5800 (Range of variation 4.9 – 17.7 %, Table 3) and the NM-BAPTA method on Cobas 6000 (Range of variation 4.6 – 14.5 %, Table 3), fails to reveal any significant discrepancy vis- á-vis the dry chemistry results. Also, no significant variation was found with varying degrees of paraproteinaemia. The reason for such concordance is difficult to determine but may be due to periodic revision and upgradation of the reagent constituents, especially on the Arsenazo III platform. After all, the two articles quoting pseudohypercalcaemia on Arsenazo III platforms were a couple of decades old! Variation in results of inorganic phosphate due to presence of paraproteins is an active area of research for almost three decades [23–26]. Various researchers have reported both falsely increased and falsely decreased results, mostly on measurements carried out on single serum specimens. In the opinion of the current author, such variation should not be prefixed as hyper- or hypo-, but rather be characterized as a variable variability, because of the irreproducible nature of the mechanism of interference. Despite extensive reportage of such variations, the present study has failed to produce significant variations between inorganic phosphate results on wet chemistry and dry chemistry platforms (Table 3). Reason for such concordance is difficult to pin-point; it may be surmised that the active intervention of the OEMs over the years to react to the continued reportage of variations and thereby modify their respective methods has likely yielded desirable results. It may be pertinent to mention here that though most of the wet chemistry results of inorganic phosphate were congruent with those of the dry chemistry results, many of the reaction curves in the former platforms were broken or irregular. Like the bilirubins, interferences of paraproteins on iron estimation have been an active area of research for long [27–29]. In the present study, the findings regarding iron and unsaturated iron binding capacity (UIBC) mirrored those of bilirubins, especially on the AU5800 platform. Many of the reaction curves were abnormal, with very high extinction coefficients. The range of variation of iron measurements on AU5800 was unacceptably large (15.1-158.5 %), with a significant p-value (0.0466) of the regression analysis.

Though the range of variation of iron measurements on Cobas 6000 was relatively low (7.7 - 23.7 %), regression analysis revealed a significant variation with a p-value of 0.0002. Like the bilirubins, the reactions for iron measurement takes place in extreme acidic matrices (pH 1.7 in AU5800, pH < 2.0 in Cobas 6000), and as explained by Bakker [27] and Dorizzi et al [28], such extreme manipulations of pH and ionic strengths may precipitate the paraproteins in the sample, causing turbidimetric interferences in measurement of iron concentrations. In contrast to iron, UIBC is usually measured in an alkaline environment (pH 8.1 in AU5800, pH 8.4 in Cobas 6000); consequently, comparability of UIBC results between wet chemistry and dry chemistry platforms were within acceptable limits. However, when percentage variation of UIBC results were plotted against A/G ratio, regression analysis returned significant results for AU5800 (p-value = 0.0207) but not for Cobas 6000, indicating that variation of UIBC results on AU5800 increases with decrease in A/G ratio. This is the singularly positive finding among all the measurands in this category of analyses (percent variation vs. A/G ratio).

A word or two about Bland-Altman plots may be pertinent in this discussion. Utility of BA plots in medical research is no doubt undeniable but it appears to the present author that mere visual inspection of BA plots may sometimes be misleading, a fact which was acknowledged by the authors themselves in a later article [32]. Applying the thumb rule that comparison data is acceptable when ~90% of the points lie between ± 2 SD of a BA plot, without analyzing the accompanying regression data, would have led to an erroneous conclusion in this study that all the measurands correlate well between the established and evaluation methods (Figures 2 and 3). This is particularly true when the sample size is small, as in the present study.

Finally, there are no qualms in acknowledging the drawbacks of this study. The first and obvious shortcoming is the sample size. Thirteen is woefully low a sample size for method comparison studies and the author acknowledges it as such. However, it must be kept in mind that the specific requirement of abnormality in the samples (presence of paraproteins) would always render gathering enough samples a tall ask. As such, the author treats this study as a sentinel survey and intends to build upon it for broader research in near future. Secondly, criticisms might arise as to why other relevant measurands were excluded from the study. The reason is twofold: firstly, there were sample volume constraints and secondly, a broader research protocol is intended to be set in near future dedicated fully to the study of paraprotein interferences. Thirdly, it might be argued that the evaluation methods should have been compared with the corresponding reference (or definitive) methods, instead of comparing with dry chemistry methods. This is a valid point but access to reference methods, mostly confined to reference laboratories, is almost out of reach for routine clinical laboratories like the one where the present study was conducted. In short, the study is not flawless, but it reasonably demonstrates important findings regarding paraprotein interferences, facts which are vindicated by a very recent study [33], which states, "...the paraproteins interfered maximally with direct bilirubin, total bilirubin, iron and Total Iron Binding Capacity (TIBC) assays."

