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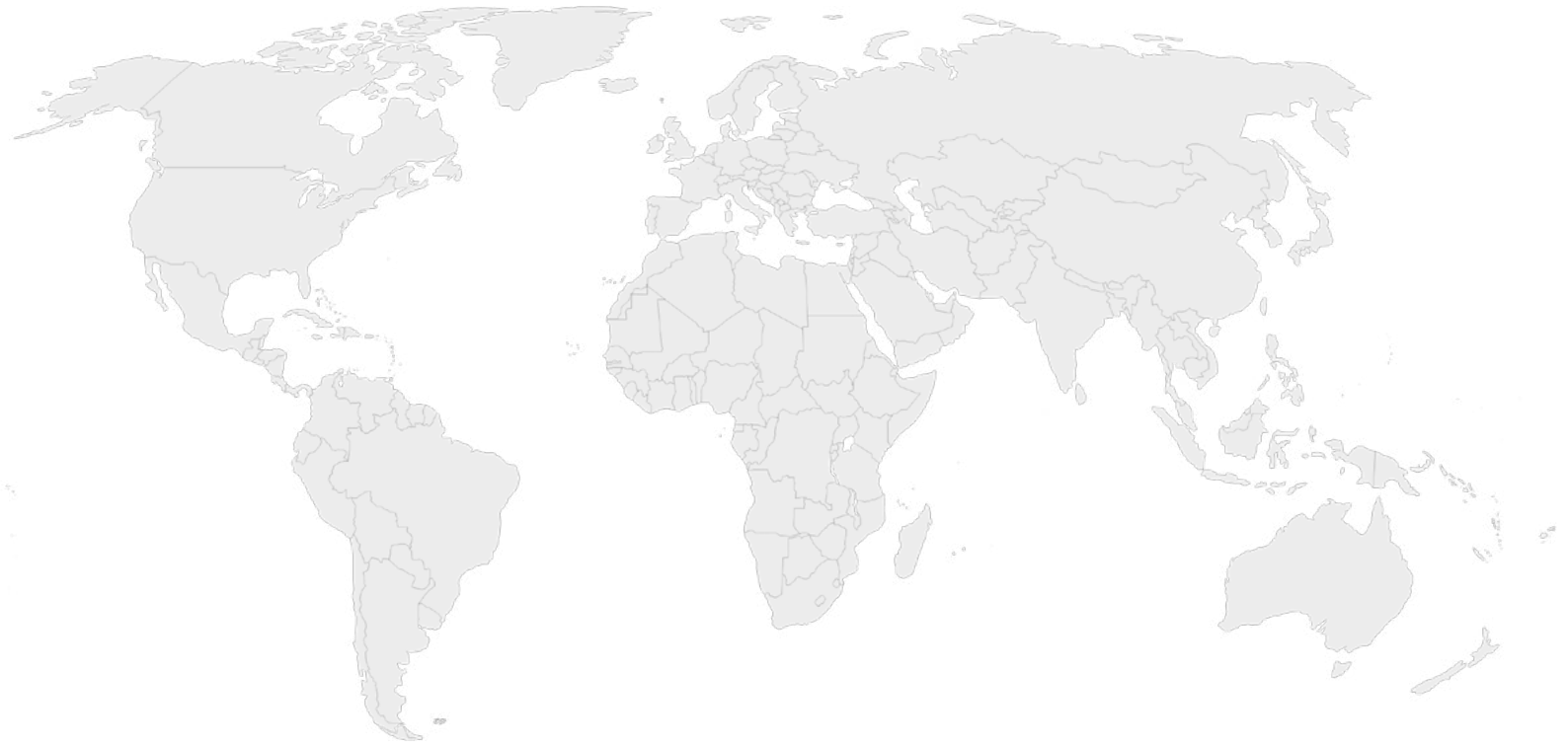

Commentary

Comment on Implosion of Grail's Galleri Cancer Screening Test?

Harjit Pal Bhattoa, Kannan Vaidyanathan
Co-Editors-in-Chief eJIFCC

The Letter to the Editor, stated that a recent prospective clinical trial using the Galleri MultiCancer Detection Test was temporarily put on hold [1]. This may give the impression that the trial will not be continued. However, the NHS website states: After the trial, we will have a much better understanding of how well the Galleri test works in the NHS. If it does work, then it could be used in the NHS in the future (like breast screening or bowel screening, but for many different types of cancer). If the Galleri test does not work well in this setting, then we will still have learned important information about what research needs to be done in the future to improve cancer screening [2].

The letter to the editor [1] contains the sentence: The Grail case has some similarities to the Theranos story, which sent some executives to jail and led to company bankruptcy. This sentence could be misinterpreted as a comparison with Theranos, and such a comparison is not accurate.

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Letter to the Editor

Gamification As a Learning Modality in Clinical Chemistry – Breaking the Glass Ceiling

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Gamification, learning, Clinical Chemistry

Gamification is the use of game-like elements, mechanics, and design principles in non-game contexts to engage and motivate individuals [1]. It involves applying elements commonly found in games, such as competition, rewards, challenges, and interactive features, to activities that traditionally may not have a game structure. The goal is to enhance engagement, motivation, and learning outcomes in various fields [2]. Gamification in the field of Clinical Chemistry, which is a branch of pathology that deals with the study of biochemical and molecular changes in the body associated with diseases, can have both benefits and limitations.

Gamification can make learning more engaging by incorporating game-like elements, such as quizzes, challenges, and interactive scenarios, which can capture the interest of learners. Games often provide rewards and achievements, motivating individuals to actively participate in learning activities and stay committed to the learning process. Games can incorporate repetition in a fun and engaging way, aiding in the reinforcement of key concepts and helping learners to remember important information. Interactive elements in games can help users actively apply their knowledge, leading to better retention compared to traditional learning methods [3]. Gamification can provide simulated scenarios that mimic real-world situations in Clinical Chemistry, allowing learners to apply their knowledge in practical contexts and develop problem-solving skills. Including leaderboards and challenges can introduce a competitive element, fostering a sense of achievement and encouraging learners to strive for excellence in their understanding of Clinical Chemistry [4].

Some learners may focus on earning rewards rather than truly understanding the material. This can result in superficial learning, where individuals memorize information only to achieve rewards rather than internalizing the knowledge [5]. Certain complex topics in Clinical Chemistry may not lend themselves well to gamification, as simplifying them for the sake of game mechanics could lead to a loss of accuracy or depth in understanding. Gamification often relies on technology, and not all learners may have access

to the required devices or internet connectivity. This can create disparities in learning opportunities. Gamification may not cater to all learning styles. Some individuals may prefer traditional methods and forcing a game-based approach might not be effective for everyone. Creating high-quality gamified content can be resource-intensive in terms of development time and costs. Institutions or educators may face challenges in implementing gamification due to budget constraints or time limitations [6].

In conclusion, while gamification has the potential to enhance the learning experience in Clinical Chemistry, it's essential to carefully consider the specific context, target audience, and learning objectives to leverage its benefits effectively. Integrating gamification as part of a diverse learning strategy rather than relying solely on it can help address some of the limitations. However, keeping in perspective the evolution of Clinical Chemistry gamification can improve knowledge retention, problem-solving skills, and overall learner motivation by applying principles inspired by the mechanics of games for the learners ranging from technicians to laboratory physicians.

Author's Disclosures

None.

Ethical Approval

Not applicable.

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Review Article

Evaluation of Metabolomics in Chemical Pathology Research in Pakistan: A Less Explored Path and New Frontier

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Keywords

Metabolomics, Chemical Pathology, research, Pakistan

Abstract

Metabolomics involves the study of unique chemical fingerprints left behind by specific cellular processes. Metabolomics encompasses the analysis of both "endogenous" compounds, such as amino acids, lipids, cofactors, nucleotides, carbohydrates, hormones, etc., and "exogenous" metabolites including drugs, toxins, environmental contaminants, pesticides, herbicides, etc. Initially, metabolic analyses heavily relied on nuclear magnetic resonance (NMR), but recent advancements in mass spectrometry (MS) and Tandem MS have expanded the horizons of research, service, and education in this field. In this overview, we delve into the domain of mass spectrometry from the perspective of Chemical Pathologists. To establish a baseline, we conducted a search using the PakMediNet search engine. Our literature review yielded a total of 1167 articles, of which 1155 were excluded, and 12 were included. Our findings indicated that while MS is utilized for method development and biomarker evaluation in the fields of basic biological sciences and the pharmaceutical industry, there are limited collaborative efforts with Chemical Pathologists for clinical applications. It is imperative to harness the advancements in MS for research and development, as this technology is a driving force behind progress in service and education. Looking ahead, areas such as newborn screening, diagnostics for inherited metabolic disorders, heavy metals analysis, and toxicology hold great potential for research in collaboration with Chemical Pathologists. To achieve these goals, the formation of working groups, establishing partnerships with institutes possessing relevant expertise, and providing funding opportunities are essential steps forward.

Introduction

The term "metabolomics" was first introduced in the early 2000s, representing a comprehensive effort to analyse the full spectrum of micro molecules, or metabolites with a molecular weight (MW) of up to 1500 Da, found within biological systems [1]. Metabolomics is a rapidly evolving field of research like genomics, transcriptomics, and proteomics. Its primary objective is to uncover the dynamic biochemical processes occurring within cells, tissues, and organisms, while also exploring exogenous" metabolites such as drugs, toxins, environmental contaminants, pesticides, herbicides, and more. Metabolites form a diverse collection of small molecules with relatively low molecular weights, encompassing lipids, amino acids, peptides, nucleic acids, organic acids, vitamins, thiols, and carbohydrates [2]. The analysis of metabolic profiles found in common biofluids like saliva, blood, urine, and faeces provides a valuable technique for assessing and predicting pathological conditions, dietary patterns, and medication toxicity. Unlike other "omics" disciplines, metabolites and their concentrations precisely reflect the underlying metabolic activities and the state of cells and tissues. This makes metabolomics an exceptionally effective approach, offering the most accurate representation of the molecular phenotype [2-4].

Metabolome profiling employs two primary approaches: targeted and untargeted methods. Targeted and semi-targeted metabolomics studies are aimed to precisely identify and quantify a specific subset of metabolites present in biological samples [5]. The goal is to provide accurate measurements of known metabolites. In contrast, untargeted studies focus on identifying and comparing as many signals as possible within a given sample set. The primary objective is to identify and categorize these signals as metabolites by utilising metabolomics databases. Untargeted studies are particularly valuable for uncovering previously unidentified metabolites, especially when these unknown compounds may serve as biomarkers in specific research areas [5]. This distinction between targeted and untargeted approaches allows researchers to choose the most suitable method based on their research objectives and the depth of metabolomic analysis required.

Metabolite exploration in biological fluids and tissues is a critical process in metabolomics research, achievable through various technological platforms such as nuclear magnetic resonance spectroscopy (NMR), Gas Chromatography-Mass Spectrometry (GC-MS), and Liquid Chromatography-Mass Spectrometry (LC-MS) [6]. Among the most common spectroscopic analytical techniques, NMR stands out for its ability to detect and analyse a wide spectrum of organic compounds in the micro-molar range without the need for derivatization. New NMR techniques (e.g., 1.2 GHz spectrometers), advances in cryoprobe, microprobe or sub-microprobe technologies, along with novel pulse sequence designs, significantly improve the sensitivity of NMR experiments and significantly decrease the lower limits of metabolite detection and quantification. In particular, the lower limit of detection for ultra-high-field NMR instruments (high nanomolar concentrations) is not too different than that of triple quadrupole MS instruments [8,9]. Mass spectrometry (MS) is gaining popularity in high-throughput metabolomics due to its greater sensitivity and is often combined with other methods such as chromatography-MS [6,7]. Due to the diversity of the metabolome, regardless of whether you choose untargeted or targeted metabolomics techniques, both approaches have their challenges. For instance, volatile organic compounds are best analysed using the GC-MS platform, while semi-polar molecules can be examined using LC-MS which can identify and quantify metabolites with high sensitivity and specificity without requiring chemical derivatization. However, it is important to note that some metabolite loss can occur during sample processing and this loss can be mitigated by combining multiple technological platforms.

Metabolomic research is still relatively new and emerging in many countries including Pakistan. Although some research has been conducted in this area, it remains in the early stages of development, requiring further investment and advancement in the field. There are significant challenges hindering the progress of metabolomic research, primarily stemming from a lack of funding and resources. One of the major impediments to the growth of metabolomic research in many countries including Pakistan is the inadequate funding available for research projects. This shortage of financial support limits the capacity of researchers to procure essential equipment and reagents necessary for conducting metabolomic analyses effectively. As a result, the potential for in-depth and comprehensive research in this field is stifled.

Another critical issue is the shortage of qualified personnel with expertise in metabolomics, which makes it difficult to conduct high-quality research and analyses in this field. Bridging this expertise gap is essential for advancing metabolomic studies in the country. The objective of this review is to identify prospective, cross sectional, experimental analytics studies carried out using Mass spectrometry techniques undertaken by Chemical Pathologists. By scrutinizing existing research, this review aims to pinpoint research gaps within the field of metabolomics. Subsequently, it will provide valuable approaches and suggestions to address these gaps. Efforts to address these challenges should include advocating for increased funding for metabolomic research, promoting collaboration with international experts and institutions, and establishing training programs to cultivate a skilled workforce in metabolomics. By addressing these issues and fostering a conducive environment for metabolomic research, scientists in the developing countries can make significant strides in advancing this emerging field and contributing to the global body of knowledge in metabolomics.

Material and Methods

In this overview, the research domain of Mass spectrometry was explored with Chemical Pathologists perspective from Pakistan. Pakmedinet was used as search engine using following keywords "Metabolomics", "Mass spectrometry", "Pakistan". The criteria

for inclusion in the shortlisting were limited to articles written by Pakistani authors, with no restrictions on the date or time of publication. Studies comprising reviews, letters to the editor, surveys, abstracts only, opinion papers, hypothesis, viewpoints, animal studies, basic sciences/nonclinical studies, article full text in language other than English and articles without any chemical pathologists listed as authors were omitted.

The two reviewers autonomously compiled the data using a predesigned pro forma enlisting the region of study publication, number of study participants, time period of recruitment, and findings of study.

Results

The databases searched revealed a total of 1167 articles. Based on the stringent inclusion criteria as depicted in Flowchart 1, 12 articles were included in the final analysis based on autonomous evaluation by two investigators with an excellent agreement of κ statistic = 0.90. Review 1articles n=157, abstracts only n=67, case reports n=116, case series n=72, frequency reports n=87, no Chemical Pathologists in authors n=656 were excluded using the predetermined criteria. Table 1 presents a comprehensive overview of the articles included in this systematic review published from 2016-2022.

Flowchart 1: Flowchart Illustrating the Author's Search Strategy for Metabolomics in Chemical Pathology – A detailed, step-by-step depiction of the process used to identify and analyse relevant studies, databases, and metabolic pathways relevant to chemical pathology research.

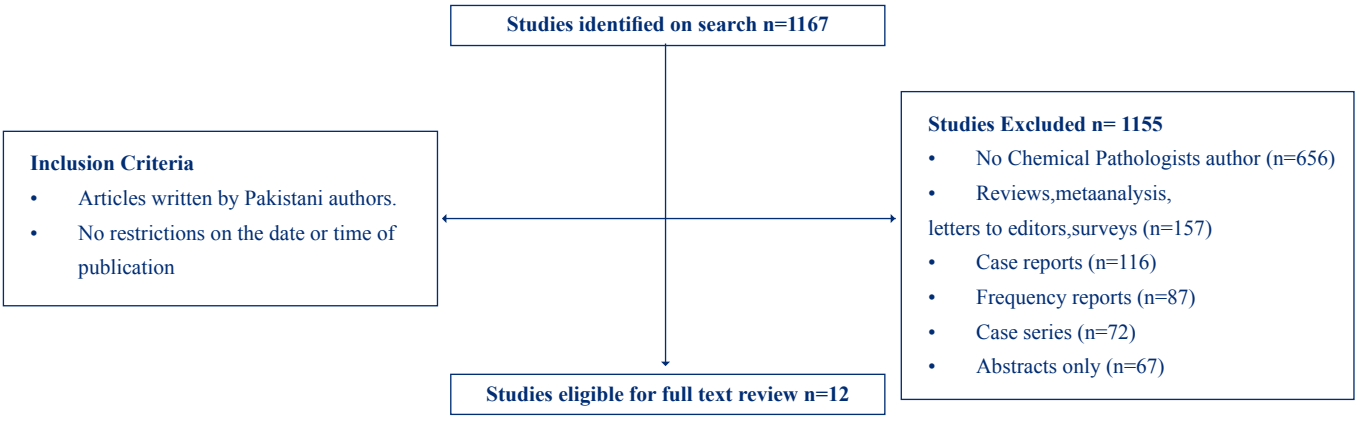

Table 1: Summary of Studies Related to the Evaluation of Metabolomics in Chemical Pathology – A concise overview of key studies, highlighting their contributions to the field.

S. No	Author(s)	Citation #	Region of Pakistan	Year of publication	Institute	Analyte measured	Technique used	Sample size (n)	Brief recommendation
1	Naz S et al.	[12]	Rawalpindi, Punjab	2021	Armed Forces Institute of Pathology, Rawalpindi, Pakistan	Vitamin D	Liquid chromatography- Tandem mass spectrometry (LC-MS)	120	LC-MS is highly sensitive, specific, cheaper method for vitamin D detection useful for guiding management of patients with malabsorption syndrome on D2 therapy
2	Naz S et al.	[13]	Rawalpindi, Punjab	2020	Armed Forces Institute of Pathology, Rawalpindi, Pakistan	Vitamin D	Liquid chromatography- Tandem mass spectrometry (LC-MS)	120	LC-MS is highly sensitive, specific method for VIT D detection and quantitation

3	Hafeez A et al.	[14]	Rawalpindi, Punjab	2018	Armed Forces Institute of Pathology, Rawalpindi, Pakistan	Organic acid	Gas chromatography-Mass spectrometry	110	Urine organic acid must be interpreted in. Context of complete clinical, nutritional, and biochemical findings for diagnosis of inherited metabolic diseases. Analytical protocols must be designed for better interpretation of results
4	Aamir M et al.	[15]	Rawalpindi, Punjab	2016	Armed Forces Institute of Pathology, Rawalpindi, Pakistan	Cannabinoid detection in hairs of addicts	Liquid chromatography- Tandem mass spectrometry (LC-MS	60	LC-MS can simultaneously detect 2 important cannabis metabolites I hair of cannabis users and hence can be used as an effective monitoring tool. While it is less time consuming and laborious, low it is plagued with low sensitivity
5	Musharraf SG et al.	[16]	Karachi, Sindh	2016	Dr Panjwani centre for molecular medicine and drug research, international centre for chemical and biological sciences, University of Karachi, Pakistan	Serum metabolites in acute leukaemia	Gas chromatography-Tandem Quadrupole Mass spectrometry	72	Serum metabolites can serve as early diagnostic biomarker for acute leukaemia detection.
6	Khan AH et al.	[17]	Karachi, Sindh	2018	Department of Pathology and laboratory medicine, AKU, Karachi, Pakistan	Kidney Stone Analysis	Fourier transform infrared spectroscopy (FTIR)	449	FT-IR has several advantages over chemical analysis including its use in smaller samples study of all stone layers, faster detection of stone crystals
7	Khan A et al.	[18]	Mardan, KPK	2018	Department of Environmental sciences, Abdul Wali khan University, Mardan, Pakistan	4 NSAIDS (Paracetamol,diclofenac,ibuprofen, codeine)and BDZ(Diazepam,Lorazepwm,Bromazepam,Temazepam) In untreated sewage	Liquid chromatography-Tandem quadrupole Mass spectrometry		Toxicity levels of mentioned chemicals must be identified and its effect in marine ecosystem, soil and groundwater should we study
8	Younas A et al.	[19]	Rawalpindi, Punjab	2020	Armed Forces Institute of Pathology, Rawalpindi, Pakistan	Cannabinoid detection in hairs of addicts	Liquid chromatography-Tandem mass spectrometry (LC-MS)	151	Hair is a better diagnostic indicator for chronic cannabis detection than urine

9	Jafri, L et al.	[20]	Karachi, Sindh	2022	Department of Pathology and laboratory medicine, AKU, Karachi, Pakistan	Gallstone constitution	Fourier transform infrared spectroscopy (FTIR)	117	Gallstone library created using FTIR allows for more synergy and less intensive gallstone analysis between different clinical laboratory
10	Dawood S et al.	[21]	Kharian, Punjab	2021	CMH kharian medical college, / National university of medical sciences, Pakistan	Drug intoxication in travel related crimes	Immunoassay (Index test), Triple Quadrupole Liquid chromatography-Mass spectrometry	77	Urine drug screening using immunoassays is a rapid, effective, and convenient way of drug intoxication in emergency however the use is limited by an array of false negative and positive results. Hence factors influencing false results must be informed to clinicians for better treatment outcome measures.
11	Aamir M et al.	[22]	Rawalpindi, Punjab	2021	Department of Clinical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan	2,4,6 Trinitrotoluene (TNT) and 4-ADNT,2 ADNT,2,4 ADNT and 2,6 ADNT screening in ordnance factory workers	Liquid chromatography-Tandem mass spectrometry (LC-MS)		LC MS can be used for simultaneous quantification of TNT and its metabolites allowing good accuracy for TNT explosion screening in industry workers of Pakistan
12	Khan ZUN et al.	[23]	Karachi, Sindh	2022	Department of Pathology and laboratory medicine, AKU, Karachi, Pakistan	Urine Organic Acids	Gas chromatography-Mass spectrometry (GCMS)	85	Five Organic acids namely, isovaleric acid, homovanillic acid, suberic acid, adipic acid, indole acetic acid serve as important discriminatory factor between children with autism spectrum disorder and developing children

Liquid chromatography- Tandem mass spectrometry (LC-MS), Fourier transform infrared spectroscopy (FTIR), Gas Chromatography-Mass spectrometry (GCMS), Trinitrotoluene (TNT).

Discussion

Metabolomics research holds the potential to revolutionize various scientific and medical domains, spanning from drug development to personalized medicine. As the amount of data produced by metabolomics studies continues to grow, there is a need for enhanced data analysis techniques. A validation study conducted by Naz S et al. [12] using LC-MS coupled with a quadrupole- tandem mass spectrometer and Electron spray Ionization to analyse serum 25 hydroxy vitamin D (D2 and D3 metabolites) in an adult population with an N=120 sample size. This study yielded impressive results, with a recovery rate of 98% and 97.5% for D3 and while the recovery rate was 97% and 98% for D2. The percentage relative standard deviation (RSD) was found to be 0.8% and 1.3% respectively, with minimal cross-reactivity with 24,25 hydroxy vit D and 25,26 di-hydroxy vitamin D metabolite than the routinely used immunoassays [12]. The utilization of this highly sensitive, specific, and minimally cross-reactive tandem MS method can offer cost-effective, and standardized results for vitamin D assessment, especially in tertiary care settings that handle substantial workloads [12]. Similar findings were replicated in a study done by Naz S et al.where they compared vitamin D testing methods and elucidated the limitations of immunoassays, highlighting the advantages of the novel LCMS/MS technique [13]. In the realm

of vitamin D analysis, metabolomic technology has emerged as a superior alternative to immunoassays. However, it's worth noting that this transition has yet to take place in Pakistan. The persistence of immunoassay usage in our country can be attributed to significant gaps that still exist in this regard. These gaps primarily stem from the limited availability of resources and the inadequate training of chemical pathologists in Pakistan. Hafeez A et al a retrospective study focusing on the diagnostic and analytical challenges involved in interpreting urine organic acid profiles in a cohort of 110 individuals over a two year-period, using gas chromatography mass spectrometry as their analytical technique [14]. Notably, within this dataset, 25% of the samples were subject to rechecking or recall by the authors due to preanalytical factors such as delayed sample arrival at the laboratory or the collection of samples randomly. The study concluded that pre-analytical control is an obligatory prerequisite for urine organic acid testing, and the profile must be interpreted in context of complete clinical, nutritional, and biochemical findings for an accurate diagnosis of inherited metabolic disorders. It is also imperative that analytical protocols be carefully designed to facilitate a more precise interpretation of the results [14]. These findings emphasize the complexity and sensitivity of urine organic acid profiling and underscore the significance of a holistic approach to ensure accurate and clinically meaningful results in the realm of metabolic disorder diagnosis.

In a study conducted by Aamir M et al, they developed and validated an LCMS technique for the detection of cannabis metabolites in the hair from chronic cannabis users. This LCMS method allowed for the simultaneous measurement of both cannabis metabolites, including THC and THC-COOH. It presented a distinct advantage over GCMS, primarily because it required fewer derivatization steps before analysis, resulting in a comparatively shorter analysis time [15]. Musharraf SG et al. utilized magnetic resonance spectroscopy (NMR) in their metabolomics research involving acute lymphoblastic leukemia (ALL) patients [16]. Using NMR technology, this case-control study compared patients with ALL to healthy controls and people with aplastic myeloid leukemia (AML). The findings revealed significant metabonomic variations between ALL and acute myeloid leukemia AML patients when compared to the control groups. These variations encompassed abnormal glycolysis, disruptions in the tricarboxylic acid (TCA) cycle, alterations in lipoprotein profiles, changes in choline metabolism, and fluctuations in fatty acid metabolism [16]. In the field of metabolomics, NMR is progressively gaining popularity as a reliable method. NMR offers precise insights into metabolite profiles, making it particularly well-suited for metabolomic studies involving biofluids. Its capability to provide a comprehensive overview of metabolites makes NMR-based metabolomics a valuable tool in this field. These studies highlight the importance of advanced analytical techniques like LCMS and NMR in elucidating metabolite profiles and their applications in diverse areas of research, from forensic analysis to disease diagnosis.

Khan AH et al.conducted a comprehensive analysis of kidney stones, employing two distinct methods: Fourier Transform Infrared (FT-IR) spectroscopy and chemical analysis. Their study encompassed a substantial sample size of 449 patients, and the comparison between these two methods yielded a reasonably good level of agreement, as reflected by a kappa statistic of 0.57 (with a 95% confidence interval ranging from 0.5 to 0.64). It's worth nothing that there were some disparities in the examination of 77 of the stones [17]. FT-IR spectroscopy emerged as a valuable method in this context. It demonstrated robustness in its ability to analyse kidney stones and was advantageous for utilizing only a small proportion of the sample. It generally allowed for the positive identification of the majority of components present in kidney stones [17]. This research underscores the effectiveness of FT-IR spectroscopy as a powerful tool for the analysis of kidney stones. Its ability to provide accurate and reliable results while conserving sample material makes it a valuable asset in the field of stone analysis and diagnosis.

Khan A et al. conducted a study focusing on the presence of pharmaceuticals, including non-steroidal anti-inflammatory drugs (NSAIDs) and four benzodiazepines/anti-depressants (ADs), in municipal wastewater in Northwest Pakistan. They utilized the Liquid Chromatography with a Triple Quadrupole Tandem Mass Spectrometry (LC-MS/MS) technique to analyze these substances [18]. The study's findings revealed significantly elevated concentrations of NSAIDs in both sewage and surface water. Specifically, substances like paracetamol and ibuprofen exhibited the highest levels. Given these findings, a comprehensive investigation into the potential toxicities of these specific chemicals is imperative. Furthermore, the study emphasizes the critical need to comprehend their potential impact on vital environmental components, including the marine ecosystem, soil quality, and groundwater systems [18]. This research highlights the importance of monitoring and addressing the presence of pharmaceuticals in wastewater, as their persistence in the environment can have far-reaching consequences. Understanding the potential risks to both human health and the environment is crucial for effective management and mitigation strategies.

Younas A et al . carried out a study aiming to assess the precision of Cannabinoids testing using LC-MS/MS in human hair and comparing it with urine samples from civil heavy vehicle drivers (total n=151) in Punjab [19]. The results of the study revealed that the diagnostic accuracy of Cannabinoids detection in hair reached 94%, whereas in urine samples, it was 83%. The Receiver Operating Characteristic (ROC) curve analysis demonstrated an area under the curve of 0.79 for urine samples and an impressive 0.96 for hair samples. These findings highlight the feasibility of hair as an alternative matrix for testing due to its non-invasive collection method, enhanced diagnostic effectiveness, and a more extended window of detection in comparison to urine [19]. This research underscores the advantages of employing hair

as a matrix for Cannabinoids testing, particularly in scenarios involving heavy vehicle drivers, where accurate and non-invasive testing methods are of paramount importance. The higher accuracy and extended detection window in hair samples make it a valuable option for such assessments.

A study conducted by Aamir M et al focused on the development and validation of an LC-MS/MS technique with the aim of simultaneously measuring Trinitrotoluene (TNT) and its metabolites in urine samples collected from workers in an ordnance factory [22]. The results of the study revealed that the utilization of LC-MS/MS with an APCI (Atmospheric Pressure Chemical Ionization) source yielded offered significant advantages in terms of speed, sensitivity, and specificity. Consequently, this approach shows promise as an effective means of screening for TNT exposure among individuals working in ordnance factory settings [22]. This research highlights the potential of LC-MS/MS with APCI as a valuable tool for monitoring and assessing TNT exposure, particularly in occupational settings where such exposure may occur. Its advantages in terms of accuracy and efficiency make it a promising method for ensuring the safety of workers in such environments. Furthermore, the application of metabolomics to analyse TNT is a novel and innovative aspect of this study, demonstrating the utility of mass spectrometry in testing and assessing exposure levels in occupational settings. In a case-control study conducted by Khan ZUN et al., the aim was to compare the urine organic acid profiles of children diagnosed with autism spectrum disorder (ASD) and typically developing (TD) children and identify biomarkers that could aid in diagnosing ASD, using GCMS [23]. The study yielded important findings, with several organic acids, including 3-hydroxyisovaleric acid, homovanillic acid, adipic acid, suberic acid, and indole acetic acid exhibiting significant differences between the two groups. These differences held promise as potential biomarkers for aiding in the diagnosis of ASD. With accuracy rate of 88.2% subjects were categorized correctly as ASD or TD group based on their urine organic acid profiles. These identified biomarkers are valuable for future research focused on understanding of the etiology of ASD in children [23]. This research highlights the potential utility of urine organic acid profiles as a diagnostic tool for ASD and underscores the significance of identifying biomarkers to aid in early diagnosis and intervention for children with this condition.