Conclusion

The present study reveals that interferences due to presence of paraproteins are fairly common especially for parameters like Direct Bilirubin, HDL-Cholesterol, Iron and Unsaturated Iron Binding Capacity on the wet chemistry platforms evaluated. Though preventing the occurrence of such interferences seems impossible, they can be detected before release of erroneous results on inspecting the reaction curves when suspicions arise regarding results discrepant with the patient demographics or clinical findings. Development of feasible algorithms which can detect irregularities of reaction curves and prevent release of the corresponding results, without significantly impacting the throughput of the system, remains a challenge.

Declarations

Ethics Approval and Consent to Participate

Ethics approval for this study was obtained from Drs. Tribedi & Roy Ethical Committee and informed and written consent was obtained from the subjects for this study, in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki.

Consent for Publication

Written informed consent was obtained from the subjects for publication of their electrophoretograms and health data.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing Interests

The author declares that he has no competing interests.

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This study has received no funding.

Author Contributions

Rajarshi Sarkar has conceptualized, collected data, analyzed data, prepared a literature review, written the manuscript and prepared the tables and figures for this study.

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Commentary

The feasibility for screening for ovarian cancer

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Keywords

Ovarian Cancer, detection, molecular test, screening, EVA test

Abstract

Introduction

The majority of the high-grade serous ovarian cancer (HGSOC) cases are diagnosed late, preventing effective treatment and therapy. We examine the feasibility of using EVA (Early oVArian cancer), a new molecular test for early HGSOC detection.

Methods

Comparison of the advantages and disadvantages of EVA with previously reported ovarian cancer tests, including CA125, was made, and the positive and negative predictive values of the tests were calculated as a measure of usefulness in the clinic.

Results

The positive predictive value of EVA and CA125 was 8.6% and 6.8% respectively, which was calculated based on the disease prevalence of 0.5%. The negative predictive value was 99.9% in both cases.

Conclusions

EVA and CA125 are unlikely to provide a meaningful population screening method for HGSOC in women at risk, since the predictive values would drive women not to perform these tests.

Introduction

In a recent issue of the journal Science Translational Medicine, Paracchini and colleagues described a new approach to the early detection of high-grade serous ovarian cancer (HGSOC), which is based on the assessment of genomic instability patterns of DNA extracted from cervical Papanicolaou (Pap) smears [1]. In this commentary, the usefulness of screening for ovarian cancer will be summarized, the plethora of biomarkers that have already been used for HGSOC diagnosis and management will be mentioned, the new test (EVA, Early oVArian cancer) will be described and attention will be drawn to the importance of the positive predictive value (PPV) of a screening test, an issue that was not covered in the aforementioned paper [1].

Ovarian cancer

Ovarian cancer is one of the most lethal gynecological malignancies, and despite its relatively low prevalence, is responsible for more deaths of middle-aged women than the approximately 10-fold more prevalent breast cancer. Part of the reason for the increased patient mortality is that the majority of HGSOCs are diagnosed late (stages III, VI) which prevents curative therapy by surgery and chemotherapy. There is convincing data demonstrating that detection of many cancers at an early stage, including ovarian cancer, leads to superior clinical outcomes (disease-free and overall survival) [2,3]. Motivated by this knowledge, the National Cancer Institute created the organization named EDRN (Early Detection Research Network), which supports discovery and validation of new biomarkers, in collaboration with researchers, charity organizations and industry [4]. EDRN has already spent more than $100 million for this task. One landmark study sponsored by EDRN on ovarian cancer screening will be briefly commented upon below, and the data will be contrasted with those of Paracchini and colleagues [1,5]. Large, prospective clinical trials of ovarian cancer screening were conducted, such as the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) [5], using either a biochemical serum test (marker CA125), transvaginal ultrasound or combination of the two tests (multimodal screening) and the Prostate, Lung, Colorectal and Ovarian (PLCO) trial in the US [6]. These trials, conducted among average-risk, asymptomatic women of ages 50-74 and 55-74 respectively, found that ovarian cancer mortality did not significantly differ between screened and unscreened women [5-7]. The U.S. Preventive Services Task Force (USPSTF) recommends population screening for only four cancer sites (colorectal, lung, breast and cervical) and ovarian cancer is not included. Thus, ovarian cancer screening with currently available methods appears to have no net benefit [8].