The concentration of research studies in Punjab underscores its role as the central hub of academic and research endeavours in Pakistan. Given its status as the most populous province, Punjab possesses a greater abundance of resources, educational institutions, and researchers, consequently leading to a higher volume of research initiatives. Sindh and Khyber Pakhtunkhwa (KPK) also feature prominently in the academic research landscape. Conversely, the absence of studies in Baluchistan highlights a crucial aspect of the nation's research and academic landscape, spotlighting the disparities in research focus across different regions and the potential implications for the development and comprehension of Pakistan's diverse locales. Efforts to promote and support research in regions with lower research activity, such as Baluchistan, can contribute to a more balanced and holistic approach to research and academic development throughout the country. Addressing these disparities can lead to a more equitable distribution of resources and research opportunities, ultimately benefiting the entire nation.

Even the expertise is quite different; the result interpretation of vitamins, steroid hormones, or immunosuppressive drugs requires a given training; for metabolomics, the training is much more complex and involves the acquisition of knowledge for the subsequent statistical approach and chemometrics. Indeed, without the latter, any metabolomic result cannot be interpreted. Hence, this underscores the dire necessity for comprehensive metabolomics training, both in Pakistan and on a global scale [24].

Conclusion

Metabolomic is an emerging field and promising avenue for chemical pathologists and researchers. Its recognition as a vital tool in medical research and diagnostics has opened exciting prospects for chemical pathologists. One of the significant advantages is its potential to revolutionize disease diagnosis and personalized medicine. By analysing the complex metabolic profiles of individuals, chemical pathologists can gain deeper insights into the underlying biochemical mechanisms of diseases. This information provides physicians with the means to make more precise diagnoses, personalized medication regimens, and an improved understanding of disease progression. Furthermore, metabolomics could provide understanding on the complex interactions between genetics, environment, and lifestyle, revealing how these factors impact health and susceptibility to illness. Chemical pathologists have a vital role for advancing medical research and improving healthcare outcomes for the diverse and dynamic population as metabolomics continues to advance in their country.

The review highlights the necessity of making a coordinated effort to close the gap between scattered MS applications in basic sciences and pharmaceuticals and their clinical integration, in cooperation with Chemical Pathologists. Interdisciplinary research and knowledge exchange can be stepped up through the creation of cooperative working groups and strategic alliances with established institutes. Additionally, creating specific funding possibilities will enable professionals and researchers to effectively progress MS-focused scholarly studies.

In conclusion, metabolomics has the potential to bring about transformative changes in healthcare and research. By fostering collaboration, supporting interdisciplinary efforts, and providing the necessary resources, the developing countries can harness the full potential of metabolomics for the benefit of their populations.

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Research Article

Maximizing Returns: Optimizing Biochemistry Lab Performance through Six Sigma application, a Yearlong Cost-Benefit Study in an Indian Laboratory

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Abstract

Introduction: One of the most essential components of a clinical laboratory's overall quality management system is quality control (QC) validation. We typically tend to use more reagents and resources than necessary in an attempt to preserve quality. Achieving higher results while using fewer resources is time imperative. We have attempted to address this issue by providing cost-benefit analysis by implementing effective QC procedures using six sigma methodology and their financial benefits.

Material and methods: Six sigma calculation of 23 routine chemistry parameters was performed over a period of one-year using bias% and cv%. New Westgard sigma rules were applied using Biorad Unity 2.0 software. A comparison was made before and after new sigma rules application including false rejection rate, probability of error detection rate, cost of all reruns, repeats, etc. Relative and absolute annual savings were computed and compared.

Results: Compared to the current rule, there was absolute savings of Indian Rupees (INR) 750105.27 when both internal failure and external failure costs were combined after the candidate rule was employed. The reduction in expenses varied with the quantity of samples examined and the quantity of QC operations carried out each day leading to an internal failure costs cut down by 50% (INR 501808.08) and external failure costs by 47% (INR 187102.8).

Conclusion: The study highlighted how quality control techniques in clinical laboratories need to be carefully planned in order to achieve significant cost reductions by lowering internal or external failure costs and effective prevention and appraisal cost planning activities prior.

Introduction

A clinical laboratory aims to produce reliable, reproducible, accurate, timely and correctly interpreted test results to aid in clinical decision-making. The analytical phase concentrates on errors ranging from 4 to 32% [1]. Often, quality is sacrificed in an attempt to save money and occasionally excessive expenditures arise from the overuse of labour, controls, reagents and calibrators [2]. Labs must prioritize cost-effectiveness while establishing and maintaining quality in all its procedures. Six Sigma is an analytical tool that can be used to find flaws in a million results. The Six Sigma scale normally ranges from 0 to 6 with a process's minimum acceptable performance being 3 sigma and values exceeding 6 being regarded as worldclass [3]. Although there are many models and techniques available globally, there isn't currently a structure in place that allows quality to be attained affordably [4].

The overall quality of a laboratory can be improved by converting sigma metrics into suitable and reliable quality control procedures. A process known as "QC validation" is used to ascertain the proper statistical QC protocols for a range of laboratory techniques [5].The idea behind it is that the best statistical quality control (QC) should have a low chance of false rejection (Pfr) and a high chance of error detection (Ped) [6]. The probability that a test run would be mistakenly designated as out of control by a quality control technique while, in reality, it is functioning correctly (i.e., there is no real error in the analytical process) is known as Pfr [7].When a genuine error arises in the analytical process Ped is the probability that a quality control procedure will accurately identify a test run as being out of control[8]. Thus Pfr is the percentage of test runs that will be refused when no errors are found and Ped is the likelihood of receiving an alert when an issue arises. Both internal and external failure costs could result from a poor application of QC methods [9]. Reprocessing control samples takes time and using more control and reagent materials as well as processing patient specimens more than once are additional costs associated with internal failure.

Incorrect diagnostic expenses and further tests performed by pathologists or physicians to confirm potentially erroneous laboratory results are examples of external failure costs [10]. Extra expenses for medical and surgical treatments are also taken into account when a patient receives an incorrect diagnosis [11]. Currently, laboratories are handling higher workloads with a wide variety of parameters with the same or fewer manpower. That being said, they still have to consistently provide high-quality results with quicker turnaround times (TATs) [12]. Choosing effective control rules for low sigma analytes and the importance of tailored QC procedures based on sigma performance are the challenges being faced by the laboratories presently [13]. In a study, sigma metrics for 21 biochemical parameters were assessed in a tertiary care hospital using external quality assurance programs and internal quality control (IQC) data. Parameters like cholesterol and glucose had high sigma levels (>6) and needed little QC work whereas stricter control guidelines were required for analytes like alkaline phosphatase with low sigma values (<3). Similar to this, CBC parameters were subjected to new westgard sigma rules at hematology labs where cut down on false rejection rates by selectively using the 13s and R4s rules improved turnaround time and financial savings. However, the challenge was striking a balance between the trade-off between sensitivity (error detection) and specificity (cutting false positives) [14]. Thus, proper planning of quality control procedures minimizes cost associated with internal failures by lowering erroneous rejections and lowers external failure costs by guaranteeing the detection of medically significant errors [15].

The purpose of this study was to ascertain combinations of QC rules and control materials which could result in more cost-effective quality control. We postulated that, while evaluating potential candidate QC rules found by QC validation, using financial analysis worksheets (six sigma cost worksheets) to calculate the costs associated with internal and external failures would be beneficial. To address this, we have attempted to apply lean six sigma and analyse the performance of 23 routine biochemistry parameters with sigma scores. Using, Biorad 2.0 unity software, the implementation of new Westgard sigma QC rules would be employed depicting number and frequency of control runs to strengthen the underperforming analytes along with a flexible quality control procedure for the outstanding and good parameteINR Following this, Internal and external failure costs would be calculated before and after implementation of Westgard QC rules and cost difference estimated in relative and absolute values with regard to reruns, repeat, control and reagent use, rework and labour use.

Material and Methods

This is a retrospective study analysis conducted on Autoanalyzer Beckman coulter AU680 based on spectrophotometry for 23 routine biochemistry parameters from September 2021 to October 2022 for a duration of one year. Third party Biorad assayed lyphocheck clinical chemistry control lot 26490(Exp11/23) was run with standard protocols as per manufacturer's instructions .It included constituting and aliquoting both low and high level of controls. The parameters that were analysed were Glucose, urea, creatinine ,total bilirubin, AST, ALT, total Protein, albumin, cholesterol, sodium, potassium, amylase and iron. Sigma metric analysis was done using MS Excel sheets. Three quality indicators Bias%, CV%, and Tea (Total allowable error) were used to calculate sigma metrics.

Bias% (inaccuracy) is the difference between the lab result and the target value [16].The target value could be the mean set by the manufacture, peer or the result of a competence exam or EQAS (External Quality Assessment Scheme). In our study, the manufacturer mean yielded the bias percentage using the formula Bias %=(Observed Value−Target Value)/Target value×100% [17].

CV% (imprecision) is the test method's analytical coefficient of variation derived from the daily IQC data. It is calculated as CV % = Standard deviation/ Laboratory mean ×100 [18]. Tea (Total allowable error) is the amount of error that can be accepted without negating the medical utility of an analytical result. TEa can be obtained from various regulatory bodies like Clinical Laboratories Improvement Act (CLIA),Biological Variation database (minimum, optimum or desirable), RCPA (Royal College of Pathologists of Australasia), Westgard site, etc. CLIA criteria for TEa has been applied here followed by BV(Biological Variation) for those [12] who did not have TEa in CLIA [19].

Sigma metric calculation

Sigma metrics were calculated from CV% (daily IQC), Bias% (manufacture mean) and TEa using formula: Sigma (σ) = (TEa%− bias%) / CV% [20]. After calculating sigma for both the levels for all the parameters,L1,L2 with different sigma scores were averaged to get a single sigma value [21].

Using Biorad Unity 2.0 software, the existing QC (Westgard Control Rule,1 2 s, 2 2 s, 1 3 s, and R4 s with n = 2) was characterized and candidate QC selections were identified and characterized [22]. A number of QC choices were determined as substitutes for the current QC, taking into account the availability of a high sigma value (> 4), low Pfr (< or = 5%) and high Ped (> or = 90%).The costs related to the current QC (1-2s,22s, R4s rule with n=2) and the candidate QC procedures were computed using the six sigma costs worksheets, specifically internal failure cost and external failure cost sheets which includes waste and rework and Quality Cost Worksheet.

Internal failure costs were broken down into three categories: the false rejection test cost (total cost of re-analyzing all patients in a test group upon finding an out-of-control QC result), the false rejection control cost (total cost of re-analyzing only control materials) and the rework labour cost [23]. In order to complete the worksheet, the information that was needed: the number of working days in a year, number of runs of the control materials per day, type of control being used, the likelihood of a false rejection, number of controls, cost of a single control material, the number of tests in each test group, estimated cost of each test, average hourly rate of employees who perform the rework and the average amount of time spent when a test needs to be repeated.

The external failure costs are the costs incurred by a laboratory to reanalyse all patients with incorrect results that were missed by quality control procedures and extra patient care cost which is the additional patient cost that arises from an incorrect test result [24]. The worksheet was completed by calculating the number of runs annually, the number of samples each run, the frequency of errors, the likelihood that an error will be detected, the estimated cost of repeating the test and the estimated cost of further patient care. Based on the frequency of the real errors recorded during the examination of the twelve months' worth of internal QC data, error frequency was determined.

For the Beckman AU auto analyzer connected to the existing QC and the candidate QC processes, two sets of costs for each single variable and each control level were computed and added up to determine the possible yearly internal, external, and total failure costs. At the end, these expenses were compared and the savings amount was determined as an absolute number (Indian rupees (INR)) and a relative savings (%).

Results

We aimed to calculate sigma scores of 23 analytes from sept' 2021 to Oct' 2022 for a period of one year run with third party Biorad controls. Results have been divided into different categories. Table 1 (1A,1B and 1C) presents the month-by-month summary of each parameter's performance characteristics together with the thorough sigma calculation

Table 1A: Sigma performance characteristics for each parameter for 12 months (Oct'2021 to Jan' 2022).

Analyte	TEa %	Level	CLIA Oct. 2021				CLIA Nov. 2021				CLIA Dec. 2021				CLIA Jan. 2022			
			CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ
Albumin	10	L1	1.08	0.84	8.481481	9.113081	3.18	2.08	2.490566	3.659917	1.95	0.3	4.974359	5.6991	2.37	2.54	3.147679	3.25402
		L2	0.94		9.744681		1.64		4.829268		1.51		6.423841		2.22		3.36036	
Alkaline Phosphatase	30	L1	3.21	6.81	7.224299	7.62426	2.59	3.2	10.34749	9.596187	2.91	8.82	7.278351	7.665791	3.23	17.9	3.74613	3.981079
		L2	2.89		8.024221		3.03		8.844884		2.63		8.053232		2.87		4.216028	
ALT	20	L1	3.09	0.82	6.20712	10.26028	4.32	1.78	4.217593	6.87843	3.52	1.03	5.389205	10.66519	3.11	1.05	6.093248	6.791683
		L2	1.34		14.31343		1.91		9.539267		1.19		15.94118		2.53		7.490119	
Amylase	30	L1	1.51	0.25	19.70199	23.25189	2.2	3.16	12.2	13.85723	1.38	-0.6	22.17391	27.90014	2.05	7.04	11.2	9.851852
		L2	1.11		26.8018		1.73		15.51445		0.91		33.62637		2.7		8.503704	
AST	20	L1	3.25	6.4	4.184615	7.363626	2.41	3.38	6.896266	7.915875	1.98	-8.17	14.22727	20.40137	3.21	2.74	5.376947	7.025157
		L2	1.29		10.54264		1.86		8.935484		1.06		26.57547		1.99		8.673367	
Bilirubin, Direct	44.5	L1	3.89	2.88	10.69923	18.60439	3.18	4.97	12.43082	18.26724	5.1	-1.79	9.076471	11.98036	4.32	0.27	10.23843	18.85524
		L2	1.57		26.50955		1.64		24.10366		3.11		14.88424		1.61		27.47205	
Bilirubin, Total	20	L1	2.4	1.71	7.620833	11.30632	2.28	1.7	8.026316	9.99355	2.57	-0.33	7.910506	11.65601	2.8	1.54	6.592857	8.128889
		L2	1.22		14.9918		1.53		11.96078		1.32		15.40152		1.91		9.664921	
Calcium	8.2	L1	1.9	1.2	3.684211	4.002599	1.64	0.55	4.664634	4.481193	0.89	-1.17	10.52809	11.19442	2.21	2.5	2.579186	2.528723
		L2	1.62		4.320988		1.78		4.297753		0.79		11.86076		2.3		2.478261	
Chloride	5	L1	1.19	0.14	4.084034	4.573267	1.39	1.28	2.676259	2.594886	1.03	0.14	4.718447	4.917118	1.63	0.15	2.97546	2.897614
		L2	0.96		5.0625		1.48		2.513514		0.95		5.115789		1.72		2.819767	
Cholesterol, HDL	30	L1	5.92	9.79	3.413851	3.328916	2.97	10.73	6.488215	5.727355	2.22	4.05	11.68919	12.08258	3.34	1.38	8.568862	8.22658
		L2	6.23		3.243981		3.88		4.966495		2.08		12.47596		3.63		7.884298	
Creatine Kinase	30	L1	1.87	2.41	14.75401	17.30147	2.17	3.28	12.31336	14.25365	2.17	1.27	13.23963	16.80776	3.3	7.08	6.945455	8.588799
		L2	1.39		19.84892		1.65		16.19394		1.41		20.37589		2.24		10.23214	
Creatinine	15.84	L1	1.39	7.72	5.841727	5.366646	1.93	0.67	7.860104	8.365724	1.86	0.41	8.295699	9.190333	2.23	8.82	3.147982	3.740658
		L2	1.66		4.891566		1.71		8.871345		1.53		10.08497		1.62		4.333333	
GGT	15	L1	1.52	2.92	7.947368	9.366541	1.27	2.07	10.1811	10.06363	1.08	-0.32	14.18519	15.79714	1.86	4.15	5.833333	5.756981
		L2	1.12		10.78571		1.3		9.946154		0.88		17.40909		1.91		5.680628	

	TEa %	Level	CLIA Oct. 2021				CLIA Nov. 2021				CLIA Dec. 2021				CLIA Jan. 2022			
			CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ
Glucose	10	L1	2.04	2.78	3.539216	3.59284	1.43	2.5	5.244755	4.753059	1.78	0.28	5.460674	6.252076	2.57	0.37	3.747082	3.985383
		L2	1.98		3.646465		1.76		4.261364		1.38		7.043478		2.28		4.223684	
Iron	20	L1	2.4	8.19	4.920833	3.805519	1.4	0.11	14.20714	10.42966	1.96	-2.58	11.52041	9.071055	2.92	6.47	4.633562	3.595609
		L2	4.39		2.690205		2.99		6.652174		3.41		6.621701		5.29		2.557656	
LDH	20	L1	7.62	0.01	2.62336	3.4751	12.13	1.4	1.533388	2.038924	9.77	1.03	1.941658	2.5283	12.1	0.49	1.612397	2.133409
		L2	4.62		4.32684		7.31		2.54446		6.09		3.114943		7.35		2.654422	
Lipase	14.2	L1	11.1	1.07	1.182883	1.529299	3.88	0.16	3.618557	4.734278	7.27	2.6	1.595598	1.90891	5.35	5.63	1.601869	2.046574
		L2	7		1.875714		2.4		5.85		5.22		2.222222		3.44		2.491279	
Phosphorus	10	L1	2.31	7.22	1.203463	1.396017	2.27	0.13	4.348018	4.679085	1.36	-0.84	7.970588	8.698338	1.64	4.31	3.469512	3.696825
		L2	1.75		1.588571		1.97		5.010152		1.15		9.426087		1.45		3.924138	
Potassium	10.98	L1	1.08	2.32	8.018519	7.445767	1.37	0.17	7.890511	7.672842	0.98	-1.14	12.36735	12.84301	1.58	4.7	3.974684	4.053131
		L2	1.26		6.873016		1.45		7.455172		0.91		13.31868		1.52		4.131579	
Protein, Total	10	L1	1.38	0.69	6.746377	6.899704	2.01	0.9	4.527363	4.216471	0.77	-0.64	13.81818	12.28283	1.58	1.25	5.537975	5.573475
		L2	1.32		7.05303		2.33		3.905579		0.99		10.74747		1.56		5.608974	
Sodium	2.73	L1	1.18	1.05	1.423729	1.605481	1.22	0.35	1.95082	1.784934	1.03	-1.42	4.029126	4.346024	1.44	1.55	0.819444	0.776182
		L2	0.94		1.787234		1.47		1.619048		0.89		4.662921		1.61		0.732919	
Urea	17.74	L1	2.19	5.18	5.73516	6.172843	2.62	0.94	6.412214	7.041723	1.82	1.47	8.93956	9.400083	2.91	4.56	4.52921	4.859093
		L2	1.9		6.610526		2.19		7.671233		1.65		9.860606		2.54		5.188976	
Uric Acid	17	L1	1.97	0.09	8.583756	9.015342	1.82	0.4	9.120879	9.120879	1.24	-2.03	15.34677	19.13724	3.17	0.07	5.340694	5.427676
		L2	1.79		9.446927		1.82		9.120879		0.83		22.92771		3.07		5.514658	

Table 1B: Sigma performance characteristics for each parameter for 12 months (Feb'2022 to May' 2022).

Analyte	TEa %	Level	CLIA Feb. 2022				CLIA March 2022				CLIA April 2022				CLIA May 2022			
			CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ
Albumin	10	L1	1.89	0.65	4.94709	4.973545	2.72	1.62	3.080882	3.293579	1.72	1.71	4.819767	4.674911	2.73	1.08	3.267399	3.115427
		L2	1.87		5		2.39		3.506276		1.83		4.530055		3.01		2.963455	
Alkaline Phosphatase	30	L1	2.82	8.77	7.528369	7.959837	2.97	8.37	7.282828	7.307516	3.67	13.58	4.474114	4.894015	2.14	17.4	5.88785	5.624776
		L2	2.53		8.391304		2.95		7.332203		3.09		5.313916		2.35		5.361702	
ALT	20	L1	3.53	1.28	5.303116	10.13956	3.25	0.23	6.083077	8.658016	3.71	3.21	4.525606	6.377999	2.44	5.12	6.098361	6.385503
		L2	1.25		14.976		1.76		11.23295		2.04		8.230392		2.23		6.672646	
Amylase	30	L1	1.42	3.88	18.39437	19.72944	1.44	2.2	19.30556	17.92659	1.19	1.59	23.87395	21.80156	1.81	3.61		16.09146
		L2	1.24		21.06452		1.68		16.54762		1.44		19.72917		1.64		16.09146	
AST	20	L1	1.96	3.32	8.510204	12.67934	2.57	3.5	6.420233	8.939283	1.87	6.17	7.395722	8.247203	1.96	6.84	6.714286	6.952771
		L2	0.99		16.84848		1.44		11.45833		1.52		9.098684		1.83		7.191257	
Bilirubin, Direct	44.5	L1	3.32	2.12	12.76506	21.30507	5.16	3.12	8.01938	11.2949	4.31	4.09	9.37587	14.94428	4.6	12.88	6.873913	10.22236
		L2	1.42		29.84507		2.84		14.57042		1.97		20.51269		2.33		13.57082	
Bilirubin, Total	20	L1	1.78	2.71	9.713483	11.66383	2.92	1.47	6.34589	7.692457	1.85	1.4	10.05405	10.28126	8.01	14.05	0.742821	1.363077
		L2	1.27		13.61417		2.05		9.039024		1.77		10.50847		3		1.983333	
Calcium	8.2	L1	1.4	0.18	5.728571	7.085338	1.82	2.76	2.989011	2.783605	1.77	4.23	2.242938	2.310092	1.75	3.16	2.88	2.669268
		L2	0.95		8.442105		2.11		2.578199		1.67		2.377246		2.05		2.458537	
Chloride	5	L1	1.41	1.2	2.695035	2.527642	1.33	2.88	1.593985	1.463659	1.41	0.22	3.390071	3.887696	1.19	0.03	4.176471	4.598336
		L2	1.61		2.360248		1.59		1.333333		1.09		4.385321		0.99		5.020202	
Cholesterol, HDL	30	L1	4.55	7.6	4.923077	4.217024	5.39	2.43	5.115028	5.411551	2.31	0.7	12.68398	9.741063	2.16	18.23	5.449074	3.749798
		L2	6.38		3.510972		4.83		5.708075		4.31		6.798144		5.74		2.050523	
Creatine Kinase	30	L1	2.73	6.02	8.783883	11.16595	2.58	6.62	9.062016	10.43505	3.77	9.05	5.557029	7.650608	3.29	9.97	6.088146	7.131828
		L2	1.77		13.54802		1.98		11.80808		2.15		9.744186		2.45		8.17551	
Creatinine	15.84	L1	4.87	0.88	3.071869	3.590879	3.66	1.59	3.893443	5.372202	2.48	3.75	4.875	5.088816	3.46	10.55	1.528902	1.69908
		L2	3.64		4.10989		2.08		6.850962		2.28		5.302632		2.83		1.869258	
GGT	15	L1	1.4	4.96	7.171429	8.869925	1.24	4.75	8.266129	8.403898	1.37	6.21	6.416058	6.964439	1.78	8.16	3.842697	4.201348
		L2	0.95		10.56842		1.2		8.541667		1.17		7.512821		1.5		4.56	

	TEa %	Level	CLIA March 2022				CLIA Nov. 2021				CLIA April 2022				CLIA Jan. 2022			
			CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ
Glucose	10	L1	2.93	1.25	2.986348	3.92373	2.91	0.4	3.298969	3.657853	2.39	2.06	3.322176	3.417725	2.81	1.53	3.014235	2.977604
		L2	1.8		4.861111		2.39		4.016736		2.26		3.513274		2.88		2.940972	
Iron	20	L1	2.14	3.04	7.925234	7.870451	2.22	0.69	8.698198	6.768899	1.64	2.35	10.7622	7.477297	3.49	12.21	2.232092	1.707991
		L2	2.17		7.815668		3.99		4.839599		4.21		4.192399		6.58		1.183891	
LDH	20	L1	9.55	0.83	2.00733	2.656251	9.05	4.56	1.706077	1.878271	9.7	10.1	1.020619	1.181951	10.49	1.58	1.755958	2.528517
		L2	5.8		3.305172		7.53		2.050465		7.37		1.343284		5.58		3.301075	
Lipase	14.2	L1	5.48	5.22	1.638686	2.132209	5.84	11.4	0.479452	0.405407	12.67	3.65	0.832676	1.322695	10.24	15.03	0.081055	0.083488
		L2	3.42		2.625731		8.45		0.331361		5.82		1.812715		9.66		0.085921	
Phosphorus	10	L1	1.06	0.44	9.018868	9.941252	1.95	0.74	4.748718	5.753921	1.29	0.83	7.108527	6.652237	1.92	0.48	4.958333	5.761925
		L2	0.88		10.86364		1.37		6.759124		1.48		6.195946		1.45		6.565517	
Potassium	10.98	L1	1.4	1.28	6.928571	7.609585	1.2	1.13	8.208333	8.541104	1.33	0.18	8.120301	9.627161	1.01	2.48	8.415842	8.681605
		L2	1.17		8.290598		1.11		8.873874		0.97		11.13402		0.95		8.947368	
Protein, Total	10	L1	1.63	1.17	5.417178	5.147815	1.42	1.95	5.669014	5.070618	1.16	0.16	8.482759	8.51964	1.55	1.57	5.43871	6.091355
		L2	1.81		4.878453		1.8		4.472222		1.15		8.556522		1.25		6.744	
Sodium	2.73	L1	1.24	1.5	0.991935	0.887687	1.29	1.4	1.031008	1.031008	1.35	0.52	1.637037	1.891334	0.98	0.33	2.44898	2.367347
		L2	1.57		0.783439		1.29		1.031008		1.03		2.145631		1.05		2.285714	
Urea	17.74	L1	2.7	2.87	5.507407	6.345491	2.52	1.14	6.587302	6.934002	2.89	4.96	4.422145	4.252606	4.18	4.06	3.272727	3.110502
		L2	2.07		7.183575		2.28		7.280702		3.13		4.083067		4.64		2.948276	
Uric Acid	17	L1	1.64	1.86	9.231707	9.43751	1.62	1.52	9.555556	9.803752	1.64	0.91	9.810976	8.664834	2.43	**2.16**	6.106996	5.603326
		L2	1.57		9.643312		1.54		10.05195		2.14		7.518692		2.91		5.099656	

Table 1C: Sigma performance characteristics for each parameter for 12 months (Jun'2022 to Sep' 2022).

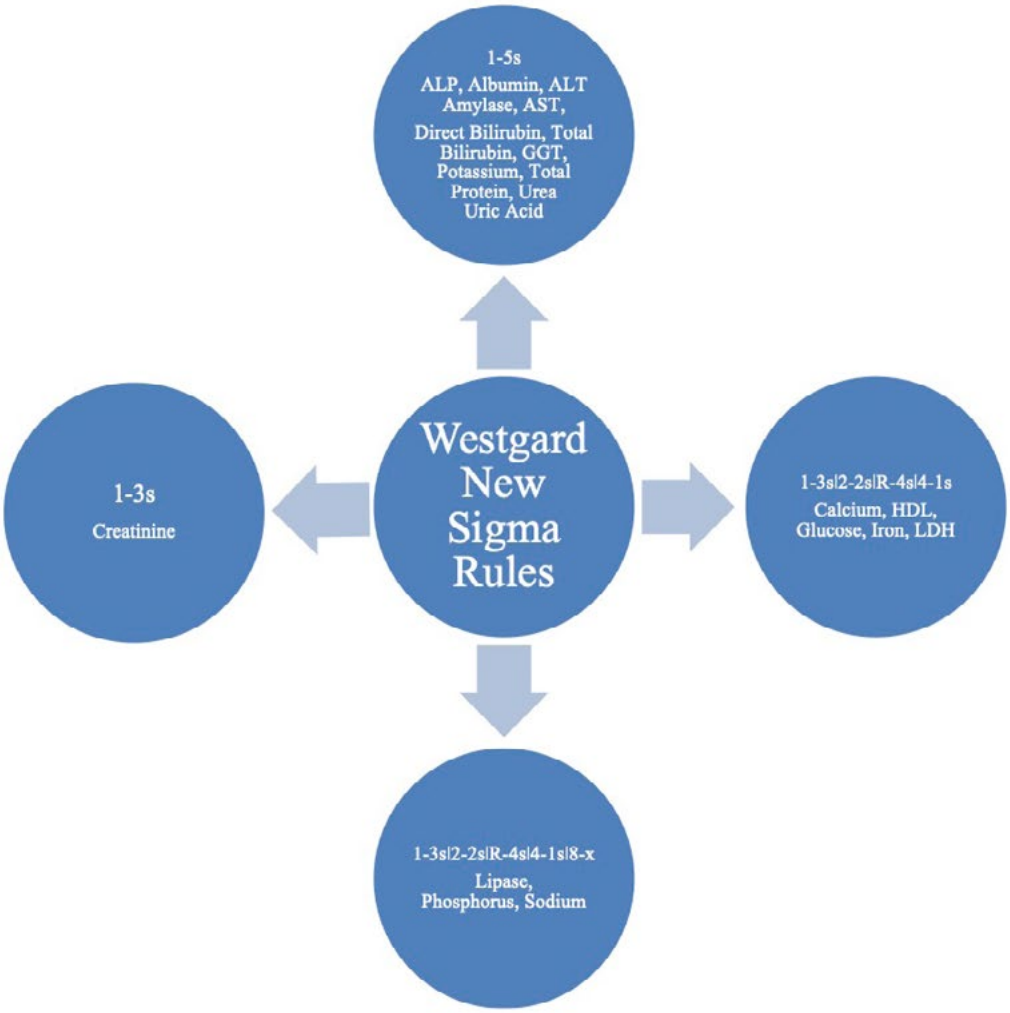
Analyte	TEa %	Level	CLIA Feb. 2022				CLIA March 2022				CLIA April 2022				CLIA May 2022			
			CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ
Albumin	10	L1	2.67	0.52	3.550562	4.09881	2.6	2.02	3.069231	2.860197	0.94	2.59	7.882979	7.003473	1.25	1.59	6.728	6.130447
		L2	2.04		4.647059		3.01		2.651163		1.21		6.123967		1.52		5.532895	
Alkaline Phosphatase	30	L1	1.76	**8.37**	12.28977	12.62093	2.8	10.39	7.003571	7.674126	1.74	9.02	12.05747	10.62961	2.13	4.29	12.07042	13.13742
		L2	1.67		12.9521		2.35		8.344681		2.28		9.201754		1.81		14.20442	
ALT	20	L1	2.25	5.84	6.293333	7.656221	2.41	3.5	6.846473	7.122788	4.34	3.08	3.898618	4.380343	3.1	0.04	6.43871	7.558485
		L2	1.57		9.019108		2.23		7.399103		3.48		4.862069		2.3		8.678261	
Amylase	30	L1	1.92	**1.1**	15.05208	17.15938	1.9	2.95	14.23684	16.13509	1.65	1.7	17.15152	18.13657	1.6	0.79	18.25625	24.34167
		L2	1.5		19.26667		1.5		18.03333		1.48		19.12162		0.96		30.42708	
AST	20	L1	3.05	10.52	3.108197	4.516598	1.71	0.43	11.44444	11.83785	2.88	3.5	5.729167	7.117161	2.76	1.46	6.717391	10.32862
		L2	1.6		5.925		1.6		12.23125		1.94		8.505155		1.33		13.93985	
Bilirubin, Direct	44.5	L1	5.61	**3.22**	7.358289	10.14936	4.5	3.55	9.1	9.8	3.64	0.22	12.16484	16.98882	3.55	1.45	12.12676	22.3702
		L2	3.19		12.94044		3.9		10.5		2.03		21.81281		1.32		32.61364	
Bilirubin, Total	20	L1	3.66	1.67	5.008197	7.992122	8.01	0.62	2.419476	4.439738	2.42	0.39	8.103306	9.439016	2.37	0.24	8.337553	9.879759
		L2	1.67		10.97605		3		6.46		1.82		10.77473		1.73		11.42197	
Calcium	8.2	L1	2.6	**5.42**	1.069231	1.199687	1.75	0.38	4.468571	4.141603	1.03	2.87	5.174757	5.054971	1.51	1.15	4.668874	5.066995
		L2	2.09		1.330144		2.05		3.814634		1.08		4.935185		1.29		5.465116	
Chloride	5	L1	1.65	1.21	2.29697	2.606177	1.19	2.3	2.268908	2.49809	1.21	0.43	3.77686	4.2441	1.2	0.01	4.158333	5.237395
		L2	1.3		2.915385		0.99		2.727273		0.97		4.71134		0.79		6.316456	
Cholesterol, HDL	30	L1	3.25	4.77	7.763077	7.624862	2.16	8.73	9.847222	6.776399	1.69	2.5	16.27219	14.35781	1.77	2.76	15.38983	15.17843
		L2	3.37		7.486647		5.74		3.705575		2.21		12.44344		1.82		14.96703	
Creatine Kinase	30	L1	2.45	9.21	8.485714	10.18286	3.29	3.84	7.951368	9.314459	3.05	5.46	8.045902	7.955643	3.17	2.04	8.820189	18.82247
		L2	1.75		11.88		2.45		10.67755		3.12		7.865385		0.97		28.82474	
Creatinine	15.84	L1	3.28	0.09	4.801829	5.154411	3.46	2.31	3.910405	4.345662	2.48	0.22	6.298387	6.211939	2.27	3.04	5.638767	5.663828
		L2	2.86		5.506993		2.83		4.780919		2.55		6.12549		2.25		5.688889	
GGT	15	L1	1.81	**11.05**	2.18232	**2.482005**	1.78	**0.51**	8.140449	**8.900225**	1.24	**2.63**	9.975806	**10.01636**	1.57	**3.01**	7.636943	**9.527995**
		L2	1.42		2.78169		1.5		9.66		1.23		10.05691		1.05		11.41905	

	TEa %	Level	CLIA March 2022				CLIA Nov. 2021				CLIA April 2022				CLIA Jan. 2022			
			CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ	CV %	Bias %	Sigma	σ
Glucose	10	L1	2.72	0.28	3.573529	4.750179	2.81	4.87	1.825623	1.803436	2.19	3.61	2.917808	3.295111	2.46	4.48	2.243902	3.521951
		L2	1.64		5.926829		2.88		1.78125		1.74		3.672414		1.15		4.8	
Iron	20	L1	3.92	3.47	4.216837	3.713273	3.49	**3.78**	4.647564	**3.556305**	1.18	**0.2**	16.77966	**12.11164**	2.52	**1.2**	7.460317	**6.091968**
		L2	5.15		3.209709		6.58		2.465046		2.66		7.443609		3.98		4.723618	
LDH	20	L1	9.27	0.32	2.122977	2.690628	10.49	1.5	1.763584	2.539498	8.02	7.75	1.527431	2.14322	12.9	2.62	1.347287	1.796382
		L2	6.04		3.258278		5.58		3.315412		4.44		2.759009		7.74		2.245478	
Lipase	14.2	L1	4.7	1.31	2.742553	2.97451	10.24	**3.05**	1.088867	**1.121556**	5.99	**2.69**	1.921536	**2.00334**	14.1	**2.28**	0.84539	**1.498507**
		L2	4.02		3.206468		9.66		1.154244		5.52		2.085145		5.54		2.151625	
Phosphorus	10	L1	1.8	0.16	5.466667	6.76612	1.92	0.53	4.932292	5.731663	1.84	4.73	2.86413	2.841156	2.01	0.19	4.880597	7.890299
		L2	1.22		8.065574		1.45		6.531034		1.87		2.818182		0.9		10.9	
Potassium	10.98	L1	1.09	1.51	8.688073	10.92043	1.01	**1.12**	9.762376	**10.07066**	0.89	**0.73**	11.51685	**12.16468**	1.04	**0.39**	10.18269	**12.65563**
		L2	0.72		13.15278		0.95		10.37895		0.8		12.8125		0.7		15.12857	
Protein, Total	10	L1	2.2	0.12	4.490909	5.134343	1.55	0.31	6.251613	7.001806	1.35	1.9	6	6.681818	1.4	**1.95**	5.75	6.501126
		L2	1.71		5.777778		1.25		7.752		1.1		7.363636		1.11		7.252252	
Sodium	2.73	L1	1.12	1.59	1.017857	1.067752	0.98	1.33	1.428571	1.380952	0.97	0.23	2.57732	2.725441	1.02	0.06	2.617647	3.243606
		L2	1.02		1.117647		1.05		1.333333		0.87		2.873563		0.69		3.869565	
Urea	17.74	L1	5.1	1.23	3.237255	3.499037	4.18	2.85	3.562201	3.385626	3.11	1.63	5.180064	5.239703	4.12	1.86	3.854369	3.917159
		L2	4.39		3.76082		4.64		3.209052		3.04		5.299342		3.99		3.97995	
Uric Acid	17	L1	3.17	1.33	4.943218	5.722646	2.43	1.2	6.502058	5.965805	2.95	1.27	5.332203	5.123914	3.17	0.36	5.249211	11.20193
		L2	2.41		6.502075		2.91		5.429553		3.2		4.915625		0.97		17.15464	

The comprehensive sigma calculation and month-by-month summary of each parameter's performance characteristics is shown in table 1A, 1B and 1C where majority of parameters were above six sigma zones such as ALT, Amylase, Direct Bilirubin and Creatine Kinase. Potassium, lipase and LDH had been performing poorly for few months (potassium in April, January 2022, lipase in October, December 2021 and January, February, March 2022, LDH in November, December 2021, January, February 2022), with less than two sigma score.

Table 2 (2A and 2B),the internal failure cost worksheet summarizes the performance characteristics for each single variable and control and contains the chosen candidate QC methods using Biorad Unity 2.0 software taking into account the availability of a high sigma value (> 4), low Pfr (< or = 5%) and high Ped (> or = 90%). The "number of tests for each group" matched the daily sample count of 300 for glucose,200 for ALT and AST and so on. Internal quality control was conducted once in the morning and evening and operations were deemed stable during that period. A cost of INR9.19 was determined for each run of the control material. After dividing the cost of a full test kit by the number of tests in a kit, the cost per measurand was projected to be different for different analytes. One test retesting took an estimated 1.0 minute and the average hourly pay of employees was calculated to be INR 150 per hour. Table 2 represents the performance characteristics of each analyte's selected candidate QC methods with the internal failure cost worksheet. New Westgard sigma rules based on sigma performance are shown in Figure 1.

Figure 1: Summarizes New Westgard Sigma Rules according to sigma performance.



With a few exceptions, such as lipase, phosphorus and calcium which call for a strict quality management strategy, the majority of the parameters in Figure 1 fall within the outstanding sigma zone with a rule of 1 5s and 1 3s.

Table 2A: Internal Failure Cost sheet.

Analyte	Sigma	New Westgard Sigma Rule	No. of control run	False Rejection (Pfr)	Ped	Estimated cost per control(in Rs)	Number of tests in each group test	Cost per test (INR)	False rejection cost test (INR)	False rejection control cost test (INR)	Patients test +control
Albumin	9.11	1-5s	2	0.03	0.95	9.19	300	4	22320.00	341.79	22661.79
Alkaline Phosphatase	7.62	1-5s	2	0.03	0.95	9.19	300	5	27900.00	341.79	28241.79
ALT	10.26	1-5s	2	0.03	0.95	9.19	300	4	22320.00	341.79	22661.79
Amylase	23.25	1-5s	2	0.03	0.95	9.19	50	15	13950.00	341.79	14291.79
AST	7.36	1-5s	2	0.03	0.95	9.19	300	5	27900.00	341.79	28241.79
Bilirubin, Direct	18.60	1-5s	2	0.03	0.95	9.19	100	6	11160.00	341.79	11501.79
Bilirubin, Total	11.31	1-5s	2	0.03	0.95	9.19	100	5	9300.00	341.79	9641.79
Calcium	4.00	1-3s|2-2s| R-4s|4-1s	4	0.02	0.95	9.19	50	5	3100.00	455.72	3555.72
Chloride	4.57	1-2.5s	2	0.03	0.9	9.19	250	5	23250.00	341.79	23591.79
Cholesterol, HDL	3.33	1-3s|2-2s| R-4s|4-1s	4	0.02	0.95	9.19	100	45	55800.00	455.72	56255.72
Creatine Kinase	17.30	1-5s	2	0.03	0.95	9.19	150	18.09	50468.31	341.79	50810.10
Creatinine	5.37	1-3s	2	0.01	0.92	9.19	300	3.5	6510.00	113.93	6623.93
GGT	9.37	1-5s	2	0.03	0.95	9.19	50	9	8370.00	341.79	8711.79
Glucose	3.59	1-3s|2-2s| R-4s|4-1s	4	0.02	0.95	9.19	400	3	14880.00	455.72	15335.72
Iron	3.81	1-3s|2-2s| R-4s|4-1s	4	0.02	0.95	9.19	50	19	11780.00	455.72	12235.72
LDH	3.48	1-3s|2-2s| R-4s|4-1s	4	0.02	0.95	9.19	50	15	9300.00	455.72	9755.72
Lipase	1.53	1-3s|2-2s| R-4s|4-1s|8-x	4	0.02	0.98	9.19	50	57.60	35712.00	455.72	36167.72
Phosphorus	1.40	1-3s|2-2s| R-4s|4-1s|8-x	4	0.02	0.98	9.19	50	6	3720.00	455.72	4175.72
Potassium	7.45	1-5s	2	0.03	0.95	9.19	250	5	23250.00	341.79	23591.79
Protein, Total	6.90	1-5s	2	0.03	0.95	9.19	200	4	14880.00	341.79	15221.79
Sodium	1.61	1-3s|2-2s| R-4s|4-1s|8-x	4	0.02	0.98	9.19	250	5	15500.00	455.72	15955.72
Urea	6.17	1-5s	2	0.03	0.95	9.19	300	4	22320.00	341.79	22661.79
Uric Acid	9.02	1-5s	2	0.03	0.95	9.19	50	8	7440.00	341.79	7781.79
Total									441130.31	8544.84	449675.15

Table 2B: Reanalyzing Cost sheet.

Analyte	Rework labour cost (INR)	Reanalyzing only control	Reanalyzing tests+Control	Total cost of waste and rework (INR)	Relative Savings (%)	Absolute Savings
Albumin	2790	3131.79	25451.79	25451.79	40%	15107.87
Alkaline Phosphatase	2790	3131.79	31031.79	31031.79	40%	18827.87
ALT	2790	3131.79	25451.79	25451.79	40%	15107.87
Amylase	2790	3131.79	17081.79	17081.79	40%	9527.87
AST	2790	3131.79	31031.79	31031.79	40%	18827.87
Bilirubin, Direct	2790	3131.79	14291.79	14291.79	40%	7667.87
Bilirubin, Total	2790	3131.79	12431.79	12431.79	40%	6427.87
Calcium	1860	2315.72	5415.72	5415.72	60%	5333.59
Chloride	2790	3131.79	26381.79	26381.79	40%	15727.87
Cholesterol, HDL	1860	2315.72	58115.72	58115.72	60%	84383.59
Creatine Kinase	2790	3131.79	53600.10	53600.10	40%	33873.41
Creatinine	930	1043.93	7553.93	7553.93	80%	26495.73
GGT	2790	3131.79	11501.79	11501.79	40%	5807.87
Glucose	1860	2315.72	17195.72	17195.72	60%	23003.59
Iron	1860	2315.72	14095.72	14095.72	60%	18353.59
LDH	1860	2315.72	11615.72	11615.72	60%	14633.59
Lipase	1860	2315.72	38027.72	38027.72	60%	54251.59
Phosphorus	1860	2315.72	6035.72	6035.72	60%	6263.59
Potassium	2790	3131.79	26381.79	26381.79	40%	15727.87
Protein, Total	2790	3131.79	18011.79	18011.79	40%	10147.87
Sodium	1860	2315.72	17815.72	17815.72	60%	23933.59
Urea	2790	3131.79	25451.79	25451.79	40%	15107.87
Uric Acid	2790	3131.79	10571.79	10571.79	40%	5187.87
Total		63414.84	504545.15	504545.15		449728.08

(Day: 310.00, R:2, Avg hourly rate of employee who perform repeat/rerun (in Rs): 150.00 and Avg amount of time consumed when one run of this test must be redone (in minutes): 1.00)

The Biorad unity software provided the probability of error detection and false rejection which changed based on the variables taken into consideration and the internal QC rule chosen. A total of 310 working days were computed annually with annual statistics showing that the laboratory completed a median of 50-300 tests every day. The internal failure costs for the parameters were determined to be INR 63414.84 for re-analyzing only the control materials (false rejection control cost + rework labour cost) and INR 504545.15 for re-analyzing the controls and all patients (false rejection test cost + false rejection control cost + rework labour cost).Total false rejection cost test amounted to be INR 441130.31. It also presented the total cost reductions both in absolute savings of INR 501808.04 and a relative of 50% for each variable that was achieved by implementing the candidate QC controls that biorad unity software recommended. The internal failure cost sheet with previous rule (1 2s,2 2s,R4s) amounted to a total expenditure of INR 1006353.19.

Using the 1 5s, 1 3s, and 2.5 s rule instead of the existing 1-2 s/2 2s/R4s rule resulted in a 50% reduction in internal failure costs to summarize. However, the low-performing parameters necessitated higher QC run and frequency which came out to be 2-2s, R4s, 8x resulting in an additional costing. Thus table 2 calculates the costs related to the current QC (1-2s,2 2s,R4s rule with n = 2) and candidate QC procedures using the six sigma costs worksheets containing all the parameters with regard to the cost of each analyte according to their batch size, Ped ,Pfr, reruns ,number of reruns.

Table 3,represented the second worksheet, Quality cost worksheet to ascertain the external failure using Westgard sigma rules which offered the external QC rule selection and the variable that determined the chance of error detection and error frequency. The anticipated expenses for rerunning the test matched the cost of a patient's test. The additional patient care costs based on an incorrect test result to be INR 0 (zero) as it was not possible to calculate other extra expenses like misdiagnosis which entails the expense of both not receiving necessary therapy and cost of receiving it erroneously. Table 3 described the Westgard sigma rules to illustrate the external failure worksheet.

Table 3: Westgard sigma rules to illustrate the external failure worksheet.

Analyte	New Westgard Sigma Rule	Runs /year	Patients / run	Error frequency	Probability of error detection	Cost per test (INR)	Estimated cost (time, labour) to repeat the test	Estimated cost of extra patient care
Albumin	1-5s	310	300	0.03	0.95	4	10602	0
Alkaline Phosphatase	1-5s	310	300	0.03	0.95	5	13252.5	0
ALT	1-5s	310	300	0.03	0.95	4	10602	0
Amylase	1-5s	310	50	0.03	0.95	15	6626.25	0
AST	1-5s	310	300	0.03	0.95	5	13252.5	0
Bilirubin, Direct	1-5s	310	100	0.03	0.95	6	5301	0
Bilirubin, Total	1-5s	310	100	0.03	0.95	5	4417.5	0
Calcium	1-3s|2-2s| R-4s|4-1s	310	50	0.02	0.95	5	1472.5	0
Chloride	1-2.5s	310	250	0.03	0.9	5	10462.5	0
Cholesterol, HDL	1-3s|2-2s| R-4s|4-1s	310	100	0.02	0.95	45	26505	0
Creatine Kinase	1-5s	310	150	0.03	0.95	18.09	23972.44725	0
Creatinine	1-3s	310	300	0.01	0.92	3.5	2994.6	0
GGT	1-5s	310	50	0.03	0.95	9	3975.75	0
Glucose	1-3s|2-2s| R-4s|4-1s	310	400	0.02	0.95	3	7068	0
Iron	1-3s|2-2s| R-4s|4-1s	310	50	0.02	0.95	19	5595.5	0
LDH	1-3s|2-2s| R-4s|4-1s	310	50	0.02	0.95	15	4417.5	0
Lipase	1-3s|2-2s| R-4s|4-1s|8-x	310	50	0.02	0.98	57.60	17498.88	0
Phosphorus	1-3s|2-2s| R-4s|4-1s|8-x	310	50	0.02	0.98	6	1822.8	0
Potassium	1-5s	310	250	0.03	0.95	5	11043.75	0
Protein, Total	**1-5s**	**310**	**200**	**0.03**	**0.95**	**4**	**7068**	**0**
Sodium	**1-3s|2-2s| R-4s|4-1s|8-x**	**310**	**250**	**0.02**	**0.98**	**5**	**7595**	**0**
Urea	**1-5s**	**310**	**300**	**0.03**	**0.95**	**4**	**10602**	**0**
Uric Acid	**1-5s**	**310**	**50**	**0.03**	**0.95**	**8**	**3534**	**0**
Total							**209681.9773**	

Considering that the probability of error detection was 0.95 and 0.98 in majority of parameters and that the error frequency varied based on the rule (0.02,0.03), number of runs and run size, the total external failure cost with candidate rule was determined to be INR 209681.97. When the 1-2.5s, 1 3s, and 1 5s rules were used (with n = 2), the external failure costs which were INR396784.73 with the previous rule (1-2s,2 2s, R4s, n = 2), only slightly rose due to the marginally decreased chance of error detection (90%) and high chance of false rejection (5%) leading to INR 209681.97.Absolute savings amounted to INR 187102.8 while we encountered a relative savings of 47%.

In a laboratory that processes a higher number of samples per day and where control materials were routinely analyzed twice a day, like ours, Table 4 simulates the potential annual savings on one variable when the 1-2.5s, n = 2,1 3s,1 5s rule was used instead of the current 1-2s,2 2s,R4s n = 2. Table 4 represents cost savings of each parameter with current and candidate rule.

Table 4: Cost Savings=(External failure costs + internal failure costs) for current rule-(External failure costs + internal failure costs) of candidate rule.

Analyte	Total External+Internal Cost with Previous rule	Total Internal+External Failure Cost with Candidate rule	Difference between candidate Rule cost and the Previous Rule cost
Albumin	53021.66	36053.79	16967.86
Alkaline Phosphatase	64972.16	44284.29	20687.86
ALT	53021.66	36053.79	16967.86
Amylase	35095.91	23708.04	11387.86
AST	64972.16	44284.29	20687.86
Bilirubin, Direct	29120.66	19592.79	9527.86
Bilirubin, Total	25137.16	16849.29	8287.86
Calcium	15011.81	6888.22	8123.59
Chloride	54432.16	36844.29	17587.86
Cholesterol, HDL	171794.31	84620.72	87173.59
Creatine Kinase	113305.95	77572.55	35733.40
Creatinine	40764.26	10548.53	30215.72
GGT	23145.41	15477.54	7667.86
Glucose	50057.31	24263.72	25793.59
Iron	40834.81	19691.22	21143.59
LDH	33456.81	16033.22	17423.59
Lipase	112568.19	55526.60	57041.59
Phosphorus	16912.11	7858.52	9053.59
Potassium	55013.41	37425.54	17587.86
Protein, Total	37087.66	25079.79	12007.86
Sodium	52134.31	25410.72	26723.59
Urea	53021.66	36053.79	16967.86
Uric Acid	21153.66	14105.79	7047.86
Total	INR 1216035.16	INR 714227.13	INR 501808.04

In contrast to the previous (1-2s/2 2s/R4s, n = 2), the application of the candidate (1-2.5s/13s/1 5s, n = 2) rule resulted in a considerable saving, according to the final calculations (total of both internal and external failure cost for all variables with candidate QC procedure and previous QC procedure at 2 levels of control material).The previous internal + external cost difference from the present internal +external cost lead to a difference of (1216035.16-714227.13) INR 501808.08 and a relative savings of 41%. Table 5 demonstrates the annual savings with rerunning both patient samples and controls and only controls.

Table 5: Annual savings (INR) by rerunning both Patients and control and only controls.

Analyte	Rerunning only Control	Rerunning Patients+Control
Albumin	3131.79	25451.79
Alkaline Phosphatase	3131.79	31031.79
ALT	3131.79	25451.79
Amylase	3131.79	17081.79
AST	3131.79	31031.79
Bilirubin, Direct	3131.79	14291.79
Bilirubin, Total	3131.79	12431.79
Calcium	2315.72	5415.72
Chloride	3131.79	26381.79
Cholesterol, HDL	2315.72	58115.72
Creatine Kinase	3131.79	53600.10
Creatinine	1043.93	7553.93
GGT	3131.79	11501.79
Glucose	2315.72	17195.72
Iron	2315.72	14095.72
LDH	2315.72	11615.72
Lipase	2315.72	38027.72
Phosphorus	2315.72	6035.72
Potassium	3131.79	26381.79
Protein, Total	3131.79	18011.79
Sodium	2315.72	17815.72
Urea	3131.79	25451.79
Uric Acid	3131.79	10571.79
Total	63414.84	504545.15

According to the calculations, if the QC rule is changed from 1-2s/22s /R4s with n = 2 to 1-2.5s,1-5s,1 3s (with n = 2), there can be an annual savings of 50% (INR 504545.15) if all patient samples and controls are reanalyzed, and INR 63414.84 (49%) if just the controls are reanalysed (Table 5).

If the laboratory's policy is to seek for a potential issue, solve it and reanalyse only the control materials and not all patient samples when a QC material goes out of control, then the latter is thought to be the true lab savings.

Table 6 focuses on actual overall savings potential annual internal failure costs, external failure costs, total costs and combined data calculated for all the parameters using 1-2s/2 2s/R4s and 1-2.5s/1 3s/1 5s rules with 2 levels of quality control material. Table 6 demonstrates internal and external failure costs, total costs, and integrated data computed for all parameters using the preceding and candidate rule .

Table 6: Internal and external failure costs, total costs, and integrated data computed for all parameters using the preceding and candidate rule.

	Internal Failure Cost (INR)		**External Failure Cost (INR)**	**Total internal+External (INR)**
	Rerun(control)	Rerun (Patients+control)		
Cost with New Rule	63414.84	504545.15	209681.97	777641.96
Previous cost	124609.34	1006353.19	396784.7	1527747.23
Relative and total Savings with candidate QC compared to old QC ,12s,22s,1 3s,R4s				
				750105.27
Cost savings=(external failurecosts+internal failurecosts) for current rule -(external failure costs+internal failure costs) for candidate rule.				
Relative Cost Savings (% of total cost savings)				49%

In a high-volume laboratory like ours and a laboratory where the QC procedures were carried out twice a day, the potential annual savings were demonstrated to be proportionately higher (INR 750105.27).When rerun of controls and controls along with patient tests in internal failure were added along with extra care cost of external failure, a total of INR 777641.96 was found to be with the new rule while a total of INR 1527747.23 was observed with the previous rule reflecting an absolute saving of INR 750105.27 and a relative savings of 49.09 %. **On applying Mann Whitney U test, the value of U is 145. The Z-score is 2.61433 and the P value is 0.00453. The result is significant at P <0.05. The result is found to be statically significant.**

Discussion and Conclusion

Using the proposed 1-2.5s,1 3s,1, 5s n = 2 rule instead of the present 1-2s,2 2s,R4S, n = 2 rule for quality control, resulted in absolute cost savings of INR 501808 and relative savings of 50%. These savings have significantly impacted laboratory budgets, especially in ours which is a high-volume settings. Re-evaluating all patient samples and controls resulted in a 50% annual savings (INR 504545.15) whereas re-analyzing only control materials resulted in a 49% annual savings (INR 63414.84). Reanalyzing controls and patient samples resulted in higher internal failure costs due to the high false rejection rate. On the other hand, the probability of a false rejection dropped to 3% when the candidate 1-2.5s,1 3s,1 5s rule was used. The internal failure costs consequently dropped significantly to INR 504545.15. Comparing the candidate rule to the present 1-2s rule/2-2s, there was a significant (50%) reduction in internal failure costs due to the lower erroneous rejection rate. In order to illustrate the possible cost savings advantages of applying this QC technique in the laboratory context, this comparison showed how effective the candidate 1-2.5s/1 3s/1 5s rule is at lowering internal failure costs by eliminating false rejections. However, there was a minor rise in external failure costs, which might be attributed to a decrease in the likelihood of error detection when the 1-2.5/2-2s s rule was applied with the same quantity of QC materials. The importance of selecting appropriate QC procedures lies in its utility to enhance cost-effectiveness without compromising quality. Furthermore, fewer technician hours were needed to repeat and reanalyze out-of-control runs, freeing up time for additional laboratory activities. Nevertheless it cannot be overlooked that the poor performing parameters like LDH, lipase and sodium that had called for rigorous control measurements and runs by application of 1-3s|2-2s|R-4s|4-1s which raised concerns over the quality and quality control material use, reagent consumption, retests, reruns, rework etc. **It's possible reasons for subpar performance were investigated and addressed in terms of electrode replacement, enhanced cleaning, reagent storage, meticulous instrument maintenance and more personnel training.** Proper validation of QC rules thus is essential for minimizing both internal and external failure costs, thereby improving the overall quality of laboratory results. Additionally, the importance of selecting appropriate QC procedures to enhance cost-effectiveness is the need of time as there is a the necessity of integrating new QC procedures into existing laboratory workflows.

To enhance QC, a study on POCT glucose monitoring devices applied Westgard Sigma Rules and sigma metrics. Devices with sigma >6 required fewer quality control inspections, maximizing resource use, while low-performing devices (sigma <3) were identified for prompt calibration and maintenance. The difficulties were that low-performing analyzers required regular recalibration and device replacement, which momentarily raised expenses [25]. Similarly, using the revised Westgard sigma rules, laboratories from various nations assessed thyroid function tests (TFTs) in a multi-center evaluation of QC performance adopting tailored QC strategies according to sigma levels which enhanced Ped (Probability of Error Detection) and

decreased erroneous rejections. It was difficult to reach an agreement on acceptable sigma levels across several sites, though. However, inter-lab standardization and overall test accuracy were enhanced by cooperative efforts [26].