Biomarkers for ovarian cancer

Hundreds of potential blood-based biomarkers for ovarian cancer detection and management have been evaluated. The latest advance is multi-cancer detection by "liquid biopsy" which involves molecular analysis of circulating tumor DNA [9,10]. These tests have not yet been validated for clinical use, and prospective trials are ongoing [9,11]. An ambitious project sponsored by EDRN [7] examined 49 of the most promising serum HGSOC biomarkers for which a reliable assay was available, for their potential to diagnose asymptomatic/preclinical ovarian cancer by using the PLCO blood collection. Specimens were collected at diagnosis, within 6 months after the time of diagnosis and >6 months after diagnosis (up to 18 months). Top performing markers included CA125, human epididymis protein 4 (HE4), transthyretin, CA15.3, and CA72.4, with sensitivity at 95% specificity ranging from 0.73 to 0.40, which declined when drawing of blood occurred >6 months after diagnosis [7]. In the study, CA125 remained the single-best biomarker for specimens collected at any time, despite the relatively low sensitivity and specificity of CA125 in pre-diagnostic samples still being problematic for screening (see below).

The EVA test and its performance for ovarian cancer screening

Malignant HGSOC cells that originate from tumors, mostly in the fallopian tube and ovarian epithelium [11], are shed into the tubes and end up at the uterine cervix, intermixed with non-cancerous cells and other debris, where they can be harvested with a Pap smear collection method and isolated DNA. This process is reminiscent of the liquid biopsy [9,10], whereby tumor cells and circulating free DNA are shed into the systemic circulation. The isolated DNA can be analysed for ovarian cancer-related mutations, such as mutations of the gene p53 and other genes. The authors chose instead to analyze genomic instability by profiling copy number variation through low-pass whole-genome sequencing, which supports a more specific assay than by profiling tumor-associated mutations. As a measure of the overall genomic instability, they used the copy number profile abnormality score (CPA), defined in their paper [1]. The CPA reflects a comprehensive quantification of unbalanced genomic traits (gains and losses), and the higher the CPA value, the greater the genomic instability. In this setting, at a specificity of 96%, the sensitivity was 75.4% [1]. These reported numbers are almost identical to the best blood-based ovarian cancer biomarker, CA125 (sensitivity of 73% at 95% specificity) [12]. An important limitation of the EVA test is that the test is equivocal in about 14% of the patients [1]. An advantage of EVA, however, is that it is claimed to detect cancer up to 9 years before diagnosis, although two patients changed status (one from positive to equivocal and one from equivocal to positive) in longitudinal samples [1].

Positive predictive value and negative predictive value (PPV, NPV)

The authors do not mention the positive and negative predictive value (PPV, NPV) of their test [1]. These performance parameters are the most important for screening tests for any disease. In addition to a well-performing test, the criteria for screening for any disease has been defined by Wilson and Jungner in 1968 [13]. Below, we will only focus on the screening test.

The asymptomatic patient who undergoes screening for a disease does not understand terms like sensitivity and specificity, which describe the effectiveness of the test in groups of diseased and non-diseased subjects (but not for the testing individual). The individual being tested is interested to know what their chance is to have or not have the disease if their test is positive or negative. PPV represents the chance of having the disease if the individual is test-positive. NPV represents the chance of not having the disease if their test is negative. Below, the PPV and NPV of the EVA and CA125 tests will be calculated for comparison, under a hypothetical but realistic scenario whereby women are between the ages of 50-74 in a population, and the prevalence of ovarian cancer is 0.5% (as described in reference [5]). Please refer to Table 1 for numerical values.

Table 1: PPV and NPV of EVA test, CA125 and 3 hypothetical tests with fixed sensitivity and variable specificity, as shown.

Test name	EVA Test	CA125	Hypothetical test #1	Hypothetical test #2	Hypothetical test #3	EVA Test modified*
Sensitivity, %	75%	73%	90%	90%	90%	75%
Specificity, %	96%	95%	98%	99%	99.50%	96%
Population	100,000	100,000	100,000	100,000	100,000	100,000
Ovarian cancer prevalence #1	0.50%	0.50%	0.50%	0.50%	0.50%	5%
Diseased women	500	500	500	500	500	5,000
Non-diseased women	99,500	99,500	99,500	99,500	99,500	95,000
Test positive diseased women (true positives, TP)	375	365	450	450	450	4,500
Test negative non-diseased women (true negative, TN)	95,520	94,525	97,510	98,505	99,002	91,200
Test positive non-diseased women (false positives, FP)	3,980	4,975	1,990	995	498	3,800
Test negative diseased women (before false negatives , FN)	125	135	50	50	50	500
Positive predictive value(TP)/ (TP+FP)x100, %	8.6	6.8	18	31	47	54
Negative predictive value(TN)/ (TN+FN)x100, %	99.9%	99.9%	99.9%	99.9%	99.9%	99.4%