In addition to controls, reagents, repeats and rework, the price of calibration also has an impact on laboratory expenses. Vaneeta et al. investigated the use of excess quality control material where the laboratory saved almost 13,051 Canadian dollars (CAD) (43%) as a result of the annual cost of calibrators being lowered from CAD 30,568.42 (2019–20) to CAD 17,517 (2020–21) [27]. Another by Francesco Cian showed that by applying the candidate 1-2.5s, n = 3 rule in place of the already used 1-2s, n = 3 rule resulted in savings of 75% annually (GBP 8232.5) on reassessing every patient sample along with the controls, whereas reanalyzing just the control materials resulted in savings of 72% annually (GBP 822.4) [28]. Our laboratory was already using 12s/2 2s/R4s before switching to candidate sigma rules therefore the relative cost savings were up to 50%. Teams using six sigma methodology need to always remember that quality has a price. The costs related to upholding high standards across an organization or process are included in the cost of quality (CoQ) [29].Before results are delivered, internal failure costs such as problems with samples, recollection, invalid instrument runs, expired reagents and delays in turnaround time like reruns, retestings, repairs, or equipment downtime have to be identified and fixed inside the laboratory. We can boost the profit margin if we can eliminate the cost of failure. Laboratories would become more error-free and lean if we eliminate both internal and external failures. A process operating at 4 sigma would use 15-20% of revenue and produce 6000 errors [30]. Less than 1% of revenue is lost in fixing extremely rare errors that occur when the lab process goes from 4 to 6 sigma [31]. Users who get inaccurate reports, recalls, customer complaints resulting from incorrect results, misdiagnosis, etc. are the ones who identify external failure costs outside of the laboratory. For handling failure, the majority of labs dedicate most of their budgets and resources. Sadly, this is the region that needs the most attention. It is best to minimize incurring internal and external failure costs as much as possible and to focus more on preventive and appraisal costs. When a lab has poor quality, it loses 25% of its revenue fixing the problems [32]. Repeat tests, revise results, replace samples and redo are examples of low-quality actions that incur significant costs. An attempt should be made to avoid wasteful practices like overprocessing, overproduction, supply management, patient waiting for test results and improper use of scales through effective preventative and assessment activities. This study is just one which underscores the need of choosing a suitable quality control method and evaluating the financial effects of various quality control regulations. **Labs seeking to implement similar methodologies may include specific actionable recommendations such as adopting process improvements like standardization, automation and lean practices along with identifying root causes using tools like Pareto analysis and Fishbone diagrams. It is possible to monitor and decrease variability by improving quality control through the use of statistical process control, stronger standards and more QC tests. Method validation, appropriate equipment maintenance, calibration and frequent personnel training and competency evaluations are essential for accuracy and dependability. Continual benchmarking against laboratory standards, risk management techniques like FMEA and error-proofing techniques all help to sustain continual quality improvement.**

Future perspectives and limitations

Understandably, there are limitations as well of this model as we have used the manufacturer mean to calculate the bias percentage. More research can be done utilizing the bias% from the EQAS (External Quality Assessment Scheme), peer or lab mean. **It can be challenging to create a statistical quality control strategy that fits the unique requirements of the laboratory .More resources such as training and quality control materials could be needed to implement a scientifically based quality control system.** This study can be applied not only in clinical biochemistry laboratory but also for assessing QC procedures in other areas of immunoassay, molecular or genetic laboratories. Further long-term effects of improved QC procedures on patient outcomes and laboratory efficiency can be explored.

Competing interests

The authors declare that they have no competing interests to disclose.

Declaration of conflict of interest

The authors affirm that this study is free from any conflicts of interest.

Ethical Approval

This investigation was carried out in compliance with the institutional research committee's ethical guidelines.

Abbreviations

CLIA-Clinical Laboratory Improvement Act, EQAS-External Quality Assessment Scheme, IQC-Internal Quality Control, QC-Quality Control

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Research Article

Investigation of Dyslipidemia and Lipid Profile Ratios Among Patients in Tertiary Care Hospitals

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Abstract

Dyslipidemia, characterized by imbalances in lipid profiles, has emerged as a multifaceted health challenge in the population. Factors such as urbanization, dietary shifts, and genetic variations contribute to the rising prevalence of abnormal lipid levels. This condition significantly amplifies the risk of cardiovascular diseases, a leading cause of mortality. This study focused on dyslipidemia, risk factors, prevalence, evaluating the ratio of cholesterol to HDL (High density lipoproteins), triglyceride to HDL, LDL (Low density lipoproteins) to HDL, Cholesterol to LDL, triglyceride to LDL,HDL to LDL along with their comparative analysis and age-related patterns of dyslipidemia. A total of 100 were collected, comprising 64 males and 36 females. The findings revealed a significant prevalence of dyslipidemia, reaching 86%. Breakdown of lipid profiles showed specific prevalence rates for cholesterol (29%), triglycerides (50%), HDL (48%), and LDL (31%). Alarmingly, out of the 100, only 14 individuals had a normal lipid profile, indicating a high incidence of dyslipidemia in the region. Additionally, the study highlighted an age-dependent increase in the likelihood of developing dyslipidemia. The high prevalence of dyslipidemia is an important public health problem. Enhanced public health preventive measures should be implemented to better diagnose and comprehensively treat dyslipidemia.

Introduction

Lipids, including cholesterol and triglycerides, are absorbed from the intestines, and transported throughout the body by lipoproteins to support energy production, steroid synthesis, or bile acid formation. Key components in these processes are cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides, and high-density lipoprotein (HDL). Dyslipidemia can arise from an imbalance in any of these factors, resulting from either organic or nonorganic causes [1]. Dyslipidemia refers to the disturbance in lipid levels, including cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides, and high-density lipoprotein (HDL). Factors such as diet, tobacco exposure, and genetics can contribute to this condition, potentially leading to severe cardiovascular complications [2]. Consequently, individuals with dyslipidemia commonly exhibit elevated levels of LDL cholesterol and triglycerides, accompanied by decreased HDL cholesterol levels in blood tests. This imbalance heightens the risk of fatty plaque deposition within blood vessels, consequently increasing the likelihood of heart-related issues. Dyslipidemia represents an abnormal metabolic state characterized by a sustained elevation in plasma lipid concentrations. This condition manifests in three distinct presentations: hypercholesterolemia (elevated cholesterol), hypertriglyceridemia, and mixed hyperlipidemia (elevated levels of both triglycerides and cholesterol). The typical trend observed in total cholesterol levels involves an increase from birth to 2 years of age, followed by stabilization. Subsequently, there is a resurgence in levels leading up to a peak just before puberty. Post adolescence, these levels experience a slight decline [3].

Genetic inheritance plays a role in dyslipidemia, with examples including familial combined hyperlipidemia, familial hypertriglyceridemia, and familial hyper abetalipoproteinemia. Additionally, several factors contribute to this condition, such as high BMI, alcohol consumption, and waist circumference. Moreover, dyslipidemia can also be secondary, arising from other medical conditions like diabetes, hypothyroidism, Cushing's syndrome, inflammatory bowel disease, and severe infections. Hence, dyslipidemia can be categorized into primary (predominantly genetic) and secondary (resulting from lifestyle or underlying medical conditions) [4]. Physiological factors such as age and gender significantly impact plasma lipid levels across various species. Gender differences in lipid and lipoprotein levels are additionally influenced by age. Notably, findings from a Study indicate that aging is linked to a gradual rise in plasma LDL-C levels in both men and women aged 20 to 60 years. Beyond the age of 20, there is a progressive increase in plasma LDL-C concentrations for both men and women [5]. Dyslipidemia results from changes in the body that lead to an excessive production of triglycerides and LDL cholesterol or a decreased production of HDL. This alteration can be categorized into two main types based on the cause: Primary Dyslipidemia are caused by genetic factors; it is often observed in families where other members also experience dyslipidemia. Secondary Dyslipidemia arises due to lifestyle factors or other concurrent diseases. It is the more common type and may result from situations such as a sedentary lifestyle, high-fat diet, Type 2 diabetes, obesity, excessive alcohol consumption, chronic renal failure, chronic liver disease, hypothyroidism, smoking, and eating disorders like anorexia or bulimia [6]. Various health behaviors, including tobacco use, physical inactivity, nutrition, and obesity, can impact lipid levels. Nutrition-related risk factors involve insufficient consumption of fruits, nuts/seeds, and vegetables, or excessive intake of saturated fats. Dyslipidemia can also be linked to familial disorders, with autosomal dominant mutations, particularly in LDL receptors, causing most cases of familial hypercholesterolemia and resulting in elevated LDL-C levels. While less common, other mutations in the cholesterol pathway have been identified [7,8]. Dyslipidemia is typically asymptomatic and is often diagnosed incidentally or through routine screening. However, in severe cases, patients may exhibit symptoms related to complications such as coronary or peripheral artery disease. These symptoms can include leg pain, chest pain, dizziness, palpitations, swelling in lower limbs or veins (e.g., neck or stomach), and fainting [9].

An alarmingly high dyslipidemia prevalence of 96% has been reported in Pakistan. The highest prevalence of hypercholesterolemia is found in Punjab at 41.6%, while the lowest prevalence is observed in Baluchistan at 22.7% [10]. Major contributors to secondary dyslipidemia include alcohol overuse, a sedentary lifestyle with a high intake of saturated fat, cholesterol, and trans fats. Additionally, certain medical conditions, such as diabetes mellitus, chronic kidney disease, primary biliary cirrhosis, and other cholestatic liver diseases, are associated with secondary dyslipidemia [11]. Cardiovascular disease stands out as the most significant complication of dyslipidemia, leading to outcomes such as sudden cardiac death, acute myocardial infarction, or stroke [12]. Dyslipidemia is a prevalent characteristic of diabetes, and both type 1 and type 2 diabetes show an association between atherosclerotic cardiovascular disease and serum cholesterol and triglyceride levels. In individuals with diabetes, the risk of coronary heart disease (CHD) is elevated at any given serum cholesterol level, and the association with hypertriglyceridemia is more pronounced than in the general population [13, 14].

Obesity, a global pandemic in the contemporary world, is intricately linked to dyslipidemia, primarily influenced by insulin resistance and pro-inflammatory adipokines [15]. Furthermore, dyslipidemia is recognized as a risk factor for peripheral artery disease, stroke, and dementia in older adults. Chronic kidney disease (CKD) significantly heightens the risk of cardiovascular disease (CVD) [16]. Other disorders: Lipid disorders play a role, both directly and indirectly, in the development of several diseases, including type 2 diabetes mellitus, various common cancers, polycystic ovary syndrome (PCOS) in females, and mental illnesses such as bipolar disorder, schizophrenia, stress, and physical inactivity [17]. Additionally, dyslipidemia contributes to prostatic growth and contractility, serving as significant risk factors for the onset of benign prostatic

hyperplasia [18]. The process of lipid metabolism is intricate, involving numerous pathways, enzymes, proteins, and factor [19,20]. In accordance with earlier literature, males exhibit higher rates of dyslipidemia, potentially due to additional risk factors such as smoking, alcohol consumption, and hypertension [21,22].

Matrial and Methods

Sample Collection

A total of 100 samples were collected. The target population for this study included population of Islamabad and Rawalpindi Pakistan. Venipuncture technique was used to draw the blood sample. The patients were with fasting for at least 9 to 12 hours before the blood test. Fasting is necessary for accurate measurement of certain lipid parameters, such as triglycerides and LDL cholesterol.

The patient's identity was verified to ensure that the sample is correctly labeled.

Sample Processing

The blood sample was transferred into the serum separator tube (SST), also known as a plain redtop tube. The SST contains a gel that, after centrifugation, forms a barrier between the serum (the liquid component of blood) and the blood cells. This allows for the easy separation of serum for lipid profile analysis. No anticoagulant was used in the SST because anticoagulants can interfere with the lipid measurements. Each sample tube was labeled with the patient's information, including name and date of birth, to ensure proper identification. Serum was obtained after centrifugation of sample by Centrifuge at 5000 rpm for 5 minutes [23].

Analysis

In the laboratory, the blood samples were analyzed via clinical chemistry analyzer Cobas e-311, for various lipid components, including total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. The analyzer required calibration using specific calibrators to ensure accurate and reliable results. The serum samples were placed, obtained after centrifugation, into the sample rack or tray. The Cobas 311 automatically processed the samples, pipetting the required volume into the reaction cuvette or well. Reagents specific to lipid profile testing were added to the samples. These reagents reacted with the lipids present in the serum to produce measurable signals. The mixture of samples and reagents went through an incubation period, allowing the chemical reactions to take place [24].

Measurements

Cobas e-311 measured the absorbance or fluorescence produced by the reaction, translated it into quantifiable values for each lipid parameter, including total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides [25].

Statistical Analysis

The statistical analysis was performed by using IBM SPSS statistics to calculate the lipid profile parameters. Normal values for Cholestrol, Triglycerides, HDL and LDL are displayed in Table 1.

Table 1: Normal values of Lipids profile.

Parameters	Normal Values
Cholesterol	<200 mg/dl
Triglycerides	<150 mg/dl
HDL	>35 mg/dl in males >45 mg/dl in females
LDL	<130 mg/dl

Results

In this study, total 100 samples were collected, in which 64 were males and 36 were females. After this study, we concluded that dyslipidemia is becoming quite common in the population of Islamabad and Rawalpindi with the prevalence of 86%. The prevalence of Cholesterol, triglycerides, HDL, and LDL was 29%, 50%, 48% and 31%, respectively. In 100 samples, only 14 samples displayed normal results of cholesterol, triglycerides, HDL, and LDL. it means out of 100 only 14 people were having normal lipid profile which revealed the high prevalence of dyslipidemia in Islamabad and Rawalpindi. It was also observed that with the increase of age, the chances of getting dyslipidemia became higher.

Figure 1: Reveals 14 normal cases who had normal values of all four parameters cholesterol, triglycerides, HDL, and LDL, while 86 samples had abnormal values of all four parameters.

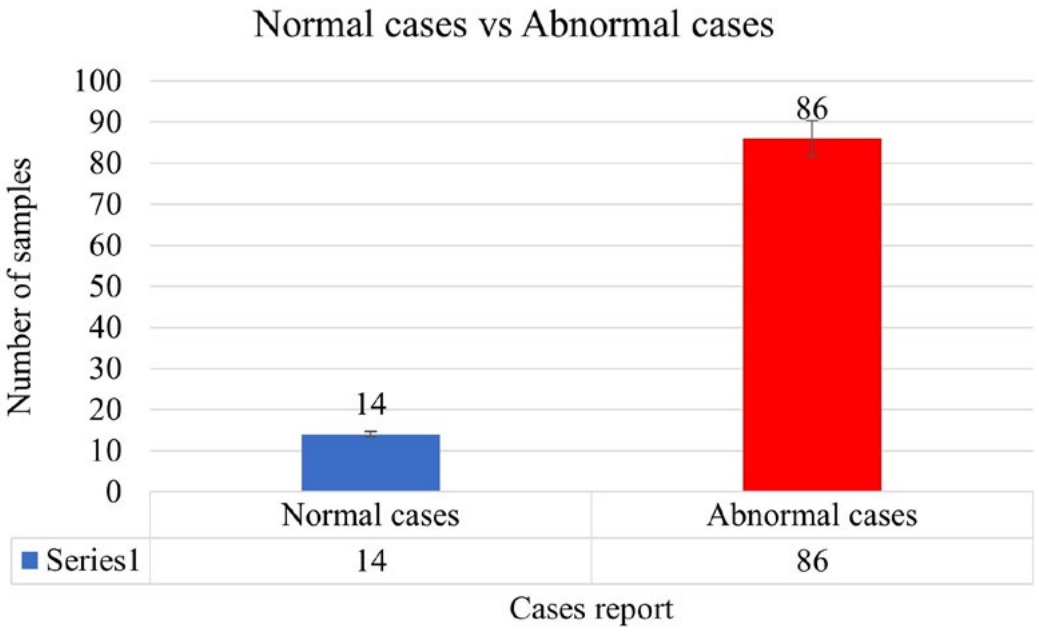


Figure 2: Represents total, normal and abnormal cases in males and females. According to gender wise distribution total males were 64 and females were 36, from which the males and females having abnormal lipid profile were 55 and 31, respectively and males and females having normal lipid profile were 9 and 5, respectively.

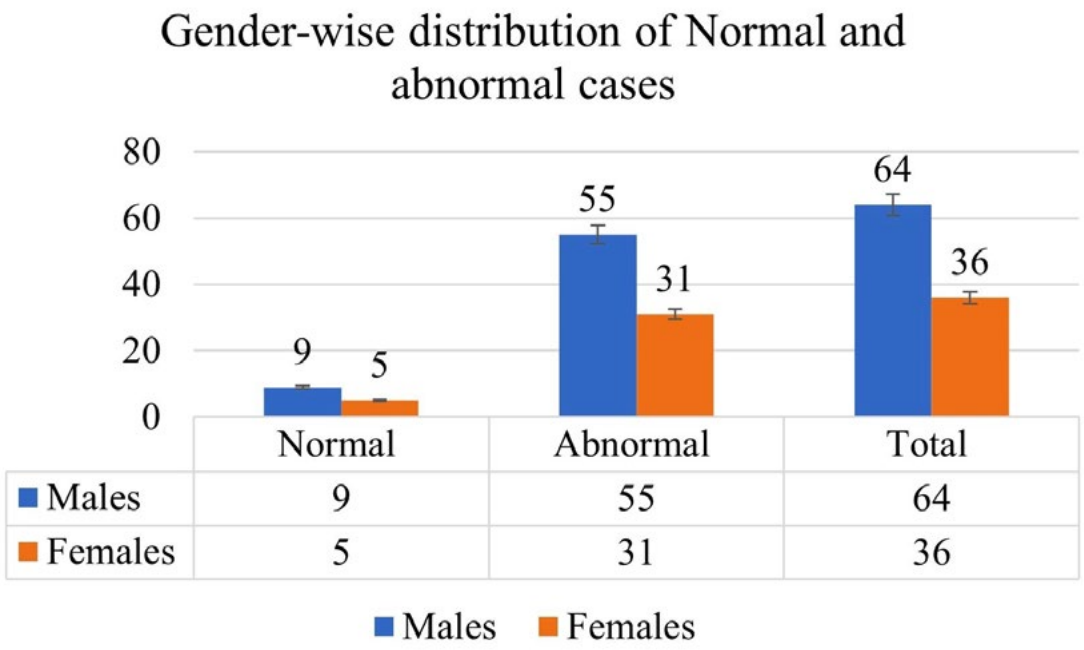


Figure 3: revealed the lipid profile analysis revealed that 29% of the sampled individuals exhibited elevated cholesterol levels, 50% experiencing heightened triglyceride levels, 48% having low levels of high-density lipoprotein (HDL), and 31% presenting elevated low-density lipoprotein (LDL) levels.

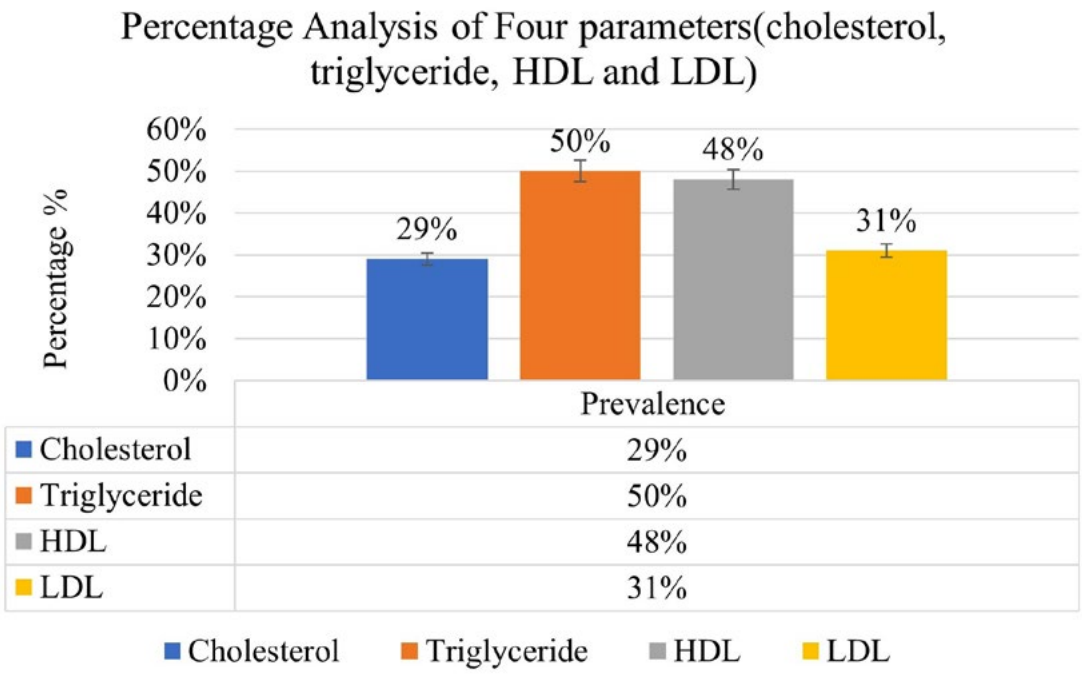
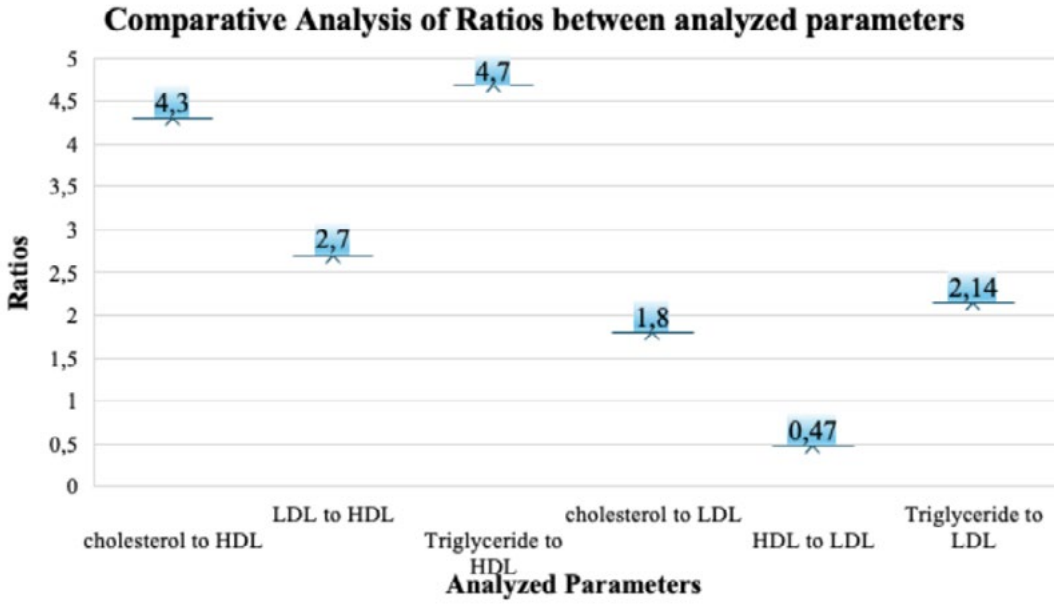


Figure 4: displayed the comparative analysis between ratios of all four parameters.



The cholesterol to HDL Ratio was 4.3, higher than the ideal ratio (<3.5), indicating a higher risk of cardiovascular disease. The LDL to HDL Ratio: 2.7, it was Higher than the ideal ratio (<2), indicating a higher risk of cardiovascular disease. Triglyceride to HDL Ratio: 4.7, Higher than the ideal ratio (<2), indicating a higher risk of insulin resistance and cardiovascular disease. Cholesterol to LDL Ratio: 1.8 Within the normal range (1.3-2.1), indicating a balanced cholesterol profile. HDL to LDL Ratio: 0.47, Lower than the ideal ratio (>0.5), indicating a higher risk of cardiovascular disease. Triglyceride to LDL Ratio: 2.14 Higher than the ideal ratio (<1.5), indicating a higher risk of cardiovascular disease.

The LDL to HDL Ratio: 2.7, it was Higher than the ideal ratio (<2), indicating a higher risk of cardiovascular disease. Triglyceride to HDL Ratio: 4.7, Higher than the ideal ratio (<2), indicating a higher risk of insulin resistance and cardiovascular disease. Cholesterol to LDL Ratio: 1.8 Within the normal range (1.3-2.1), indicating a balanced cholesterol profile. HDL to LDL Ratio: 0.47, lower than the ideal ratio (>0.5), indicating a higher risk of cardiovascular disease. Triglyceride to LDL Ratio: 2.14 Higher than the ideal ratio (<1.5), indicating a higher risk of cardiovascular disease.

Comparative Analysis

Population had higher than ideal ratios for Cholesterol to HDL, LDL to HDL, Triglyceride to HDL, and Triglyceride to LDL, indicating a higher risk of cardiovascular disease. The HDL to LDL ratio is lower than ideal, indicating a higher risk of cardiovascular disease. Only the Cholesterol to LDL ratio is within the normal range. These ratios suggest that our population may have a higher risk of cardiovascular disease and insulin resistance.

Discussion

In current study 100 samples were collected, consisting of 64 males and 36 females. The findings of the study suggested a notable increase in the prevalence of dyslipidemia of 86%, similar findings Liu LY et al., (2023) conducted a study including more than 20,000 samples, and research found out that the overall prevalence of dyslipidemia with a significantly higher occurrence in men (23%) compared to women (7.2%) [26].While a contradict study by Fatmi et al. (2020) in Karachi echoes the concerning trend, reporting dyslipidemia prevalence of 68.5% in males and 79.4% in females [27].

In present study the lipid profile analysis revealed concerning figures, with 29% of the sampled individuals exhibiting elevated cholesterol levels, 50% experiencing heightened triglyceride levels, 48% having low levels of high-density lipoprotein (HDL), and 31% presenting elevated low-density lipoprotein (LDL) levels, moreover, similar findings by Hussain et al., (2023) conducted a study, revealed a high prevalence of mixed dyslipidemia (92.26%) and isolated dyslipidemia (5.24%), elevated LDL-C emerged as the most common lipid disorder (84.25%), while hypercholesterolemia was the least common [28]. In another study by A study conducted by Basit et al., (2020) across Pakistan has reported an alarmingly high prevalence of dyslipidemia at 96%. Hypercholesterolemia, across Pakistan, has been reported to be highest in Punjab and lowest in Baluchistan, with a prevalence of 41.6% and 22.7%, respectively [29].

In current study comparisons between the ratios of each of the four parameters. A higher risk of cardiovascular disease is indicated by the cholesterol to HDL ratio of 4.3, which is higher than the recommended ratio of <3.5. A higher risk of cardiovascular disease is indicated by the LDL to HDL ratio of 2.7, which is higher than the optimal ratio of less than 2. Triglyceride to HDL Ratio: 4.7, increased than the optimum ratio (<2), indicating an increased risk of insulin resistance and cardiovascular disease. A balanced cholesterol profile is indicated by a cholesterol to LDL ratio of 1.8, which is within the normal range of 1.3-2.1. The ratio of HDL to LDL is 0.47, which is below the optimal value of >0.5 and suggests an increased risk of cardiovascular disease. increased than the optimal ratio (<1.5), the triglyceride to low-density lipoprotein ratio (2.14) indicates an increased risk of cardiovascular disease. Similar findings by Behnam Tajik et al., 2022 For coexisting T2D-CHD, the HRs were 1.89 (95% CI, 1.03–2.46) for apoB/apoA1, 1.85 (95% CI, 1.04–3.29) for triglycerides/HDL-C, 1.69 (95% CI, 1.01–2.31) for non-HDL-C/HDL-C, and 2.02 (95% CI, 1.01–3.07) for total cholesterol/HDL-C. On the other hand, the incidence of coexisting T2D-CHD was negatively correlated with serum LDL-C/apoB ratios [HRs 0.50 (95% CI, 0.28–0.90)]. Our exposures did not appear to be associated with any other CMM problems. Finally, it was found that a higher risk of T2D-CHD coexistence was independently correlated with raised triglyceride, VLDL-C, total cholesterol/HDL-C, TG/HDL-C, apoB/apoA1, and decreased LDL-C/apoB [30]. In another study by Zubair et al., (2020) specifically excluded diabetic and hypertensive patients from their study, identifying dyslipidemia in 68.1% of diabetic individuals and 71.6% of hypertensive patients, establishing a statistically significant connection between these conditions and abnormal lipid profiles [31].

Current study contributes valuable insights into the prevalence of dyslipidemia in Islamabad and Rawalpindi, aligning with the broader narrative seen in studies across Pakistan. The collective evidence underscores the urgent need for comprehensive public health strategies to address dyslipidemia, incorporating lifestyle modifications, dietary changes, and targeted interventions for at-risk populations.

Conclusion

The high prevalence of dyslipidemia in the population of Islamabad and Rawalpindi necessitates urgent attention as it poses a significant public health challenge. The data presented underscores the pressing need for enhanced preventive measures aimed at both diagnosis and comprehensive treatment of dyslipidemia in this region. The numbers we found highlight the seriousness of the situation. Out of 100 people we looked at, 86 had dyslipidemia. This means many people are at risk of heart-related problems. When we looked closer, we saw that distinct types of cholesterol problems are quite common here. Looking into the future, we need to understand more about why people here have these cholesterol problems. It could be related to their lifestyle. Also, checking if there is a genetic influence on these issues can help us plan better ways to manage and prevent them. We also need to make sure people in these areas know more about how to keep their cholesterol in check. As we move ahead, it would be useful to keep studying this problem over time to see if things are getting better with the steps we take. This way, we can keep adjusting our plans to make sure people in Islamabad and Rawalpindi have better heart health in the future.

Data Availability Statement
Data will be provided on request.

Declaration of conflict of Interest
Authors do not have any conflict of interest.

Ethical Approval
Ethical approval was obtained by the research panel.

Authors contributions
Muhammad Bilal Habib contributed to conceptualization, data curation, formal analysis, investigation, validation, methodology, visualization, writing – original draft. Noreen Sher Akbar and Ghanwa Batool, role in data curation, writing – review, editing and visualization.

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Research Article

A National e-Survey of Adult Reference Intervals of Routine Chemistry Analytes Used by Laboratories across Pakistan: A Step Towards Harmonization

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Keywords
survey, Pakistan, reference intervals, clinical chemistry, harmonization

Abstract

Objectives: To identify the variation of reference intervals, reporting units used for key blood chemistry parameters in laboratories across Pakistan and to understand the factors contributing to these discrepancies.

Methodology: A comprehensive e-questionnaire developed using google forms covering key blood chemistry parameters (Electrolytes, fasting glucose, glucose random urea, creatinine and lipid profile), reference intervals, reporting units, and laboratory practices was administered via email to the Pathologists. Frequency and percentages were calculated for each response and descriptive results were also evaluated.

Result: A total of 38 responses were received five responses were excluded due to incomplete forms. The responses from 33 laboratories revealed substantial variability in reference intervals (RIs) for routine blood chemistry parameters, underscoring a significant lack of standardization. 66.66% laboratories had not developed specific RIs, relying instead on manufacturer-provided RIs, with infrequent reviews or updates. Challenges were prevalent due to non-harmonized RIs, leading to patient and physician counseling issues. Primary obstacles included funding deficiencies and limited access to healthy samples.

Conclusion: These findings emphasize the critical need for national regulatory guidelines to standardize RIs, thereby enhancing the reliability and accuracy of laboratory diagnostics in Pakistan.

Introduction

Reference intervals (RIs) are integral part of all clinical chemistry laboratory reports. These numerical values are crucial for appropriate interpretation of laboratory results. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has worked relentlessly to provide recommendations for the establishment of RIs helping ensure the quality and accuracy of laboratory testing [1,2]. National Committee for Clinical Laboratory Standards (NCCLS) has published it's guidelines in 2000 [3]. The third edition of C28-A3 guideline (2008) is the latest document for establishment of RI. These guidelines stress on use of a strict evidence based approach by taking samples from local population, with stratifications based on factors such as age and gender, using appropriate statistical method. IFCC Committee on Reference Intervals and Decision Limits (C-RIDL) has proposed new methodologies as a part of global effort to standardize RIs [4]. However, the process of developing RIs is too expensive and labor intensive, a less extensive process that requires only verification of RIs provided by external sources is acceptable. In addition to this the entire process of development or and verification of RIs should be documented and reviewed periodically [5].

Pakistan is a developing country where laboratories have limited resources. Most of Pakistani clinical laboratories opt for RIs mentioned in kit inserts or guidelines that are formulated for Western populations. In addition to this, a wide variation is noted in RIs, reporting units and reporting patterns. These inconsistencies create confusion when comparing results from different laboratories. The regularization of RIs reporting standards is essential to improve reliability, better communication among healthcare providers, and more consistent medical decision-making.

In this nationwide survey, we aim to assess problems faced by laboratory professionals for establishment of RI, find out the extent of the variation in RIs and reporting units used in laboratories across Pakistan for key blood chemistry parameters. By understanding these factors, we can then work towards problem solving and propose recommendations for standardizing reference intervals and reporting practices leading towards better healthcare in Pakistan.

Method

The survey was conducted by the section of Chemical Pathology, Department of Pathology and Laboratory Medicine, the Aga Khan University (AKU), Karachi, Pakistan. We successfully obtained an exemption from the ethical review committee with number 2024-9946-29537. The survey was designed for clinical laboratories performing routine blood chemistry.

A comprehensive e-questionnaire with 30 items was developed using google forms, covering key blood chemistry parameters (Electrolytes, fasting glucose, random glucose, urea, creatinine and lipid profile), reference intervals, reporting units, and laboratory practices. In the first section, four questions were about basic laboratory information. The second section comprised of five questions related to establishment of RIs, three questions were about hurdles and challenges. In the last section, there were 18 questions about units and ranges of key blood chemistry parameters. Within the sections, five questions were multiple choice, allowing the participants to select as many options as they deemed appropriate. Three questions had Yes/No options only. Nine questions were about the units reported while nine were about the specific ranges for each analyte. Importantly, e-mail addresses or internet protocol addresses were not collected.

A list of laboratories performing key blood chemistry parameters (electrolytes, fasting glucose, glucose random urea, creatinine, and lipid profile) across Pakistan was acquired from the marketing department of the clinical laboratory of AKU. Three independent consultant Chemical Pathologists reviewed list, and laboratories were invited by snowball technique, one laboratory invited other laboratory to participate. The inclusion criteria were devised as 1) the laboratory housing a Consultant Pathologist 2) the laboratory is reporting all the parameters being studied in this survey.

The e-questionnaire was administered via email to the participants. Electronic consent for participation was acquired on the initial page of the survey. Participation in the survey was voluntary. The responses were transcribed into Microsoft Excel. Frequency and percentages were calculated for each response and descriptive results (if specified) were evaluated.

Results

A total of 38 responses were received from laboratories across Pakistan. Five responses were excluded due to incomplete forms. Out of the 33 respondents, the participant laboratories were from four provinces and nine cities of Pakistan. The province of Sindh had maximum participation with 17 (51.51%) responses, followed by Punjab 13 (39.39%). All 17 responses from Sindh were from Karachi city only, as presented in Figure 1. As far as lab demographics are concerned 15 (45.45%) were in tertiary care hospitals while only 4 (12.12%) were small community hospital laboratories. Nine (27.27%) laboratories reported an approximate sample workload as more than 1000 samples daily, while another 9 (27.27%) indicated workload between 500-1000, followed by 8 (24.24%) laboratories with 100-250 samples, as shown in Figure 2.

In response to Yes/No questions regarding establishment of RIs, majority of the laboratories 22 (66.66%) had never developed laboratory specific RIs for any of routine blood chemistry parameters. While all the laboratories reported that RIs they used were adjusted for age and gender when necessary. All but one laboratory encountered challenges due to lack of harmonization

of RIs used for routine chemistry analytes. Many laboratories had to individually counsel patients and physicians about the cause of these differences.

In response to multiple-choice questions, it was observed that 5 (15.15%) laboratories never reviewed or updated the RIs, while 24 (72.72%) laboratories claimed to do so when there was a change in analytical method. Manufacturer-provided RIs from assay kits were most commonly used by laboratories across Pakistan. Direct measurement from local samples was rare, and there was no use of Big Data methods. The primary hurdle for establishment of laboratory specific RIs was found to be a lack of funding, followed by limited access to healthy population samples, while lack of time was the least reported issue. The majority of the respondents suggested that national guidelines from regulatory bodies would help in standardizing RIs in Pakistan, as illustrated in Figure 2.

Details of different units and RIs for routine blood chemistry parameters are presented in Table 1, Table 2 and Figure 3.

Figure 1: Map of Pakistan with numbers and locations of laboratory responses.

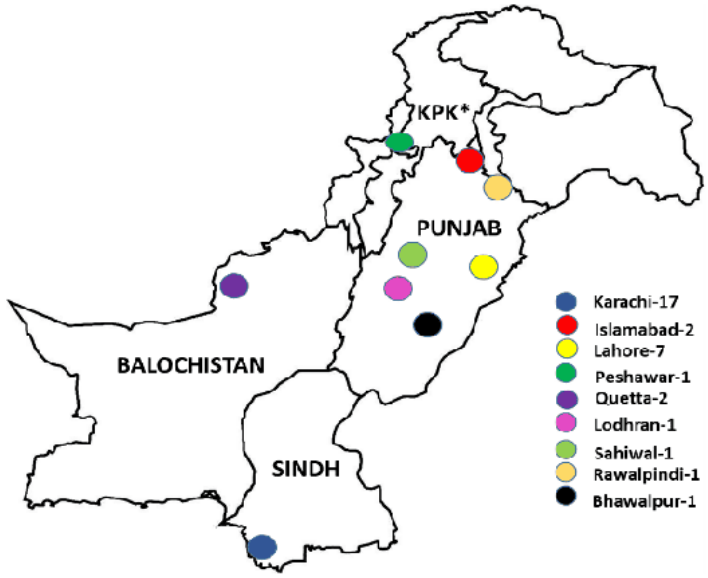

Figure 2: Participant laboratory's demographics and issues faced by laboratories in establishment of reference intervals.

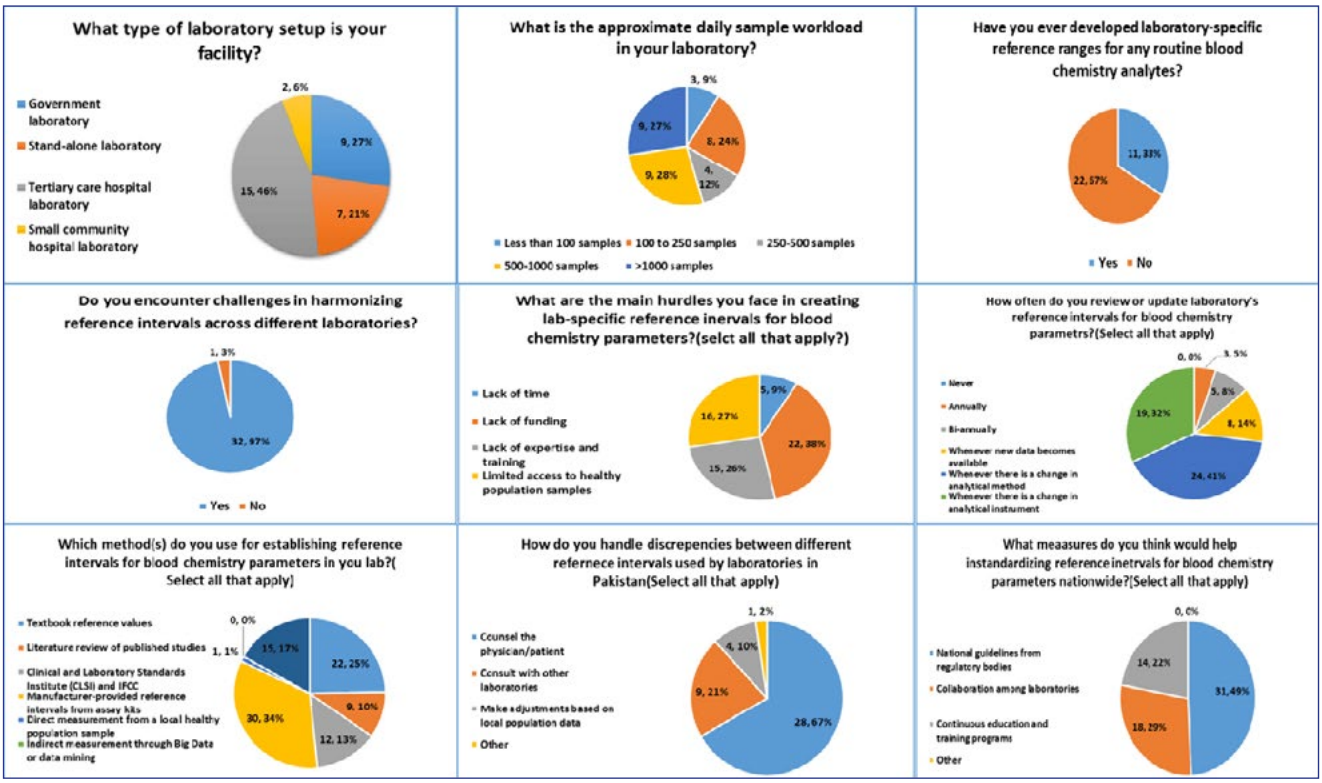

Figure 3: Reference intervals of sodium, potassium and chloride used by different laboratories across Pakistan (concentration unit mEq/L).

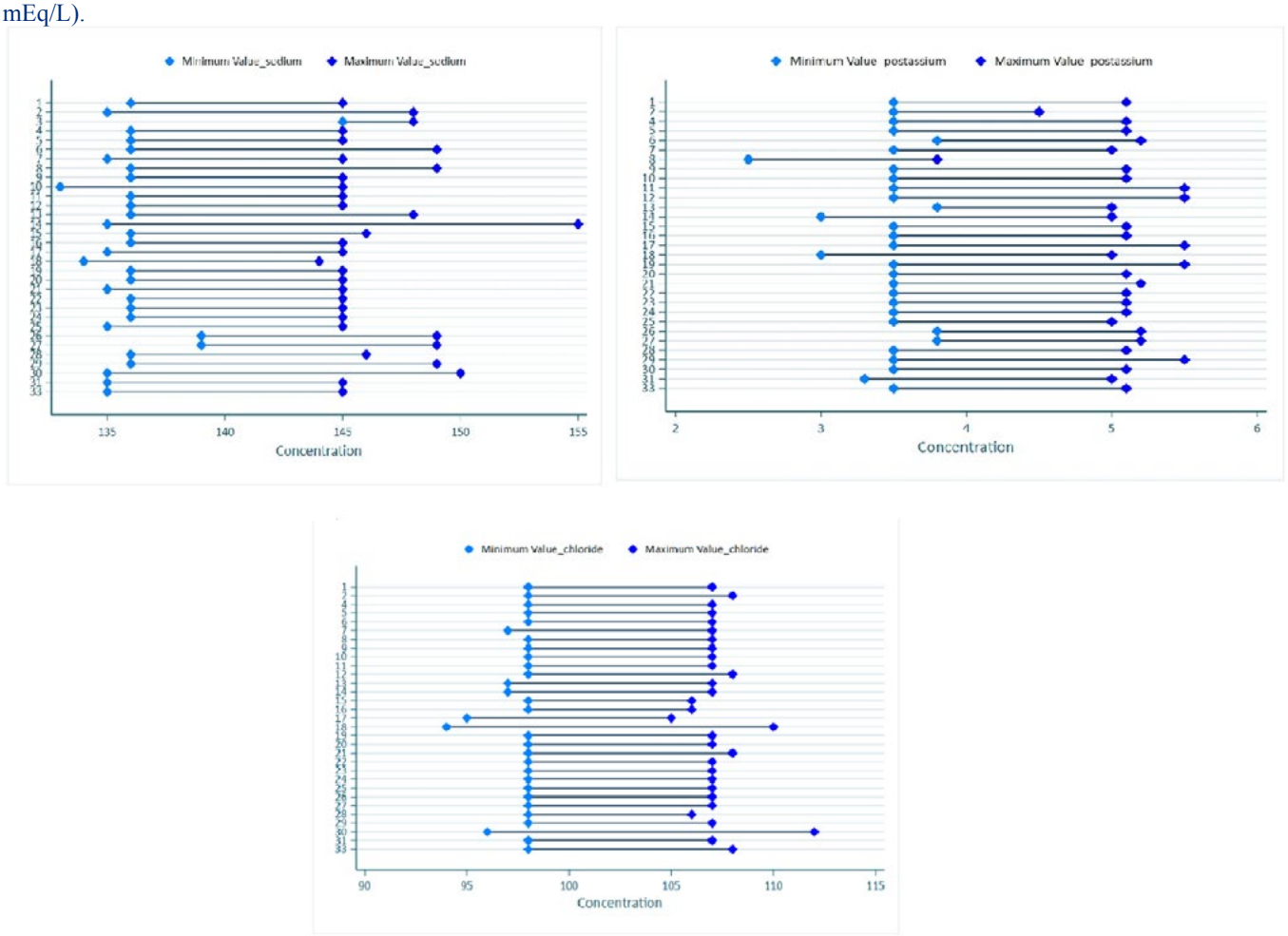

Table 1: Reference ranges and units of glucose, urea and creatinine used by different clinical laboratories across Pakistan.

Respondent lab number	Fasting Glucose, unit	Random Glucose, unit	Serum Urea, unit	Serum Creatinine, unit
Lab no 1	65-100 mg/dl	80-160 mg/dl	6-20 mg/dl	M1.0-1.7 mg/dl F: 0.6-1.3 mg/dl
Lab no 2	70-100 mg/dl	70-140 mg/dl	10-50mg/dl	0.6-1.3 mg/dl
Lab no 3	70 to 99 mg/dl	70-160 mg/dl	10-26mg/dl	0.8- 1.3 mg/dl
Lab no 4	<100 mg/dl	< 140 mg/dl	17-49mg/dl	NB 0.3-1, infant 0.2-0.4, Child 0.3-0.7, Adult M 0.9-1.3, Adult F 0.6-1.1 mg/dl
Lab no 5	Normal <100 Pre diabetes:100 -125 Diabetes:>= 126 mg/dl	<140 mg/dl	6-20mg/dl	Adult M:0.9 - 1.3 mg/dl Adult F :0.6 - 1.1 mg/dl
Lab no 6	Normal FBS 65-100, Impaired FBS >100-<126 Provisional diagnosis of diabetes mellitus >126 mg/dl	<140 mg/dl	10-50mg/dl	0.5-1.5 mg/dl

Respondent lab number	Fasting Glucose, unit	Random Glucose, unit	Serum Urea, unit	Serum Creatinine, unit
Lab no 7	Normal Fasting <100 prediabetes 100-125 Diabetes >126 mg/dl	<140 mg/dl	10-50 mg/dl	0.6-1.5mg/dl
Lab no 8	Normal: 65-100 IFG: 101-125 Provisional diagnosis of diabetes >126 mg/dl	<200 mg/dl	10-50 mg/dl	M 0.6-1.3 F 0.5-1.0 mg/dl
Lab no 10	70-99 mg/dl	<140 mg/dl	15-39 mg/dl	0.5-1.5 mg/dl
Lab no 11	< 100mg/dl	< 200 mg/dl	16.6-48.5 mg/dl	M 0.72-1.25 F 0.57 - 1.11mg/dl
Lab no 12	80 – 110 mg/dl	80 – 180 mg/dl	10-50 mg/dl	0.6-1.3 mg/dl
Lab no 13	70-110 mg/dl	80-200 mg/dl	10-50 mg/dl	M 0.7-1.3 F 0.6-0.9 mg/dl
Lab no 14	70-100mg/dl	<200 mg/dl	10-50mg/dl	M: 0.6 -1.2 F: 0.5-1.1 mg/dl
Lab no15	70-100 mg/dl	70-200mg/dl	6-20mg/dl	M 0.9 1.3 F 0.6-1.1 mg/dl
Lab no16	70-100 mg/dl	70-160 mg/dl	17-43mg/dl	M 0.70 - 1.2 F 0.5 - 0.90 mg/dl
Lab no 17	70-110 mg/dl	140-200 mg/dl	15-45 mg/dl	0.3-1.1 mg/dl
Lab no 18	Normal <100 Impaired 100-125 Diabetes >126 mg/dl	<200 mg/dl	10-50mg/dl	M 0.6-1.2 F 0.5-0.9 mg/dl
Lab no 19	80-110 mg/dl	<140 mg/dl	<40mg/dl	<1.2 mg/dl
Lab no 20	<100 mg/dl	<200mg/dl	12-40 mg/dl	M 0.9-1.3 F 0.6-1.1 mg/dl
Lab no 21	70-110 mg/dl	<140mg/dl	20-45mg/dl	F 0.6-1.1 mg/dl
Lab no 22	70-110 mg/dl	<140mg/dl	10-50mg/dl	M: 0.7-1.2 F: 0.5-1.0 mg/dL
Lab no 23	Normal < 100 IFG 100- 125 DM >126 mg/dl	<200 mg/dl	Adults 13-43 > 60 yrs 17-49 mg/dl	0.5-1.2 mg/dl
Lab no 24	70-99 mg/dl	70-140 mg/dl	9-22mg/dl	M 0.72- 1.25 mg/dl F 0.57- 1.11 mg/dl
Lab no 25	60-100 mg/dl	80-140mg/dl	10-40mg/dl	0.90–1.30mg/dl
Lab no 26	<100mg/dl	70-100mg/dl	10-50mg/dl	0.6-1.1mg/dl M 0.7-1.3 F 0.6-1.1 mg/dl
Lab no 27	Normal <100 IFG 100- 125 DM >126 mg/dl	80- 140mg/dl	17-49mg/dl	M: 0.9-1 mg/dl F: 0.6-1.1mg/dl
Lab no 28	60-100mg/dl	60-200 mg/dl	2.2-7.1 mmol/l	0.5-1.1mg/dl
Lab no 29	Normal <100 Prediabetes 100-125 Diabetes >126mg/dl	DM >200 mg/dl	15-55mg/dl	M 0.75-1.18 mg/dl F 0.55-1.02 mg/dl

Respondent lab number	Fasting Glucose, unit	Random Glucose, unit	Serum Urea, unit	Serum Creatinine, unit
Lab no 30	45 - 99 mg/dl	70 - 140 mg/dl	10-50mg/dl	M: 0.64 - 1.2 F: 0.42 - 1.06 mg/dl
Lab no 31	3.3-5.6mmol/l	<10 mmol/l	Adults: 2.1-7.1 mmol/l	M: 62-120 umol/L F: 60-105 umol/L
Lab no 32	Hypoglycemia <70 Normal 70-99 Pre-Diabetic 100-126 DM >126 mg/dl	Low:< 70 Normal:70-200 High :>200 mg/dl	Low< 10 Normal 10-50 High >50 mg/dl	M:Low <0.73, Normal 0.73-1.18 High >1.18, F: Low< 0.55 Normal 0.55-1.02 High >1.02 mg/dl
Lab no 33	Normal <100 Prediabetes 100-125 Diabetes >126mg/dl	Normal >70 DM >200mg/dl	20-44mg/dl	M:0.9-1.18 F:0.7-0.9 mg/dl

M: male, F: female, DM: diabetes mellitus, IFG: impaired fasting glucose, FBS: fasting blood sugar. NB: newborn

Table 2: Reference ranges and units of lipid profile used by different clinical laboratories across Pakistan.

Respondent lab number	Serum Cholesterol, unit	Serum Triglyceride, unit	Serum HDL cholesterol, unit	Serum LDL cholesterol, unit	Serum VLDL cholesterol, unit
Lab no 1	< 200 without known CAD < 160 with known CAD mg/dl	Normal :<150 Borderline :150-199 High :200-499 Very High: >500 mg/dl	> 40 mg/dl	Optimal: <100 Above Optimal: 100-129 Borderline High:130-159 High: 160-189 Very High: >190 mg/dl	<30 mg/dl
Lab no 2	Desirable: <200 Borderline high: 200-239 High :>240 mg/dl	Normal :<150 Borderline: 150-199 High :200-499 Very high: >500	Low :<40 Desirable: >60 mg/dl	Optimal :<100 Near Optimal: 100-129 Borderline: 130-159 mg/dl	<30 mg/dl
Lab no 3	< 200 mg/dl	< 150 mg/dl	>40 mg/dl	< 130 mg/dl	<30 mg/dl
Lab no 4	Without known CAD< 200 With known CAD< 160 mg/dl	70-150 mg/dl	>35 mg/dl	Without CAD: <150, With CAD: <100 mg/dl	upto 40 mg/dl
Lab no 5	Desirable:<200 Borderline high:200-239 High:>239 mg/dl	Normal:<150 Borderline High:150 - 199 High:200 – 499 Very High:>499 mg/dl	>35 mg/dl	Optimal:<100 Near/above optimal:100 - 129 Borderline High:130 - 159 High:160 – 189 Very High:>189 mg/dl	Not reported
Lab no 6	< 200 without known CAD < 160 with known CAD mg/dl	46-236 mg/dl	Without CAD >40 With known CAD >60 mg/dl	Desirable without CAD <130 Optimal with known CAD <100 mg/dl	Not reported

Respondent lab number	Serum Cholesterol, unit	Serum Triglyceride, unit	Serum HDL cholesterol, unit	Serum LDL cholesterol, unit	Serum VLDL cholesterol, unit
Lab no 7	Normal :<200 Borderline high :200-239 High:>240 mg/dl	Normal: <150 Borderline high: 150-199 High: 200-499 Very high: >500 mg/dl	Optimal: >60 Intermediate :40-60 Low :<40 mg/dl	Optimal: <100 Near Optimal: 100-129 Borderline High :130-159 High :160-189 Very High: 190 mg/dl	Not reported
Lab no 8	Without known CAD < 200 With known CAD < 160 mg/dl	70-150 mg/dl	>35 mg/dl	Without known CAD <150 mg/dl	<30 Calculated mg/dl
Lab no 9	Normal: < 200 Borderline: 201- 239 High :240>mg/dl	Normal :150 Borderline: 151-199 High: 200-499mg/dl	Low risk factor: <40. Desirable >60 mg/dl	Normal: <100 Borderline: 130-159 High: 160-18 Very high: >190mg/dl	<30 normal mg/dl
Lab no 10	<200 mg/dl	Normal:<150 mg/dl	>35 mg/dl	Upto 150 mg/dl	Not reported
Lab no 11	Normal: <200 mg/dl	Normal:<150 mg/dl	< 100 mg/dl	> 60mg/dl	Not reported
Lab no 12	<200 mg/dl	50-150 mg/dl	M:35-55 F:35-65 mg/dl	<100 mg/dl	5.0-30 mg/dl
Lab no 13	140-200 mg/dl	50-200 mg/dl	M:35-55 F:35-65 mg/dl	<150mg/dl	02-30 mg/dl
Lab no 14	Desirable: <200 mg/dl	<150 mg/dl	M: <45 F: <55.0	Desirable: <100 mg/dl	0-25 mg/dl
Lab no15	Desirable:<200 Borderline High: 200-239 High: >239 mg/dl	Normal:<150 Borderline: 150-199 High: 200-499 Very high >500 mg/dl	Low risk factor: <40 Desirable: >60 mg/dl	Desirable: <100 Borderline high: 100-129 High: 130-189 Very high: >189 mg/dl	Not reported
Lab no16	160 - 200 mg/dl	<150 mg/dl	>45 mg/dl	<100 mg/dl	5.0 - 30.0 mg/dl
Lab no 17	<200 mg/dl	<150 mg/dl	M 35-65 F 35-80 mg/dl	100-160 mg/dl	02-30 mg/dl
Lab no 18	<200 mg/dl	50-150 mg/dl	> 40 mg/dl	<100 mg/dl	Not reported
Lab no 19	<200 mg/dl	<150 mg/dl	M>40, F >30 mg/dl	<100 mg/dl	<30 mg/dl
Lab no 20	<200 mg/dl	<150 mg/dl	M > 30 F >34mg/dl	<100 mg/dl	<30 mg/dl
Lab no 21	Desirable: < 200, Moderate: 200-240 High risk :> 240 mg/dl	Desirable: 45- 150 Borderline: 151- 200 High Risk: 201- 500 mg/dl	M desirable: > 60 F desirable 40- 59 Risk, M 50- 59 risk, F: < 40-30 high risk, M< 50 high risk F <30 mg/dl	Desirable: < 100 Above Optimal: 101-130 Borderline High: 131-160 High: 161-200 mg/dl	< 30: Desirable, > 30 mg/dl High

Respondent lab number	Serum Cholesterol, unit	Serum Triglyceride, unit	Serum HDL cholesterol, unit	Serum LDL cholesterol, unit	Serum VLDL cholesterol, unit
Lab no 22	Normal: <200 Borderline high: 200-239 High: >240 mg/dl	Normal: 100-130, Borderline: 130-160 High> 160mg/dl	35-50mg/dl	50-150 mg/dl	Not reported
Lab no 23	Desirable :> 200 Borderline :200- 239 High :> or equal to 240 mg/dl	<150 mg/dl	Less than 40 (low)mg/dl	<100 mg/dl	Not reported
Lab no 24	Less than 200 mg/dl	Less than 150 mg/dl	Greater than 40	Less than 130 mg/dl	Less than 30 mg/dl
Lab no 25	<200 mg/dl	<150 mg/dl	>45 mg/dl	<130 mg/dl	Not reported
Lab no 26	<200 mg/dl	<200 mg/dl	>40 mg/dl	100-129 mg/dl	<40 mg/dl
Lab no 27	Desirable: <200 Borderline: 200-239 High:>239 mg/dl	<150 normal mg/dl	M> 40. F> 50 mg/dl	Optimal: <100 Above Optimal :100-129 Borderline High: 130-159 High: 160 -189 Very High: >190 mg/dl	Calculated
Lab no 28	140-200 mg/dl	50-150 mg/dl	40-70 mg/dl	70-100 mg/dl	Not reported
Lab no 29	Desirable without CAD <200 Optimal with CAD < 160 mg/dl	<150 mg/dl	without CAD: >40 with CAD: >=60 mg/dl	Desirable without CAD: <130 Optimal with CAD <100 mg/dl	Not reported
Lab no 30	< 200 mg/dl	< 200 mg/dl	35 - 65 mg/dl	< 150 mg/dl	Not reported
Lab no 31	Desirable <5.2 mmol/L	0.4 to 1.6 mmol/L	>1.3 mmol/L	<2.59 mmol/L	0 to 0.78 mmol/L
Lab no 32	Desirable: <200 Borderline high: 200-240 High: >240 mg/dl	Normal :<150, Borderline high: 150-199 High: >200-499 Very High >500 mg/dl	Low <39, Normal > 40 mg/dl	Desirable :<100 Above Desirable:100-129 Borderline high:130-159 High:160-189 Very High: >190 mg/dl	Desirable: <129, Above Desirable: 130-159, Borderline high: 160-189, High: 190-219 Very High: >220 mg/dl
Lab no 33	Desirable :<200 Borderline high: 200-240 High: >240 mg/dl	Normal:<150 Borderline : 150199 High: 200 -499 Very high: >500 mg/dL	Low < 40 Normal >50	Near optimal: 100 - 129 Borderline high: 130 -159 High: 160 -189 Very high: >190 mg/dl	<40 mg/dl

M: male, F: female, HDL:high density lipoprotein, LDL: low density lipoprotein, VLDL: very low density lipoprotein, CAD: coronary artery disease

Discussion

Reference intervals reported along with each analyte are considered as important as the actual observed value [6]. Due to differences in lifestyle components, dietary habits and genetic makeup it is recommended to use region specific, or laboratory validated RIs in clinical laboratory reports [7]. However, RIs among Pakistani population are not clearly defined and most laboratories rely heavily on RIs established for Caucasian population. Harmonization of RIs is essential to ensure standardized health care.