#1,2,3.For women of ages 50-74 as per [5]
*Altered numbers of the EVA test based on a hypothetical scenario

In a population of women ages 50-74 old, the prevalence of ovarian cancer is about 0.5% [5]. The screening test EVA has a sensitivity of 75% at a specificity of 96%. Among 100,000 women in the screened population, there are 500 women with ovarian cancer and 99,500 women with no cancer. The test will identify 375 women who have cancer (true positive, TP) (500 x 0.75) and it will miss 125 diseased women (false negative, FN) (500 x 0.25). The test will also be negative for 95,520 women (true negative, TN) (99,500 x 0.95) and 3,980 women will be positive (false positive, FP) (99,500 x0.04). The PPV of the EVA test will then be (375(TP)/(375(TP) + 3,980 (FP))) x100=8.6%. By using similar calculations for the classical and best ovarian cancer biomarker (CA125) with sensitivity of 73% and a specificity of 95%, the PPV of CA125 is 6.8%. The NPV of the EVA and CA125 tests, using the formula NPV=(TN)/(TN+FN) x100, the NPV is 99.9%. For discussion purposes, it is also useful to calculate the PPV and NPV of a test with a fixed hypothetical sensitivity of 90% (which is acceptable for a good screening test) and hypothetical specificities higher than the EVA and CA125 tests, such as 98%, 99% and 99.5% (Table 1). The PPV and NPV of the EVA test was calculated with a modified disease prevalence of the screened population from 0.5% to 5%. The latter scenario could fit with women who are at higher risk of ovarian cancer such as family predisposition, symptoms, or presence of abdominal masses of unknown pathologies or with more prevalent cancers such as breast cancer. As mentioned, screened individuals for ovarian cancer or any other disease are interested about their own risk, and not parameter/risks that are associated with groups of patients (such as sensitivity and specificity). The PPV and NPV are the most informative indicators that explain the generated results of testing. Importantly, the risk of a woman having ovarian cancer before the test is done is equal to the disease prevalence, in this case 0.5%. Before screening, these women also have a high chance (99.5%) of not having the disease. When the test is performed, the risk is elevated from 0.5 % to about 8% for EVA and to about 7% for CA125 if the test is positive. When the test is negative, the chances of not having the disease is decreased

from 96 or 95% to 99.9% (Table 1). With such low PPV of EVA and CA125 tests (7-8%) there is doubt that many women will choose invasive laparoscopic or other surgical interventions to confirm or exclude presence of HGSOC. In Table 1, the PPV is calculated for a hypothetical, more specific test (specificities of 98%, 99% and 99.5%) at a fixed sensitivity of 90%. In this case, the PPVs increase to 18%, 31% and 47%, respectively, and laparoscopic verification is likely acceptable to these women. The EVA test could have a PPV of >50% in a screened population with prevalence of 5% (Table 1).

Conclusion

The conclusion from the aforementioned discussion is that the EVA and CA125 tests are not effective and likely not acceptable for population screening of average risk women of 50-74 years old for HGSOC. Despite their similar performance for screening, the CA125 test, invented 40 years ago [12], has important advantages over the EVA test. It is known that the performance of diagnostic tests is reduced when they migrate from the discovery lab to the clinic. This notion remains to be verified for EVA. While the CA125 test is a simple ELISA-type assay in serum, EVA is more technically demanding, slower and likely much more expensive. EVA gives equivocal results in about 14% of patients and predictably it will be less precise and less reproducible than a well-established ELISA assay for CA125. This raises concerns regarding its clinical applicability, with results requiring sufficient management in a clinical setting, including using potential follow-up tests or protocols that have been discussed elsewhere [14]. Furthermore, the implementation of EVA-like tests in clinical practice will require appropriate equipment, personnel training, direct and indirect costs of running the test and time, which, taken with the previously mentioned caveats, may prove unfeasible for widespread adoption. There is a light at the end of the tunnel, however. A potential distinct advantage of EVA is its hypothesized ability to diagnose ovarian cancer 9-11 years before clinical diagnosis. If this is confirmed, it could facilitate cures of the disease with early interventions, despite its low PPV.

Author's Disclosure statement

MKC and EPD have no conflicts to report.

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