In our survey, we found that most of the laboratories were affiliated with tertiary care hospitals with a large workload of more than 500 samples daily. Despite this high volume, it was alarming to note that two thirds of the laboratories had never established their own RIs for any routine blood chemistry parameters. The standard recommendation is to select a minimum of 120 healthy reference subject for establishment of RIs. This process is very tedious, so a simpler method of RIs verification can be done with only 20 healthy subjects [5]. While all of the respondents were using age and gender adjusted RIs, many faced difficulties due to variabilities in RIs used by different laboratories. These inconsistencies can potentially lead to confusion and errors in diagnosis and treatment. There is an urgent need for consolidated efforts for establishment/verification of population specific RIs.

Establishment of RIs is a difficult, expensive and time intensive project and laboratories in lower-middle income countries like Pakistan face several difficulties in the process [8]. Financial constraints was reported as the primary barrier, cited by 22 respondents. Funds are required for reference population selection, kits, and staff training. The second biggest problem was found to be a lack of expertise and training. Training in data interpretation and data analysis is critical for reliable RIs. The respondents also found it difficult to get samples from healthy population as they mostly dealt with patients suffering from one disease or another. Time constraints were the least cited but still relevant issue. All this highlights the urgent need for national level consolidated efforts for monetary support, training programs and better sample collection strategies.

It was interesting to note that none of the laboratories reported that they never reviewed or updated their RIs. This indicates that all laboratories have a recognition for updated RIs. However, most of the laboratories only updated RIs whenever a new analytical method was introduced, followed by any change in analytical instrument. Very few laboratories had a routine of annual RIs review. This lack of periodic review may be insufficient for accurate reporting.

The laboratories used RIs from various sources. Manufacturer-provided RIs from assay kits were most widely used. These values are specific to instrument and reagent used, and not formulated to population specific needs. Textbook RIs are readily available and were found to be next most popular option. Less than half laboratories reported using (CLSI), IFCC national or international guidelines, this may be due to a general lack of awareness about their availability. Only one laboratory employed the direct method for establishment of RIs using local healthy population. This method is least used as it needs a significant time and effort. Although many laboratories had very large sample volume, none of the laboratories opted to big data or indirect data mining. Big data employs multiple statistical tools for calculation of RIs [9]. The absence of its use may reflect a lack of infrastructure, expertise, or access to large datasets required for effective implementation. These limitations must be addressed for enhancing clinical laboratory reporting.

Many patients need serial monitoring of blood chemistry parameters for disease and treatment monitoring [10,11]. Patients may get the same tests from different laboratories over the years. 32 (96.70%) laboratories faced challenges due to variations in RIs of same analyte. Managing such discrepancies required multifaceted approach. The most common approach, used by laboratories was to counsel the physician or patient about the causes of such variations. Only a small portion of laboratories consulted with other laboratories or made adjustments based on local population data. Such discrepancies in unnecessary confusion for patient and extra work for laboratory professionals.

When asked about their opinion for various measures to standardize RIs across Pakistan, an overwhelming majority believed that national guidelines from regulatory bodies would be instrumental in standardizing RIs nationwide. A significant portion recognized that collaboration among laboratories and continuous training programs for better standardization of RIs. This points towards urgent need for consolidated nationwide efforts across government and public sectors to establish guidelines RIs for Pakistani population. These guidelines will provide a benchmark for laboratories to follow, promoting consistency and quality assurance in diagnostic practices.

When we analyzed our responses for RIs of serum sodium we found that majority of the laboratories [12 (54.50%)] use the range 136-145. This indicates a strong preference for this reference interval. Other laboratories also reported roughly similar RI for serum sodium. The most frequently reported range of potassium is 3.5-5.1, with 11 (33.33%) adhering to this range. Other ranges include 3.5-5.5 mEq/L [5 (15.15%) laboratories], 3.0-5.0 mEq/L [4 [12.12%] laboratories), 3.8-5.2 mEq/L [4 [12.12%] laboratories), and 3.5-4.5 mEq/L [1 [3.03%] lab)]. The most frequently reported range for serum chloride is 98-107 mEq/L or mmol/L [21 (63.63%) laboratories]. Other ranges were roughly similar with two outliers, which were 95-105 mmol/L and 96-112 mmol/L. There is a notable split between mEq/L and mmol/L, with both being used almost equally across the board. Electrolytes play a vital role in controlling the acid base balance, nerve conduction, muscle contraction and enzyme

activity within the body. Electrolyte disorders are commonly encountered in clinical practice [12, 13]. Different RIs and units used by various laboratories can lead to confusion and potential diagnostic errors.

The most frequently reported range for fasting glucose was 70-100 [8 (24.24%) laboratories]. Other reported ranges include 65-100 [2 (6.06%) laboratories], 70-99 [2 (6.06%) laboratories], and 70-110 [2 (6.06%) laboratories]. Only a few laboratories adopted a more detailed reporting approach with specific classification like Normal [10 (30.30%) laboratories)], Pre-diabetes [5 (15.15%) laboratories], impaired fasting glucose [4 (12.12%)], provisional diagnosis of diabetes [3 (9.09%)] and Diabetes [7 (21.21%) laboratories]. There was variation among RI for normal and pre-diabetic/impaired fasting glucose; however, diabetes was labelled at > 126 mg/dl by all laboratories. For random glucose the most frequently reported range was <140 mg/dL, reported by [8 (24.24%) laboratories] followed by <200 mg/dL [5 (15.63%) laboratories]. There was variability in the upper and lower limit of random glucose reporting, with some laboratories providing upper limits as high as 200 mg/dL and lower limit as 60mg/dl. One laboratory gave detailed RIs as Low< 70, Normal 70-200 High >200 mg/dl and one lab reported Normal >70 diabetes >200 mg/dl. Only one lab used the unit as mmol/l while all the rest used mg/dl as the reporting unit. Several guidelines are available for diagnosis and monitoring of diabetes mellitus that depend upon levels of fasting and random glucose [14, 15]. Variations in RIs for fasting and random glucose levels across different laboratories can have significant implications for the diagnosis, management and monitoring of diabetes and potentially cause patient safety issues.

The most frequently reported RI for serum urea was 10-50 mg/dL, reported by 14 (42.42%) laboratories. There was a variation in the upper limit ranging from 22-50 mg/dl. Two laboratories reported in mmol/l rest used mg/dl as the unit. Only one laboratory reported as low, normal, High, while the rest used only a single RI with no further specifications. For serum creatinine 20 (60.60%) stratified RI for gender, 1 (3.03%) stratified according to age and 1 (3.03%) stratified as low, normal and high. Considerable variation was noted in lower limit (0.3-1.0mg/dl) and upper limit (1.0-1.7 mg/dl). The commonly used reporting unit was mg/dl. Serum urea levels are used to assess the state of hydration and kidney function in body in the body. Differences in RIs for urea and creatinine can lead to variability in diagnosing renal dysfunction or dehydration. In addition to assessment of renal functions, creatinine levels are crucial for dosing medications that are renal excreted. Variations in RIs can result in either underdosing or overdosing medications, particularly in drugs with narrow therapeutic indices [16, 17].

While analyzing lipid profile, a wide variation in RI was noted. In addition to this, only 5 (15.15%) laboratories mentioned RI stratified according to coronary artery disease (CAD), 10(30.30%) laboratories stratified serum cholesterol results as desirable, borderline high and high. Serum triglyceride (TG) was reported as normal/desirable, borderline, high /very high by 10 laboratories. Most commonly reported RI for normal was 150 mg/dl. For serum High-density lipoprotein (HDL) various terms like optimal/ desirable, intermediate, low, low risk factor, risk, high risk. 8 (24.24%) laboratories had gender stratification. A wide variation was noted in the reported optimal RI (>35->60mg/dl). For serum low density lipoprotein cholesterol (LDL) 4(12.12%) laboratories mentioned RI stratified according to CAD, various terms like optimal/near optimal/above optimal/ desirable, borderline/ borderline high, high, very high, low, low risk factor, risk, high risk were used. Wide variation was noted in desirable /optimal RI (>60- <150 mg/dl). 13 (39.39%) laboratories did not report VLDL.

Conclusion

The survey highlights significant challenges faced by clinical laboratories in Pakistan regarding the establishment of RIs. There is wide variation in RIs across different laboratories, which may lead to inconsistencies in diagnostic practices and cause patient safety issues. There is urgent need for national guidelines and collaborative efforts to standardize RIs, ensuring accurate and consistent diagnostic outcomes across all laboratories in Pakistan.

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Research Article

Standardizing The Biochemical Tests for Chronic Kidney Disease (CKD): Where Do We Stand? A National Survey of Laboratories Across Pakistan

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CKD, Survey, biochemical tests, standardization, Pakistan

Abstract

Introduction: CKD affects 8.6% of the global population, with South Asian countries seeing prevalence rates between 10.6% and 23.3%, including 21.2% in Pakistan. CKD's burden is further exacerbated in South Asia due to rising hypertension and diabetes. Accurate estimation of glomerular filtration rate (GFR) and albuminuria are vital for CKD management. Despite increasing CKD studies, regional testing remains underdeveloped. This survey evaluates CKD testing practices in Pakistan, aiming to propose recommendations for improving uniformity, enhancing surveillance, and advancing CKD care standards.

Methods: A cross-sectional survey was conducted by the Chemical Pathology section at Aga Khan University (AKU) using a validated questionnaire developed by International Federation of Clinical Chemistry (IFCC) which was modified for local context. The survey, distributed via Google Forms to major laboratories across Pakistan, focused on CKD testing methods. Data were analyzed using Excel (Microsoft Corporation, 2018) software.

Results: A total of 13 laboratories participated in the survey. All laboratories measured serum creatinine, while two measured serum cystatin C, eleven measured urinary protein, and ten measured urinary albumin. GFR was estimated using equations in 10 laboratories, with CKD-EPI 2021 (29%), MDRD (22%), and CKD-EPI Pak (14%) being the most commonly used. However, only six laboratories employed pediatric equations for children. Significant variability was

observed in the testing methods for serum creatinine, urinary protein, and urinary albumin.

Conclusion: Our findings emphasize the urgent need to standardize CKD testing in Pakistan. Inconsistencies in estimated GFR reporting, serum creatinine measurement and proteinuria testing highlight the need for harmonized protocols to improve diagnosis, management, and public health outcomes.

Introduction

The Global Burden of Disease Study estimated that there were 697.3 million cases of chronic kidney disease (CKD) worldwide in 2019 [1]. CKD affects approximately 8.6% of the global population, with prevalence rates in South Asian countries ranging from 10.6% to 23.3%, and about 21.2% in Pakistan [2, 3]. Although CKD is a global public health challenge, its impact is especially severe in South Asian populations due to the increasing incidence of risk factors such as hypertension and diabetes [4, 5]. Consequently, complications such as accelerated cardiovascular disease, premature mortality, and kidney failure have a significantly detrimental impact on the national economies of low- and middle- income countries [6, 7].

As CKD often progresses silently, clinicians depend heavily on clinical laboratory results for diagnosing, classifying, treating, and managing patients. The Kidney Disease: Improving Global Outcomes (KDIGO) Guidelines has classified CKD patients into six stages based on the estimated glomerular filtration rate (eGFR), which is derived from the serum concentration of creatinine, as well as three levels of kidney damage based on albuminuria [8, 9]. Thus, measurement of creatinine and albuminuria is central to the management of CKD that help in assessing the severity, risk and prognosis of patients.

Despite a growing number of studies on CKD prevalence and incidence over the past decade, global capacity for CKD testing and monitoring remains significantly less developed compared to that for hypertension, diabetes, and cardiovascular disease [7]. The first step to making progress in improving CKD monitoring activities is to ensure consistent and accurate results across clinical laboratories. Not only would this enable optimal patient care, but also lead to a high level of harmonization in regional CKD testing methodology and measurements. For effective patient care, including accurate diagnosis, referral prioritization, clinical research, and public health prioritization, laboratory results must be comparable across different times, locations, and measurement methods. This necessitates precision and agreement between laboratories, with traceability to accepted reference standards [10].

Thus, we aim to assess the current status of Pakistani clinical laboratories in standardizing CKD testing tools and assays/ equipment. Through this survey, we will evaluate the methods used for calculating eGFR and albuminuria in clinical laboratories across Pakistan. By gathering this data, we can propose recommendations to enhance uniformity in CKD testing practices, strengthen CKD surveillance by ensuring consistent documentation of laboratory abnormalities, and support a national effort to improve the standard of care in CKD management.

Materials and Methods

A cross-sectional survey was conducted by the section of Chemical Pathology, Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi after approval from the institutional ethical review committee (AKU- 2024-9947-28892). A previously validated questionnaire developed by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Committee on Kidney Disease – C-KD was used with subsequent modifications according to local context [11].

The survey was designed and circulated via a Google Forms link to the lead Pathologists of 13 major clinical laboratories across Pakistan. The survey consisted of multiple sections, first was informed consent and general information about the laboratory; followed by questions regarding testing methodology for serum creatinine, eGFR creatinine, serum cystatin C, eGFR cystatin C, urinary protein, and urinary albumin.

The survey accepted responses from April-May 2024. 13 pathologists attempted the survey, and all responses were included in the final analysis. The data was analysed using Excel (Microsoft Corporation, 2018) software.

Results

A total of 13 laboratories participated in our survey. The location of these laboratories and their general characteristics are depicted in Figure 1 and Table 1 respectively.

Figure 1: Map of Pakistan showing the cities from which the responses came, along with the frequencies of responses.

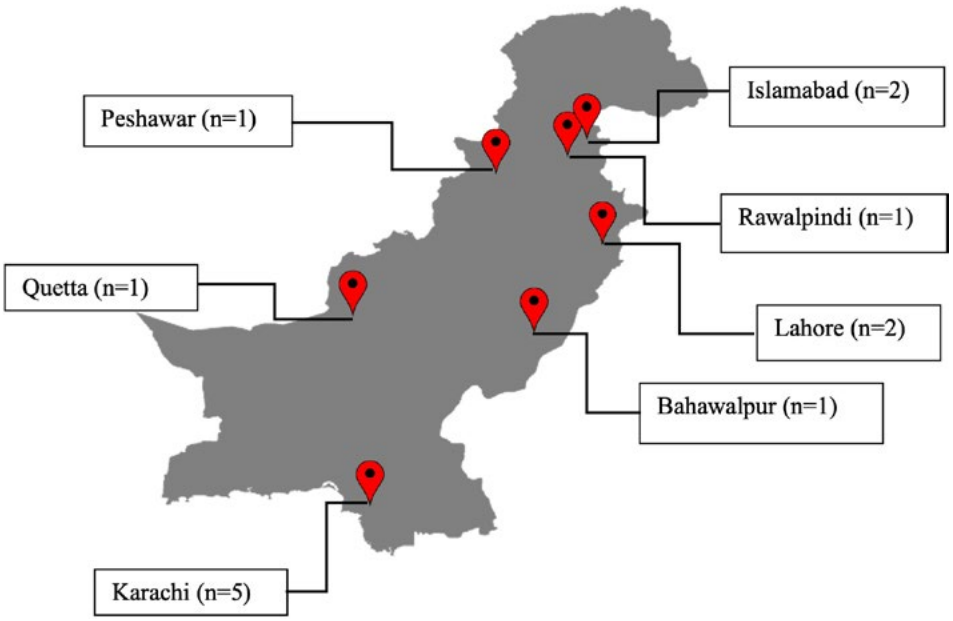


Table 1: General characteristics of laboratories who participated in the survey.

Number of laboratories				
Laboratory processes requests from:				
Specialized physicians, primary care physicians and self-referred or walk in patients	7			
Specialized physicians only	2			
Primary care physicians and self-referred or walk in patients	2			
Self-referred or walk in patients only	1			
CKD testing available at the laboratory:	**Serum creatinine**	**Serum cystatin C**	**Urinary protein**	**Urinary albumin**
Yes	13	2	11	10
No	0	11	2	3
Measurement of eGFR using equations:	**eGFR creatinine**		**eGFR cystatin C**	
Yes	10		2	
No	3		11	
Measurement of eGFR using equations in children:				
Yes	6			
No	7			
Number of serum creatinine requests received in a day:				
<100	1			
100-999	7			
1000-5000	5			
>5000	0			

Number of serum cystatin C requests received in a day:	
<100	2
100-999	0
1000-5000	0
>5000	0
Number of urinary protein requests received in a day:	
<100	6
100-999	5
1000-5000	0
>5000	0
Number of urinary albumin requests received in a day:	
<100	7
100-999	3
1000-5000	0
>5000	0
Turnaround time for serum cystatin C measurement:	
<6 hours	0
6-12 hours	0
12-24 hours	1
24-48 hours	1
Turnaround time for urinary protein measurement:	
<6 hours	4
6-12 hours	3
12-24 hours	4
24-48 hours	0
Turnaround time for urinary albumin measurement:	
<6 hours	4
6-12 hours	3
12-24 hours	2
24-48 hours	1

Only six laboratories provide e GFR based on creatinine for children, with half of them using the original Schwartz equation and the remaining using the modified Schwartz equation.

While the use of serum cystatin C is still limited, two respondents measure serum cystatin C using immunonephelometry and immunoturbidimetry. Equations used for estimating GFR using serum cystatin C are depicted in Figure 3.

The different methods used for measuring serum creatinine (SCr) and equations for estimating GFR using SCr are shown in Figures 2A and 2B.

Figure 2A: Different methods used for measuring serum creatinine.

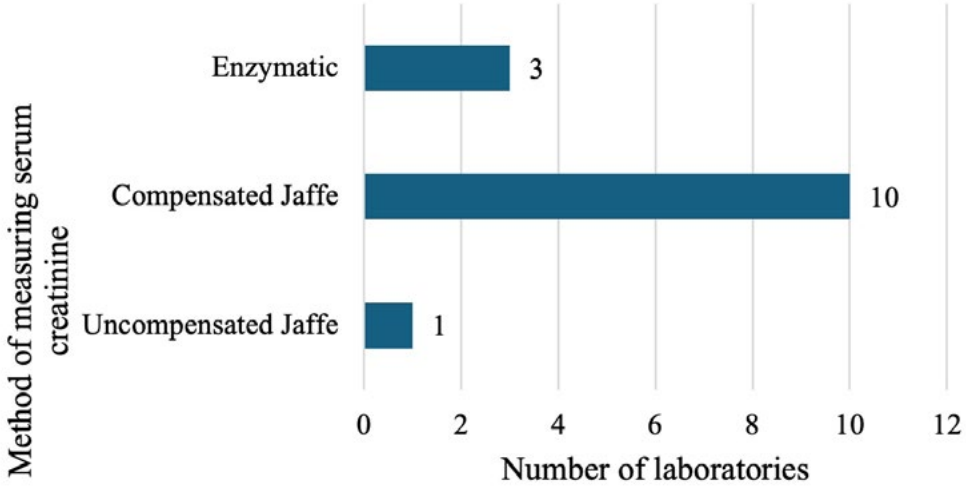


Figure 2B: Variations in the type of eGFR creatinine equations used.

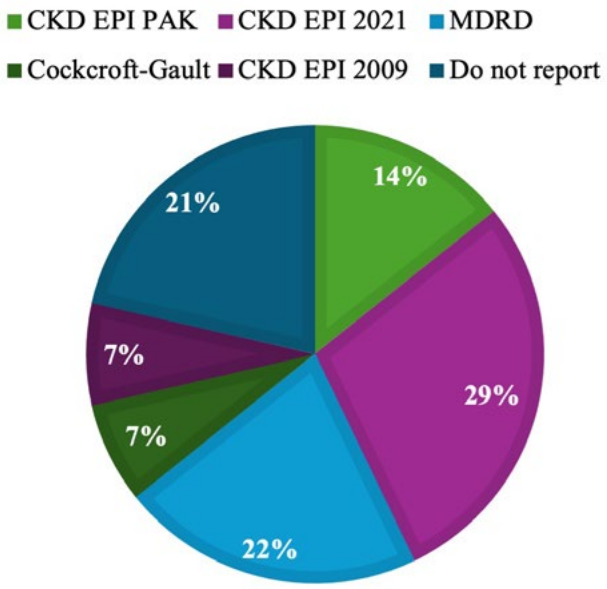
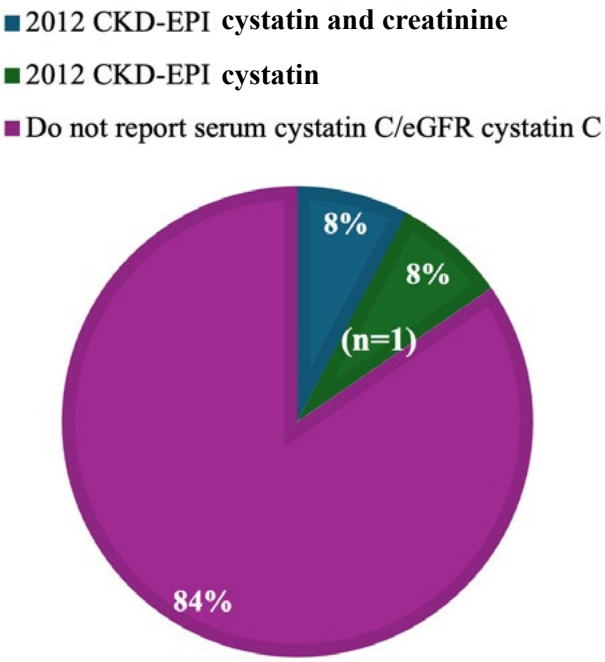


Figure 3: Use of serum cystatin C and eGFR cystatin C.



Regarding the reporting of eGFR creatinine or eGFR cystatin C, almost one-third (n=4) of the laboratories automatically report it with every result of serum creatinine or serum cystatin C while four report it only when requested by the physician.

Regarding the presentation of eGFR results, more than half of the respondents (n=8) report the exact value while just one laboratory only reports the numerical value when it is <60ml/min/1.73m2. Eight laboratories also report eGFR with reference values along with some type of commentary to facilitate clinical interpretation.

	The GFR measurement obtained is always reported, regardless of the value (n)	**The numerical value is only reported when GFR is <60ml/min/1.73m2 (n)**	**Not applicable (n)**
How are the eGFR results presented?	**8**	**1**	**4**

	Yes (n)	**No (n)**	**Not applicable (n)**
Are the eGFR results presented with reference values?	**8**	**1**	**4**
Are eGFR results presented with some type of commentary facilitating clinical interpretation?	**8**	**2**	**3**

The reasons for not reporting eGFR varied with laboratories stating that the formulas used for calculating GFR are not sufficiently validated to warrant their routine use (n=1), the clinical departments have not requested it (n=1), the digital database system of the laboratory does not allow it (n=1).

The methods and urine samples used to measure proteinuria and albuminuria are depicted in Figures 4 and 5, respectively.

Figure 4A: Variations in the type of preferred urine sample to measure urinary protein.

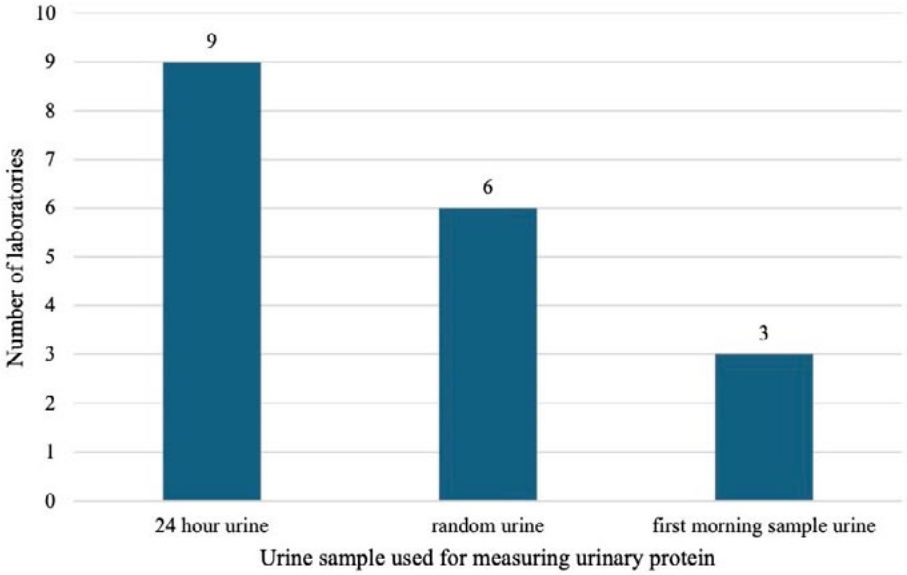


Figure 4B: Methods of urinary protein estimation.

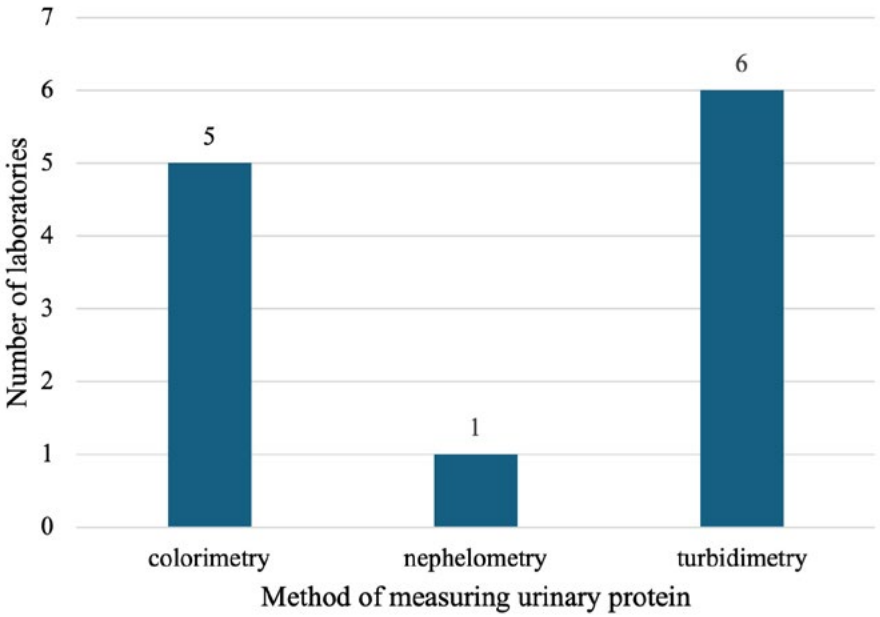


Figure 5A: Preferred urine sample for urinary albumin estimation.

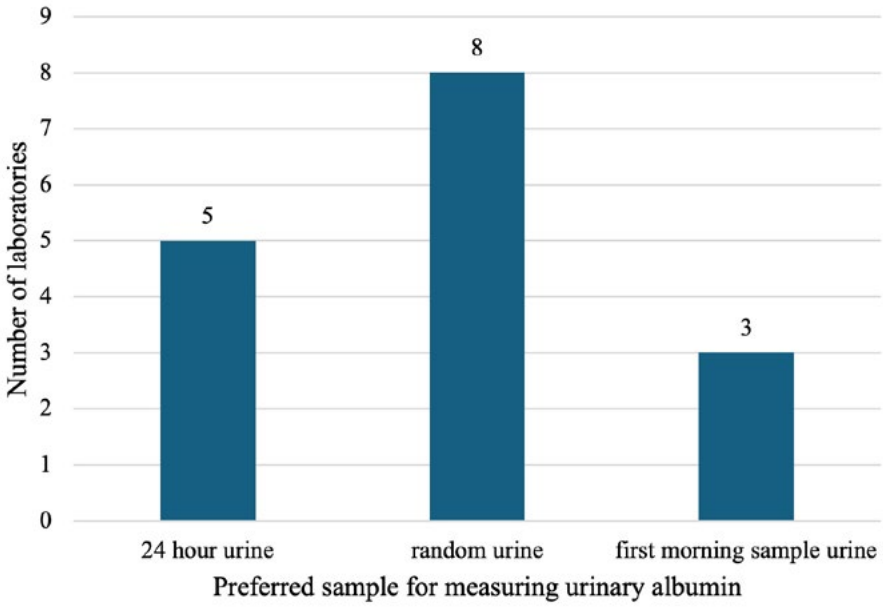
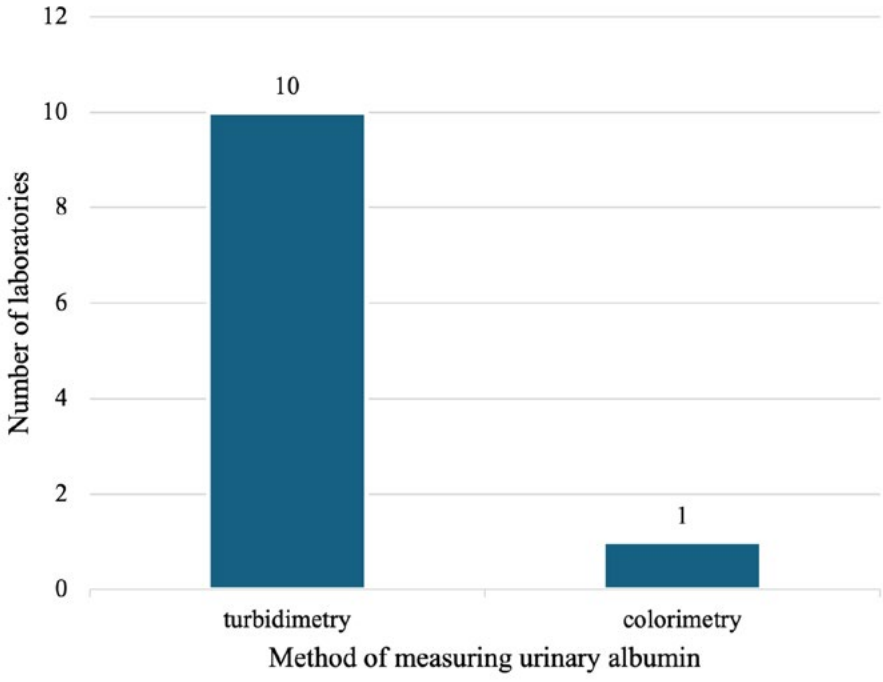


Figure 5B: Methods of urinary albumin estimation.



The analyzers and reference cut-offs used in the measurement of proteinuria and albuminuria are shown in Table 2.

Table 2: Variations in the analyzers and corresponding reagents used in the testing of urinary protein and urinary albumin.

Test	Analyzer	No. of laboratories	Reference Cut-offs
Urinary protein	**Abbott** **Siemens** **Roche**	**3** **2** **6**	**<150mg/dl, <300mg/day, <150mg/day** **<100mg/day, <15mg/dl** **<100mg/day, <300mg/mmol, <150mg/day, <300mg/day**
Urinary albumin	**Abbot** **Siemens** **Roche**	**2** **2** **6**	**<30mg/day, <30mg/g** **30mg/day, >4000 mg/day** **30, <20 mg/L, <30mg/24h** **<30mg/g creatinine, microalbuminuria 30 to 300 mg/g,>300 mg/g macroalbuminuria, <20 mg/L, >34mg/mmol, 30mg/day, >4000 mg/day**

Discussion

Our findings highlight prominent inconsistencies and deviations from recommended guidelines in CKD testing practices across the country. While measuring GFR is the gold standard for assessing kidney function, it is labor-intensive, time-consuming, and expensive, limiting its widespread use. To address these challenges eGFR calculations have been widely adopted, utilizing over 50 predictive equations, primarily based on serum creatinine (SCr) or cystatin C. The National Kidney Disease Education Program (NKDEP) recommends including eGFR with every SCr test, as it provides a more accessible assessment of kidney function and helps identify chronic kidney disease (CKD) without added costs or inconvenience [12, 13]. However, despite nearly 80% of surveyed laboratories estimating GFR using SCr equations, less than half offer reflex reporting of eGFR with every SCr test, indicating a need for improvement.

The majority of surveyed laboratories use the Jaffe technique for SCr measurement due to its low cost. However, variations in SCr measurement methods significantly impact both nephrology research and routine clinical practice. Standardizing SCr measurement, with a preference for the enzymatic method due to its lower variability, is essential for generating more reliable GFR estimates [14, 15].

Cystatin C, an alternative marker for estimating GFR, offers advantages over creatinine due to its reduced influence from muscle mass, diet, and ethnicity. Only 15% of our respondents use cystatin C, which is understandable given its higher costs, assay variability, and incomplete understanding of non-GFR factors affecting its concentration. Consequently, KDIGO and NICE guidelines recommend using cystatin C alongside creatinine primarily for the confirmation of CKD [16, 17].

Various eGFR creatinine equations are being used across Pakistani laboratories, with CKD-EPI 2021 (29%), MDRD (22%), and CKD-EPI Pak (14%) being the most common. Literature suggests that the CKD-EPI Pak equation is more accurate and precise for estimating GFR in the Pakistani population [3], highlighting the need to harmonize its implementation across laboratories in the region. Additionally, less than half of the laboratories are using pediatric equations to estimate GFR in children which warrants immediate attention.

Although most laboratories provide clinical commentary to aid in interpreting GFR results for non-nephrology specialists, many still report exact GFR values. Guidelines recommend expressing GFR values greater than 60 ml/min/1.73m² as “>60 ml/min/1.73m²” due to the limited precision and accuracy of equations at higher values [18]-a practice currently followed by only one Pakistani laboratory.

Interpreting survey results on proteinuria and albuminuria is more challenging, as most participants conducted these tests but reported results in different units, with varying cut-offs even among laboratories using the same analyzers. Methods of measuring proteinuria and albuminuria vary broadly and there is also no consensus on the optimal urine sample type for measuring these markers.

Harmonizing CKD testing is critical, especially given the increasing burden of CKD risk factors like diabetes and hypertension in Pakistan, as well as the rise in CKD of unknown origin (CKDu) in regions close to the equator, including South Asia. Since CKDu often presents with mild or absent proteinuria, simple urinalysis is ineffective for screening, underscoring the need for standardized clinical testing guidelines [19]. Such guidelines can improve clinical practice, care coordination, and drive quality improvement and population health initiatives [20, 21].

Conclusion

In conclusion, our findings underscore the urgent need for standardization and adherence to recommended guidelines in CKD testing practices across Pakistan. Despite the widespread adoption of eGFR calculations, significant gaps remain in

the reflex reporting of eGFR, the standardization of SCr measurement methods, implementation of more accurate equations like CKD-EPI Pak, and utilization of pediatric equations. The inconsistencies in proteinuria and albuminuria testing additionally emphasize the necessity for harmonized practices. Addressing these discrepancies is vital to improving CKD diagnosis and management, particularly in the face of rising CKD risk factors in the region. By standardizing testing protocols, we can enhance clinical practice, improve patient outcomes, and support broader public health efforts.

Conflict of interest statements

The authors declare no conflict of interests.

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None.

Ethical Approval

The study was done in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki, after approval from the institutional ethical review committee (AKU- 2024-9947-28892).

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Research Article

Reference intervals of thyrotropin, thyroid hormones, and thyroid autoantibodies in adult and older individuals according to iodine status

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Keywords

Reference range, age-specific reference intervals, thyrotropin, thyroid hormone, thyroid autoantibody, iodine status

Abstract

Background-Aim: Several factors, including ethnicity, age, iodine status, and assay method, can influence thyroid test results. This study aimed to establish reference intervals (RIs) for thyroid parameters in adults and older individuals, considering their iodine statuses.

Methods: A cross-sectional study at a single tertiary center was conducted. Participants underwent interviews, physical examinations, thyroid ultrasounds, thyroid autoantibody testing, and a spot urine iodine concentration analysis. The included participants were grouped into adult (age 18 – 59) and older (age ≥60) groups. The studies for 2.5th—97.5th values of thyroid parameters were committed to establishing RIs.

Results: A total of 357 individuals were screened, with 216 (112 adults, 54% women, 104 older, 50% women) were included in the analysis. The RIs for thyroid stimulating hormone (TSH) were as follows: 0.39 – 4.17 mIU/L for the overall group, 0.35 – 3.98 mIU/L in the adult group, and 0.42 – 4.83 mIU/L in the older group. The prevalence of adequate iodine intake (urine iodine level ≥ 200 µg/L) was 62.2% (186/299). Though RIs for TSH, Free T4, and Free T3 were slightly higher in the adequate iodine intake group, no statistically significant differences were noted. Positive anti-thyroglobulin antibodies were more prevalent in older participants (27.3% VS. 21.2%), as were anti-thyroid peroxidase antibodies (24.2% VS. 13.9%).

Conclusion: Older individuals exhibited significantly higher TSH levels and lower FT3/Total T3 levels, while FT4/Total T4 remained comparable to adults. All thyroid parameters and thyroid autoantibody levels showed no statistically significant differences between those with adequate iodine intake and those with iodine deficiency.

Background

Variations in thyroid hormone levels can arise from multiple factors, including ethnicity, gender, age, body mass index (BMI), habitat, and diet [1]. Additionally, variations in analytical techniques, laboratory environments, specimen collection, and transportation can further impact thyroid function test results [2]. The reference intervals (RIs) provided by the manufacturer are merely guidance; each laboratory should establish RIs tailored to its specific population. Several studies have shown population-specific thyroid hormone levels [2-4]. Notably, the large population study from the National Health and Nutrition Examination Survey (NHANES-III) in the United States identified significant disparities in mean TSH levels across racial and ethnic groups. Specifically, the results indicated that mean TSH levels were lower in Black individuals, followed by Mexican Americans, and then White individuals [4]. These results highlight the importance of considering ethnic factors in thyroid assessments.

Prior studies have shown discrepancies in thyroid function test results among different analyzers [5]. A comparative study conducted in Australia among various analyzers (Siemens Centaur, Roche, Architect, and Immulite) revealed minor differences at TSH levels less than 2 mIU/mL, approximately 1 mIU/L at TSH levels between 4 – 5 mIU/L, and about 2 mIU/L at TSH levels of 8 – 10 mIU/L. These variations can have a substantial impact on clinical decisions and patient management. The RIs of thyroid function tests among Thai individuals from the previous report used an analytic platform different from our center [6]. Consequently, it is essential to establish RIs for thyroid hormone tests in our center, which was the primary purpose of this study.

The changes in thyroid hormones associated with aging have been widely studied in multiple populations [5,7,8]. The findings from those past studies identified a significant increase in TSH levels, accompanied by only a minor change in FT4 levels as age progresses. However, the underlying pathophysiological mechanisms driving these changes remain unclear. Several hypotheses have been proposed, including reduced negative feedback effect due to altered pituitary-thyroid axis setpoints, decreased bioactivity of TSH from potential isoform modifications, diminished responsiveness of thyrocytes to TSH, and possibly epigenetic influences linked to environmental factors [4,9]. Thus, establishing age-specific thyroid hormone RIs is one of several ways to avoid inappropriate diagnosis and help improve the management of thyroid disorders in the older.

Iodine serves as a critical substrate for thyroid hormone synthesis. Individuals with iodine deficiency may exhibit clinical hypothyroidism diversely, depending on the severity of the deficiency and the age of the affected individual. The World Health Organization (WHO) recommends that at-risk populations should be screened for iodine status, and those identified as deficient should receive appropriate iodine supplementation to prevent infant mortality and enhance cognitive development. Multiple studies conducted in both iodine-deficient and iodine-sufficient regions have shown a trend of elevated TSH levels in areas with sufficient iodine status [10]. While Thailand is classified as an iodine-sufficient nation [11], assessing the relationship between iodine status and thyroid hormone levels within the Thai population remains essential.

The objective of this study was to establish RIs of thyroid function test in five parameters: thyroid stimulating hormone (TSH), free triiodothyronine (free T3), free thyroxine (free T4), total triiodothyronine (TT3) and total thyroxine (TT4), and to establish RIs of thyroid autoantibodies, including anti-thyroglobulin antibodies (Anti-Tg), anti-thyroid peroxidase antibodies (anti-TPO) and anti-thyrotropin receptor antibodies (anti-TSHr) among Thai adults and older by the laboratory method used in our center. These newly established RIs are expected to be more specific to Thai adults and older patients. The second objective is to study the association between iodine status and thyroid hormones among the Thai.

Material and Method

Study population and screening

A cross-sectional study was conducted at a single tertiary center from October 2021 to October 2022. The Faculty of Medicine, Chulalongkorn University Institutional Review Board approved this study (IRB no. 637/63) in accordance with the Declaration of Helsinki (as revised in 2013). During the COVID-19 outbreak, we used a non-face-to-face participant screening procedure to reduce visits. The initial screening tool was a QR-code-linked questionnaire distributed online through our department's social media platform. The questionnaire consisted of checklists of inclusion criteria, which were Thai individuals aged ≥18 years, and exclusion criteria, which were BMI >30 kg/m2, a recent hospitalization within the past three months, a history of receiving an iodinated contrast media during the past three months, and underlying conditions affecting thyroid diseases or having been prescribed with medications affecting thyroid functions (furosemide, heparin, non-steroidal anti-inflammatory drugs). In the next step, a phone interview was conducted to verify the adherence to inclusion and exclusion criteria among potential participants who had answered the questionnaire and to arrange a visit to enroll participants in the study.

Then, in the final screening step, selected participants underwent a physical examination and thyroid ultrasound conducted by a single endocrinologist. If the individuals were found with any of the exclusion criteria, which were enlarged thyroid gland, inhomogeneous thyroid parenchyma, multiple thyroid nodules (any size), or a single thyroid nodule, size >1 cm. In diameter, they were excluded. The enrolled participants were requested to obtain blood for thyroid function and thyroid autoantibodies tests and provide spot urine collection for urine iodine concentration

analysis. Only participants with all three negative thyroid autoantibodies will be included in the study as the reference population for normal thyroid conditions. Informed consent was obtained from all individuals included in this study. The participants were sampled and grouped by non-probability sampling method with quota selection to attain an equal balance between genders and age groups (adult group aged 18 -59 years and older adult group aged 60 years and over).

Specimen processing and Laboratory analysis

The specimen collection included two tubes of heparinized blood, two tubes of clotted blood, and a cup of urine. After the specimen was collected, all samples were processed within two hours. Two tubes of heparinized blood and a clotted blood tube were centrifuged with 1,000 g for 10 minutes and immediately analyzed for TSH, FT4, and FT3 as centrifugation was finished. The analyses for TSH, free T3, and free T4 were performed with an Abbott Alinity I analyzer (Abbott Laboratories, Chicago, Illinois) by chemiluminescence immunoassay method at the central laboratory, King Chulalongkorn Memorial Hospital. All residual plasma and sera are then portioned in different sterile vials to store at -70 Celsius degrees for further analysis. Clotted blood tubes are simultaneously processed to the Center for Medical Diagnostic Laboratories (CMDL), Faculty of Medicine Chulalongkorn University, to perform Anti-TSHr with Roche Cobas e601 (Roche diagnostics, Basel, Switzerland) by electrochemiluminescence immunoassay method. The test is performed within 3 hours. The remaining parameters, including total T3, total T4, anti-TPO, and anti-Tg, were analyzed within 30 days with -70 Celsius degree-stored sera samples thawed at room temperature for 30 minutes, removed fibrin, mixed until homogenized, and centrifuged with 1,000 g for 10 minutes. The spot urine samples collected from participants were stored at -70 Celsius degree for further analysis of urine iodine concentration with the ammonium persulfate digestion method (Sandell-Kolthoff reaction) at the Nuclear Chemistry laboratory, Faculty of Medicine, Siriraj Hospital, within 90 days.

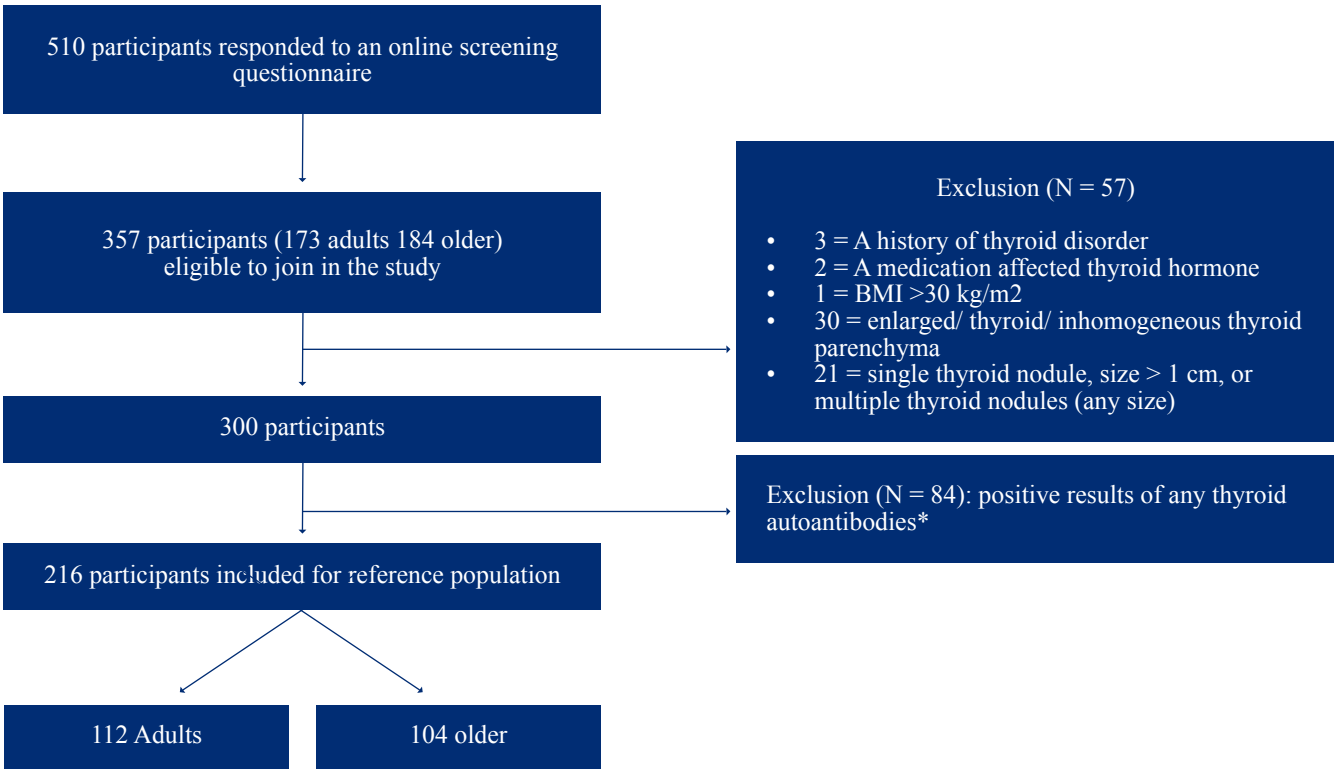
Statistical Analysis

According to the Clinical and Laboratory Standards Institute (CLSI) volume EP-28, at least 120 data were used to analyze and define the reference intervals. The percentiles of 2.5 and 97.5 were used for the lower and upper limits of the reference intervals. Each variable data was tested for normal distribution by the Shapiro-Wilk test method. In normal distributed data, mean ± SD and unpaired student t-test were used for a descriptive analysis—a non-parametric test, such as medians with IQR, and the Kruskal-Wallis test was used in non-normal distributed data. The reference range was compared among groups using the Wilcoxon rank-sum test. All analyses were a two-sided test with $\alpha = 0.05$ by the Stata Statistic program version 16.1.

Results

Five hundred and ten participants responded to the online questionnaires. After checking those answered questionnaires for the inclusion and exclusion criteria, the telephone interview was managed to respond to the eligible participants. Finally, 357 participants agreed to join an enrolment visit at our center. During the visit, six participants were revealed to meet exclusion criteria: a history of thyroid disorders in four participants, one was taking medication affecting thyroid hormone levels, and one had a BMI exceeding 30 kg/m2. During the thyroid ultrasonographic study, 51 participants were excluded due to abnormal thyroid ultrasound (30 with enlarged thyroid glands/ inhomogeneous thyroid parenchyma and 21 with thyroid nodules, size > 1 cm). The presence of thyroid nodules (any size) observed from ultrasonographic screening was 23.5% (84/357) in all, 15.6% (27/173) in the adult group, and 31.0% (57/184) in the older group. Then, three hundred participants were enrolled to collect blood samples and spot urine samples. The eighty-four participants with positive titer for any thyroid autoantibodies were further excluded, and the final number of included participants as a reference population was 216 (112 adults and 104 older) (Figure 1).

Figure 1: Flow diagram of study enrolment.



* thyroid autoantibodies: anti-thyroglobulin, anti-thyroid peroxidase, and anti-TSHr antibodies

Baseline characteristics

The positive titer of thyroid autoantibodies was defined as a higher titer than the upper reference
limit by the manufacturer (anti-Tg positive titer >4.11 IU/mL, anti-TPO positive titer >5.61 IU/mL, anti-TSHr positive titer ≥1.75 IU/L). The prevalence of positive anti-Tg, anti-TPO, and anti-TSHr antibodies was 24.3% (73/300), 19.0% (57/300), and 5.7% (17/300), respectively. There was a 21.2% VS. 27.5% prevalence of positive anti-Tg (P = 0.202) in adult and older group, respectively. The prevalence of positive anti-TPO was significantly higher in adults than in the older group: 13.9 % VS. 24.2%, P = 0.027, respectively (Figure 2). The positive titer of anti-TSHr was more likely prevalent in the adult group (8.0%) than in the older group (3.3%), with a non-statistically significant P-value (P = 0.080). Moreover, the positive anti-Tg and anti-TPO were significantly higher in women than men, with a P-significant value (Figure 3).

Among 300 enrolled participants, one did not collect samples for urine iodine analysis. The prevalence of adequate iodine intake (urine iodine level 100 – 199 µg/L) was 62.2% (186/ 299), mild iodine deficiency (urine iodine level 50 – 99 µg/L) was 24.1% (72/299), moderate deficiency (urine iodine level 20 – 49 µg/L) was 12.7%. (38/299) and severe iodine deficiency (urine iodine level <20 µg/L) was found in 1.0% (3/299). Moreover, the above-requirement iodine status (urine iodine level 200 - 299 µg/L) was 19.7% (59/299), and the excessive iodine status (urine iodine level ≥300 µg/L) was 8.3% (25/299).

Among 216 reference populations, the median age of all participants, adult, and older groups was 58.5 years, 33.5 years, and 65.5 years old, respectively. Women were 52 % in all groups, 55% in the adult group, and 50% in the older group. The mean BMI was 23.2 kg/m^2 in all and significantly higher in the older group (24.0 kg/m^2) than in the adult group (22.5 kg/m^2) with P-value <0.001. All baseline characteristics of the participants in the adult and older groups are shown in Table 1. Each laboratory parameter's analytical methods, including a limit of detection, quantification, and performances, following the CLSI EP15-A3 guideline, are shown in Supplementary Table 1. The total imprecision coefficient variation for TSH, Free T3, Free T4, total T3, total T4, anti-Tg, anti-TPO, and anti-TSHr were 1.7%, 4.7%, 2.7%, 3.3%, 1.2%, 3.0%, 2.2% and 4.0% respectively. Only samples with negative for all thyroid autoantibodies were included in the analysis of RIs.

Figure 2: Prevalence of positive thyroid autoantibodies between the adult and older groups.

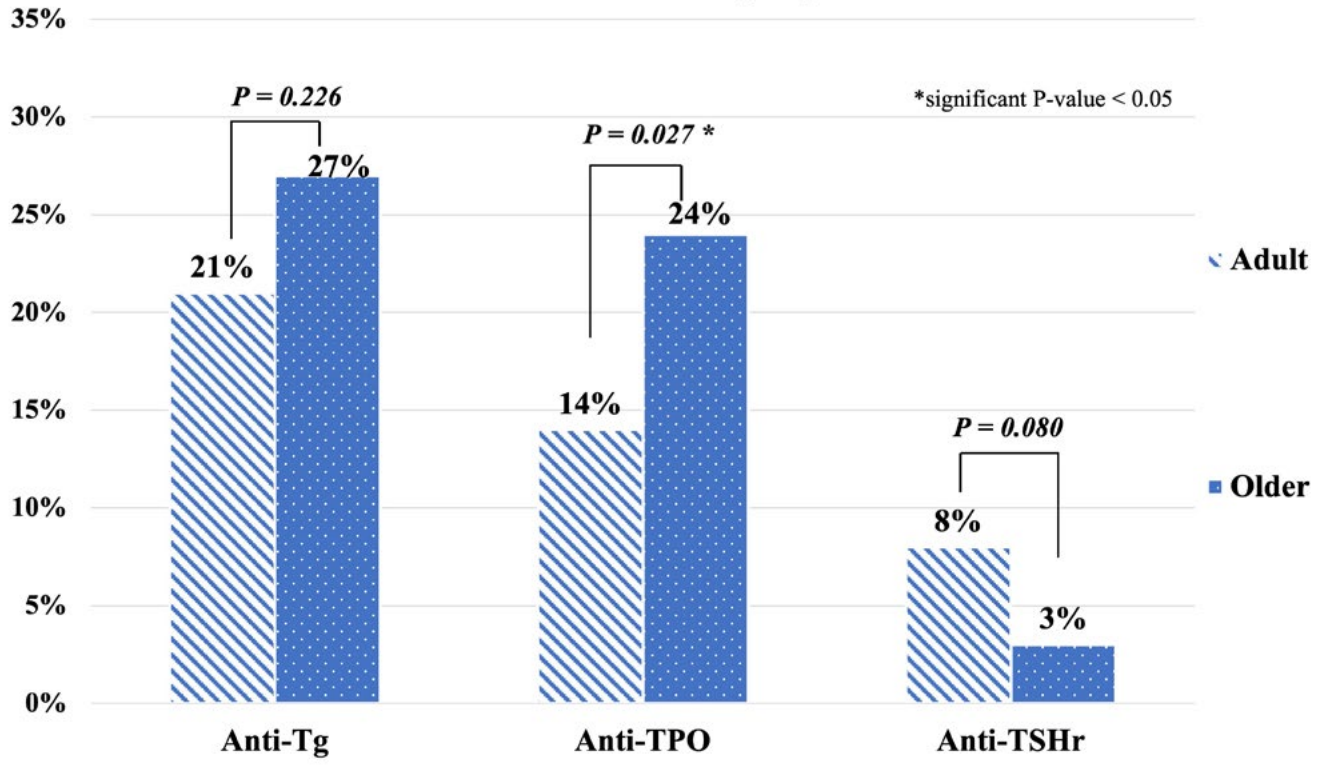


Figure 3: Prevalence of positive thyroid autoantibodies between gender.

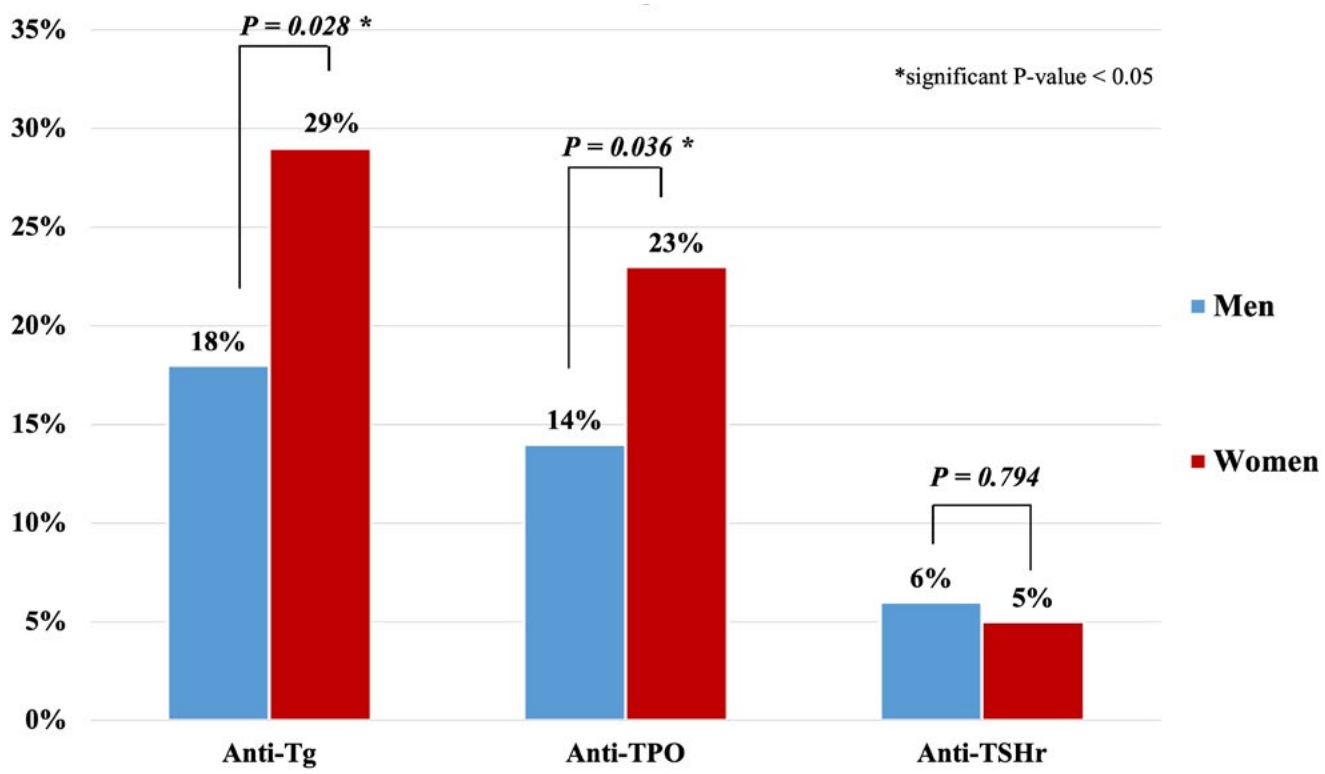


Table 1: Baseline characteristics of all included participants (N = 216).

Variables	All (N= 216)	Adults (N = 112)	Older (N = 104)	P-value
Age, year (median, IQR)	58.5 (33.0 - 65.0)	33.5 (27.0 - 47.5)	65.5 (63.5 - 68.0)	<0.001
Woman, n (%)	113 (52.3)	61 (54.5)	52 (50)	0.512
BMI, kg/m2 (mean ± SD)	23.2 ± 2.9	22.5 ± 2.8	24.0 ± 2.9	<0.001
Current smoker, n (%)	5 (2.4)	3 (2.7)	2 (1.9)	1.00
Family history of thyroid disorders, n (%)	20 (9.3)	16 (14.3)	4 (3.9)	0.006

Reference Intervals

The results of RIs of TSH in all subjects (n = 216) were 0.39 – 4.17 mIU/L, in the adult group (n = 112) were 0.35 – 3.98 mIU/L, and in the older group (n = 104) were 0.42 – 4.83 mIU/L. The upper reference limit of the TSH, free T4, total T4, free T3, and total T3 in all subjects and each group was lower than the manufacturers. Compared with the adult group, the RIs of TSH were higher, and the RIs of FT3 and TT3 were lower in the older group with statistically significant P-values. For FT4, TT4 and other thyroid autoantibodies (anti-Tg, anti-TPO, and anti-TSHr) were comparable between the adultand older groups. The RIs of TSH, free T4, total T4, free T3, total T3, and thyroid autoantibodies in each age group compared to the RIs from the manufacturer's package insert were illustrated in Table 2.

Table 2: Reference intervals of TSH, Free T4, Total T4, Free T3, Total T3, TgAb, TPOAb, and Anti-TSHr in all subjects, adult group, older group, and from the manufacturer's package insert.

Group	N	TSH (mIU/L)	Free T4 (ng/dL)	Total T4 (µg/dL)	Free T3 (pg/mL)	Total T3 (ng/dL)	TgAb (IU/mL)	TPOAb (IU/mL)	Anti-TSHr (IU/L)
All	216	0.39 - 4.17	0.75 - 1.09	4.46 - 9.38	2.10 - 3.27	59 – 115	0.35 - 3.13	0 - 2.28	0 - 1.67
Adult	112	0.35 - 3.98*	0.76 - 1.06	4.23 - 9.49	2.15 - 3.36*	60 – 118*	0.23 - 3.08	0 - 2.31	0 - 1.69*
Older	104	0.42 - 4.83	0.74 - 1.15	4.23 - 9.46	1.99 - 2.94	52 – 109	0.37 - 3.35	0 - 3.22	0 - 1.59
Manufacturer*	-	0.35 - 4.94	0.70 - 1.48	4.87 - 11.72	1.58 - 3.91	35 - 193	0 - 4.11	0 - 5.61	<1.75

*The reference interval with a statistically significant difference (P-value <0.05) between adult and older groups. (TSH: P = 0.025, Free T3: P = 0.013, Total T3: P = 0.041, antiTSHr: P<0.001)
*The reference interval is from the manufacturer's package insert. TSH, Free T4, Total T4, Free T3, Total T3, Anti-Tg, and Anti-TPO tests were performed with the Abbott Alinity I analyzer (Abbott Laboratories, Chicago, Illinois) using chemiluminescence immunoassay. The anti-TSHr test was performed with Roche Cobas e601 (Roche Diagnostics, Basel, Switzerland) using an electrochemiluminescence immunoassay.

Table 3: Reference intervals of TSH, Free T4, Free T3, Anti-Tg, Anti-TPO, and Anti-TSHr between genders.

Group	N	TSH (mIU/L)	Free T4 (ng/dL)	Total T4 (µg/dL)	Free T3 (pg/mL)	Total T3 (ng/dL)	Anti-Tg (IU/mL)	Anti-TPO (IU/mL)	Anti-TSHr (IU/L)
All	103				2.21 – 3.36*				
Men	113	0.35 – 3.72	0.76 – 1.12	3.95 – 9.04	2.00 – 2.87	57.32 – 117.07	0.27 – 3.21	0 – 3.46	0 – 1.66
Women		0.40 – 4.82	0.74 – 1.08	4.66 – 9.87		59.22 – 109.43	0.35 – 3.19	0 – 2.04	0 – 1.72
Adult	51								
Men	61	0.34 – 3.51	0.76 – 1.07	3.86 – 8.90	2.19 – 3.48*	59.48 – 121.80	0.14 – 3.07*	0 – 3.48	0 – 1.68*
Women		0.36 – 4.58	0.74 – 1.09	4.52 – 10.42	2.05 – 2.89	59.35 – 121.93	0.10 – 2.00	0.32 – 3.52	0 – 1.73
Older	52								
Men	52	0.30 – 4.57	0.73 – 1.17	3.80 – 9.31	1.90 – 3.16*	49.59 – 114.04	0.38 – 3.49	0 – 3.95	0 – 1.58
Women		0.70 – 6.08	0.73 – 1.12	3.77 – 9.69	1.98 – 2.87	50.93 – 104.68	0.35 – 3.68	0 – 4.02	0 – 1.67

*The reference interval with a statically significant difference (P-value <0.05) between men and women.

The RIs of TSH and thyroid hormones between genders were analyzed, and no statistically significant differences were observed, except for the FT3 values. The upper reference limit of the FT3 was higher in men than women in all participants, including adult and older groups, with statistically significant P-values. For RIs of thyroid autoantibodies between genders, the RIs of anti-Tg were statistically higher, and the RIs of anti-TSHr were significantly lower in adult men (Table 3).

Since one did not collect for urine iodine concentration test, thus 215 participants were categorized into 132 participants with adequate iodine intake (urine iodine ≥100 µg/L) and 83 participants with iodine deficiency (urine iodine <100 µg/L). The mean urine iodine concentration in an adequate iodine intake group was significantly higher than in the iodine deficiency group, 183.5 µg/L, and 50.8 µg/L, respectively, with P<0.001 (Table 4). The RIs of TSH, Free T4, and Free T3 in the adequate iodine intake group were slightly higher than those of the iodine deficiency group, with no statistical significance. The RIs of thyroid autoantibodies, including anti-Tg, anti-TPO, and anti-TSHr, between the adequate iodine status and iodine deficiency groups demonstrated similar results, as listed in Table 4.

Table 4: The median, 2.5th, and 97.5th percentile levels of thyrotropin, thyroxine, triiodothyronine, and thyroid autoantibodies according to iodine status.

Group	All (n = 215)[a]	Iodine deficiency (Urine iodine <100 µg/L, n = 83)	Adequate iodine intake (Urine iodine ≥100 µg/L, n = 132)	P-value[b]
UIC (µg/L)	129.7 (75 – 204.6)	59.8 (48.2 – 82.8)	183.5 (136.7 – 250.1)	<0.001
TSH (mIU/L)	1.15 (0.39 – 4.17)	1.23 (0.36 – 3.87)	1.08 (0.42 – 4.66)	0.918
FT4 (ng/dL)	0.92 (0.75 – 1.09)	0.92 (0.73 – 1.07)	0.93 (0.77 – 1.12)	0.493
FT3 (pg/mL)	2.61 (2.10 – 3.27)	2.60 (2.02 – 3.32)	2.63 (2.19 – 3.27)	0.665
Anti-Tg (IU/mL)	0.95 (0.35 – 3.13)	0.85 (0.31 – 3.05)	1.04 (0.35 – 3.23)	0.074
Anti-TPO (IU/mL	0.56 (0 – 2.28)	0.53 (0 – 3.39)	0.56 (0 – 2.16)	0.717
Anti-TSHr (IU/L)	0.98 (0 – 1.67)	1.0 (0 – 1.64)	0.95 (0 – 1.68)	0.750

UIC: urine iodine concentration, reported as median (IQR), the other data reported as median (2.5^{th} – 97.5^{th} percentile) value; one missing value of urine iodine level in a total of 216 participants; [b] P-value <0.05 as significant value.

Discussion

This study showed a comprehensive evaluation of establishing the reference intervals (RIs) of thyroid hormones in adults and the older. The method for recruitment of the reference population was extensive to enroll subjects with the most likely normal thyroid status, according to the National Academy of Clinical Biochemistry (NACB) guideline [12]. The study enrolment included history taking, physical examination, thyroid ultrasonographic study, serologic testing for thyroid autoantibodies, and spot urine iodine concentration examination. Our RI results of adults' and the older's TSH, thyroid hormones, and thyroid autoantibodies were narrower than those from the manufacturer's. The RI of TSH among the older group was higher and had a wider range than in the adult group, with a significant P-value (P-value = 0.025). The thyroxine hormones (FT4 and Total T4) were comparable between the adult and older groups, whereas the RIs of the FT3/Total T3 were lower in the older group with statistically significant P-value. The RIs of TSH and FT4 were similar between genders, but the FT3 level showed a statistically significant lower in women, corresponding to the previous results in the Thai population [6]. This study also demonstrated a comparable range of RIs of TSH, thyroid hormones, and thyroid autoantibodies between the adequate iodine intake group and the iodine deficiency group.

Several past studies, including a meta-analysis study [10], found variable TSH ranges that could be affected by several factors, including study population and analytical assay methods. The study population of disease-free individuals without exclusion for positive thyroid autoantibodies tended to have a higher TSH level [13]. The study, excluding unknown thyroid disease with either thyroid autoantibodies or thyroid ultrasound, resulted in a narrower range of TSH [13-16]. The RI studies reported by different analytical platforms from various ethnicities are listed in Table 5.

Table 5: Comparison of TSH and Free T4 reference intervals, reported by previous studies from various ethnicities and laboratory methods.

Study author Country, year	Thyroid-Ab testing[a]	Thyroid USG[b]	Sample size	TSH range (mIU/L)	Analyzer platform	Analytical method
This study Thailand, 2024	Yes	Yes	216[a]	0.39 – 4.17	Alinity I, Abbott	CLIA
Lu Y [14] China, 2023	Yes	Yes	1,114[a]	0.70 – 4.93	Architect, Abbott	CLIA
Hickman [15] Australia, 2017	Yes	No	1,177	0.43 – 3.28	Architect, Abbott	CLIA
Kim [13] Korea, 2015	No Yes	No Yes	18,043 7,686[a]	0.62 – 7.20 0.72 – 6.80	DiaSorin S.p.A.	CLIA
Kratzsch [16] Germany, 2005	Yes	Yes	453a	0.40 – 3.77	ELECSYS, Roche	ECLIA
Sriphrapradang [6] Thailand, 2014	Yes	No	2,545	0.34 – 5.11	Cobas e411, Roche	ECLIA

[a] study population with the exclusion of abnormal thyroid autoantibodies, [b] study population with the exclusion of abnormal thyroid ultrasound. ECLIA: electrochemiluminescence immunoassay, CLIA: chemiluminescence immunoassay.

Most thyroid parameters analyzed in our study showed no significant differences between genders, except for a statistically significant higher FT3 level in men than women. In China, Lu et al. identified higher TT3 in men than women, and higher TSH levels were observed in women [14]. The study of the Korean population clearly showed that women exhibited higher levels of TSH [13]. However, some studies indicated only minor differences in TSH levels between genders [4,17]. Additionally, higher levels of thyroid autoantibodies were found in women than in men, according to our study's results and some other studies [13]. Thus, gender-specific RIs will likely differ among various ethnic groups, highlighting the importance of developing tailored RIs that apply to specific subpopulations.

A trend of higher TSH levels with increasing age was observed in our study, which aligns with findings from previous reports [6-8, 18]. However, the rise in TSH levels among the older participants from our study was less pronounced than in an earlier study conducted on the Thai population. This discrepancy may be because most participants in the older group were aged 60 to 70, with only 17% (18 out of 104) being over 70. This limitation highlights that our older participants may only partially represent the broader population of older individuals.

Dietary iodine is necessary for thyroid hormone synthesis. It is absorbed and transported to the thyroid gland, where it undergoes oxidation and organification, ultimately contributing to the synthesis of T3 and T4. In most adults, TSH secretion is increased if iodine intake falls below 100 µg/day [19]. The thyroid gland has autoregulatory mechanisms to handle iodine intake involving the sodium iodide symporter. When dietary iodine is adequate, the thyroid typically absorbs less than 20% of it. However, this absorption rate may increase significantly, exceeding 80% during chronic iodine deficiency [19]. The compensation mechanism for low iodine levels is increasing the thyroid gland's size and activity to capture more iodide.

A spot urine specimen for measuring urine iodine concentration is a standard method for assessing iodine status in the population, according to WHO/UNICEF/ICCIDD [20]. This method is easy to collect and affordable. The limitation of this method is the falsely low iodine status if subjects had too little fluid ingestion [20]. In this study, we found the prevalence of adequate iodine intake in two-thirds (62.2%, 186/299), the prevalence of iodine deficiency was 37.8% (113/299), which was primarily mild to moderate iodine deficiency level, and only three individuals (1%) with severe iodine deficiency. However, among the adequate iodine intake group, almost half of them, 45.2% (84/186), were above the requirement of iodine intake.

The result of this study shows that the median levels of TSH are higher in individuals with iodine deficiency than those with adequate iodine intake group. However, the 2.5th and 97.5th percentile levels are higher in the group with adequate iodine intake, although these differences are not statistically significant. Our study's other thyroid parameters, including FT4, FT3, Anti-Tg, Anti-TPO, and Anti-TSHr antibodies, also demonstrated no significant difference among different iodine status groups. In contrast, the results from the Danish and Northeastern German populations with iodine deficiency found a significantly lower trend of TSH levels in the lower iodine status group [21,22]. The other study showed no difference in TSH and thyroid hormone levels among different iodine statuses, as shown in the results of this study [23]. The observed discrepancies may arise from differences in the reference populations and varying levels of iodine deficiency in each study. Moreover, the RIs provided by

manufacturers were primarily derived from healthy individuals in iodine-sufficient areas. Of note is that the manufacturers did not evaluate for iodine status among their reference populations. Therefore, established specific RIs for subpopulations in an iodine-deficient area should be considered.

The prevalence of positive titer of any thyroid autoantibodies from this study was 28% (84/300), which corresponded to the results from the previous report in the Thai population that found about 23.5% of positive titer of any thyroid autoantibodies [6]. In China, Zhai et al. reported a lower prevalence of anti-Tg and anti-TPO positive in 12.7% (1,889/14,985) and 11.5% (1,728/14,985), respectively [24]. In Caucasians, the report by the Rotterdam study in the Netherlands [4] showed a comparative prevalence to the result from Chinese, with an anti-TPO positive in 12.1% (1136/9402). By gender, the more prevalent positive anti-Tg and anti-TPO antibodies were observed in women of our study in both the adult and older groups. For anti-TSHr, the positive rate was found to be similar.

The limitation of our study is the relatively small number of reference population. The sample size in each age group did not reach the recommended number of 120, partly due to the unexpectedly high positive rates of thyroid autoantibodies in participants who had to be excluded. The RIs for thyroid function tests established in this study are derived from a specific single-center population and cannot be generalized to other populations. However, they serve as a reliable reference for Thai individuals at centers utilizing the same analytical methods.

Conclusion

This study provided established reference intervals for the full panel of thyroid testing, including TSH, Free T4, T4, Free T3, T3, anti-thyroglobulin (Anti-Tg), anti-thyroid peroxidase (Anti-TPO), and thyrotropin receptor antibody (anti-TSHr) in adult and older individuals among Thai. Compared to adults, the RIs in the older showed higher TSH levels, lower FT3/TT3 levels, and comparable levels of FT4/TT4. Lastly, there were no statistically significant differences among RIs of TSH and thyroid hormones between subjects with discrepant iodine status.

Conflict of interest

Abbott Laboratories provided most of the reagents for the thyroid hormone analysis in this study, except for the anti-TSHr and urine iodine concentration tests. However, the laboratory company was not involved in any study methods, protocols, analyses, or manuscript writing.

Acknowledgment

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Supplementary Table 1.

Parameter	Instruments	Method	Unit	Measuring Interval	Total imprecision		LOD	LOQ
					%CV (Conc)	%CV (Conc)		
TSH	Alinity I (Abbott)	CMIA	mIU/L	0.0083 - 100	1.7% (0.642)	2.7% (24.739)	0.0036	0.0083
Free T3	Alinity I (Abbott)	CMIA	pg/mL	1.5 - 20	4.7% (2.02)	2.1% (10.01)	0.95	1.25
Free T4	Alinity I (Abbott)	CMIA	ng/dL	0.42 – 5.00	2.7% (0.93)	3.8% (2.99)	0.28	0.42
Total T3	Alinity I (Abbott)	CMIA	ng/dL	40.0 – 600.0	3.3% (72.64)	2.9% (257.42)	5.0	30.0
Total T4	Alinity I (Abbott)	CMIA	µg/dL	3.00 – 24.00	1.2% (6.93)	2.6% (13.89)	0.55	2.17
Anti-TG	Alinity I (Abbott)	CMIA	IU/mL	3.00 – 1000.00	3.0% (11.8)	4.4% (45.87)	0.11	0.33
Anti-TPO	Alinity I (Abbott)	CMIA	IU/mL	3.00 – 1000.00	2.2% (15.40)	2.0% (54.57)	0.03	0.21
Anti-TSHr	Cobas e601 (Roche)	ECLIA	IU/L	0.8 – 40.0	4.0% (4.42)	2.2% (18.1)	0.80	1.10

LOD: Limit of detection, LOQ: Limit of quantification, CMIA: Chemiluminescent microparticle immunoassay, ECLIA: Electrochemiluminescence immunoassay method

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Research Article

Beyond Heart Failure: role of NT-Pro-BNP in Diabetes mellitus Patients with Preserved Ejection Fraction

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Keywords

Glycemic Control and Diabetes, HDL-C, Glycosylated Haemoglobin (HbA1C), Left Ventricle Ejection Fraction, Diabetes Mellitus Type 2, NT-Pro-BNP

Abstract

Background: Despite advancements in medical care, including coronary interventions and medications, cardiovascular-related mortality and morbidity remain disproportionately high among patients with diabetes mellitus. A significant factor contributing to this issue is the presence of asymptomatic macrovascular and microvascular angiopathies in many diabetic patients. These vascular complications are often detected at later stages, resulting in the failure of treatment strategies to effectively prevent the progression of heart failure and mitigate worsening conditions. Given this background, our research aims to explore the potential of the biochemical marker NT-pro-BNP (N-terminal pro b-type natriuretic peptide) in the early detection of left ventricular diastolic dysfunction in diabetic patients who maintain a preserved ejection fraction. Left ventricular diastolic dysfunction is a condition where the left ventricle has difficulty relaxing and filling with blood, which can precede the development of heart failure. Identifying this dysfunction early could be crucial in preventing major adverse cardiac events (MACE) such as heart attacks, stroke, and cardiovascular-related death.The focus of our study is to determine whether NT-pro-BNP, which is typically elevated in heart failure, can serve as an early marker for diastolic dysfunction in this specific patient population. By identifying diabetic patients at risk earlier, interventions could be tailored more effectively, potentially improving outcomes and reducing the incidence of severe cardiovascular events.

Study population and methods: This study was conducted at a tertiary care medical care hospital in Madurai, Tamil Nadu, India, with a sample population of 500 patients who had preserved ejection fraction. The participants were divided into two groups: 169 diabetic patients and 150 non-diabetic patients. As part of the baseline assessment, routine clinical chemistry analysis and 2D echocardiograms were performed. Additionally, the biomarker NT-pro-BNP, which is associated with heart failure, was measured using the electrochemiluminescence method.

Result: Among the diabetes and non-diabetes groups, the biomarker NT-pro-BNP were significantly different and the serum concentration of NT-pro-BNP was found to be higher in poor glycemic control type 2 diabetes mellitus patients. Serum NT-pro-BNP screening and 2D echocardiogram showed the best predictor of left ventricular diastolic

dysfunction and hospital stay due to major adverse cardiac events in type 2 diabetes mellitus patients.

Conclusion: Our study highlights the clinical significance of NT-pro-BNP among (left ventricular diastolic dysfunction) type 2 diabetes mellitus with preserved ejection fraction > 60 %.

Introduction

Diabetes is the second most common noncommunicable disease burden even in developing countries like India with most of the population falling under moderate workers [1]. Silent myocardial infarction and asymptomatic cardiac remodelling including cardiac myopathies are quite common in diabetes. Because of the availability of advanced cardiac interventions, the mortality associated with heart failure and cardiovascular related complications requiring hospitalization has been increased over the past decade. This has paved the way for emergence of the newer heart failure biomarkers profile over the years to help the treating clinicians in diagnosis as well as prognosis [2]. Since the new biomarkers like NT- pro BNP are not available in all the clinical setup, these are not quite commonly used in the clinical practice. So the diagnosis and monitoring of the prognosis of heart failure is still based on the clinical signs and symptoms, followed by ECHO [2,3].

The heart failure symptoms like breathlessness, pedal edema starts developing after the compensation of cardiac output in a significant level i.e., LVEF<40% that is called as reduced ejection fraction heart failure [4]. Since most of the myocardial infarction in diabetes mellitus will be presenting as silent myocardial infarction, this goes unnoticed. All the cardiac related biomarker profile are expensive to run in a long way and still have a query to pick up the heart failure in early stage before the compromise of left ventricular ejection fraction (LVEF). This results in the search of any one biomarker that might be able to pick up the chances of an individual who will land up in future heart failure especially in diabetes mellitus patients [5]. Have this question in mind, we formulated this study to assess the clinical significance of the NT-pro-BNP analysis in preserved ejection fraction in diabetes patients

Materials and Methodology

After obtaining institutional ethical committee clearance no: VMCIEC/004/2023 in accordance with the Declaration of Helsinki, we retrospectively analysed patients admitted to a tertiary care hospital from January 2022 to July 2024. During this period, 500 patients, both inpatients and outpatients, underwent NT-pro-BNP testing along with echocardiograms. A strict exclusion criterion was applied, excluding patients with chronic kidney disease, anaemia, sepsis, or a reduced ejection fraction of less than 50%. From the total sample of 500 patients, we excluded 181 cases due to conditions such as COPD, CKD, anaemia, structural heart disease, pericarditis, or an ejection fraction less than 60%.

The remaining patients were categorized based on their glycaemic status into two groups: Group 1 (n=169, 61% male and 39% female), which included non-diabetic patients out of which 21 cases were with hypertension and Group 2 (n=150, 63% male and 37 % female), which included diabetic patients out of which 19 cases were with hypertension. The data for this study were sourced from the medical records of Medical College Hospital and Research Institute, Madurai, following approval from the institutional ethical committee.

NT-pro-BNP was analysed using the Cobas e411 by the electro- chemiluminescence method, while all the other routine biochemical parameter were analysed using the TOSHIBA 120 FR fully automated analyser. Echocardiograms was done for comprehensive assessment.

Measurement of NT-pro BNP

NT-pro-BNP levels were measured using the Cobas e411 Elecsys NT-pro-BNP immunoassay (Roche Diagnostics) [1]. The range of measurements of the same was 5 to 35 000 pg/mL with limit of detection 5 pg/mL.

Statistical analysis

Out of 319 total sample population, 169 diabetic patients and 150 non diabetic patient data were statistically tested. In this, there is a significant difference in the NT-pro-BNP level in the diabetic patients compared to non-diabetic patients. There is a positive correlation between the HbA1C level and NT-pro-BNP level in diabetic patients with preserved ejection fraction LVEF > 60.

Table 1: Basic and Biochemical characteristics of study subject.

Variables	Non-diabetes (EF>60%) In median with interquartile range (n=169)	Diabetes (EF>60%) In median with interquartile range (n=150)	p-Value
Age (in years)	59.00 (48.00 - 67.00)	59.00 (51.00 - 67.00)	0.55
FBS (in mg/dl)	88.00 (85.00 - 136.00)	155.00 (133.75 - 200.25)	**0.001**
PPBS (in mg/dl)	116.00 (89.00 - 150.00)	171.00 (125.00 - 250.00)	**0.001**
HbA1C (%)	5.6 (5.3-5.9)	9.8 (8.5-11.5)	**0.001**
NT-pro-BNP (in pg/mL)	131.30 (74.85 - 361.50)	4567.00 (2008.00 - 8925.00)	**0.001**
Cholesterol (in mg/dl)	182.50 (154.00 - 219.00)	177.00 (143.00 - 196.00)	**0.026**
Triglyceride (in mg/dl)	131.00 (89.50 - 179.25)	146.50 (111.25 - 198.00)	0.051
HDL (in mg/dl)	48.00 (43.00 - 52.00)	37.00 (33.00 - 39.00)	**0.001**
LDL (in mg/dl)	118.00 (96.00 - 152.75)	111.50 (86.00 - 135.00)	0.095
Serum creatinine (in mg/dl)	0.400 (0.300 - 0.500)	0.400 (0.300 - 0.575)	0.782
Blood urea (in mg/dl)	18.00 (14.00 - 25.00)	19.50 (15.00 - 24.75)	0.082
Systolic BP (in mm/Hg)	125.00 (115.00 - 135.00)	125.00 (115.00 - 135.00)	0.246
Diastolic BP (in mm/Hg)	80.00 (80.00 - 85.00)	80.00 (80.00 - 90.00)	0.5

The table compares median values with interquartile ranges for age, blood pressure, fasting and postprandial blood sugar levels, and HbA1C between non-diabetes (n=169) and diabetes (n=150) individuals with ejection fraction >60%. The diabetic group shows higher NT pro BNP levels than the non-diabetic group.

Table 2: Correlation of NT-pro-BNP with other parameters among the non-diabetic group and diabetic group.

Variables	Non-Diabetic group		Diabetic group	
	NT-pro-BNP		NT-pro-BNP	
	r value	p value	r value	p value
RBS	0.118	0.353	0.128	0.281
HbA1C	**.961****	**0.000**	**.863****	**0.000**
FBS	-0.016	0.853	-0.056	0.664
PPBS	**0.167**	**0.065**	**.489****	**0.000**
Cholesterol	-0.127	0.098	0.008	0.921
TGL	0.031	0.691	0.031	0.704
HDL	**-.924****	**0.000**	**-.715****	**0.000**
LDL	0.008	0.921	0.038	0.644
Age	**-.209****	**0.006**	**.194***	**0.017**

The table shows the spearman correlation (r value) and significance (p value) of NT-pro-BNP levels with glycemic status and lipid profiles in patients of non-diabetic group and diabetic group. Significant correlations $p < 0.05$ and $p < 0.01$ are indicated with an asterisk (*) and (**) respectively. NT-pro-BNP is significantly correlated with HbA1C, HDL and age in both groups in EF > 60.

Table 3: Regression analysis between non- diabetes and diabetes mellitus group with EF > 60

Group	Diabetes mellitus		Non-Diabetes mellitus	
Variables	**Standardized Beta Coefficients**	**p-Value**	**Standardized Beta Coefficients**	**p-Value**
HbA1C	**0.869**	**0.000**	**0.011**	**0.918**
Cholesterol	0.211	0.086	0.011	0.911
Triglyceride	-0.079	0.145	0.032	0.678
HDL	**0.121**	**0.021**	**-0.485**	**0.000**
LDL	-0.170	0.154	-0.089	0.313
Age	**0.119**	**0.012**	**0.163**	**0.029**
Dependent variable: NT-pro-BNP				

Table 4: Association between variables in good glycemic and poor glycemic control within diabetes mellitus group.

Variables	Diabetic group with good glycemic control with HbA1C cut off < 7 %		Diabetic group with poor glycemic control with HbA1C cut off > 7 %	
	NT-pro-BNP		NT-pro-BNP	
	r value	**p value**	**r value**	**p value**
RBS	-0.327	0.429	0.099	0.431
HbA1C	**.963****	**0.000**	**.855****	**0.000**
FBS	-0.374	0.362	-0.146	0.294
PPBS	**0.314**	**0.377**	**.504****	**0.000**
TGL	-0.174	0.477	0.006	0.944
HDL	-.717**	0.001	-.646	0.090
LDL	0.048	0.854	0.024	0.786
Age	.575*	0.010	0.075	0.393

Discussion

NT-pro-BNP as a Biomarker of Heart Failure

The role of NT-pro-BNP as a biomarker of heart failure severity is supported by studies such as those by Maisel et al. [1] and Januzzi Jr et al. [2]. These studies highlight the rapid measurement and diagnostic utility of NT-pro-BNP in identifying heart failure.

In our study, a significant positive correlation was found between HbA1C and NT-pro-BNP levels in both groups. The strength of this correlation was higher in the diabetic group (r = 0.863, p = 0.000) than in the non-diabetic group (r = 0.961, p = 0.000), suggesting that chronic hyperglycaemia, as reflected by elevated HbA1C advanced glycated end product, has a direct impact on NT-pro-BNP levels. The relationship between HbA1C and NT-pro-BNP suggests that poor glycemic control exacerbates cardiac stress, even in patients with preserved ejection fraction. These findings align with previous mentioned studies that report increased NT-pro-BNP levels in diabetic patients, likely due to the combined effects of hyperglycemia, insulin resistance, and other metabolic factors that contribute to myocardial dysfunction. It's essential to note potential molecular overlaps with insulin and cyclic guanosine monophosphate (cGMP) pathways. In diabetic cardiomyopathy, insulin resistance disrupts normal insulin signalling pathways, leading to impaired glucose uptake and utilization [3]. This disruption may contribute to cardiac dysfunction and remodelling, enhancing the release of NT-pro-BNP as a compensatory mechanism [4].

Association with Diabetic Cardiomyopathy

Studies by Jia et al. [5] and Marwick et al. [6] provide evidence for the association between NT-pro-BNP elevation and diabetic cardiomyopathy. These authors emphasize the distinct nature of cardiac dysfunction in diabetes, independent of other cardiovascular risk factors.

Molecularly, chronic hyperglycemia and insulin resistance activate pathways such as the renin-angiotensin system (RAS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, leading to increased oxidative stress [7]. This oxidative stress contributing to cardiac remodelling and dysfunction characteristic of diabetic cardiomyopathy, possibly augmenting NT-pro-BNP release [8] supported our findings that in the

diabetic group, NT-pro-BNP also correlated significantly with postprandial blood sugar (PPBS, r = 0.489, p = 0.000) and negatively with HDL (r = -0.715, p = 0.000). These correlations suggest that both glycemic variability and dyslipidemia contribute to cardiac strain in diabetic patients. The negative correlation between HDL and NT-pro-BNP in both groups indicates that lower HDL levels, a marker of poor lipid control, are associated with higher NT-pro-BNP, reinforcing the role of dyslipidemia in cardiovascular risk.

Subclinical Heart Dysfunction in Diabetes

This study highlights a significant difference in NT-pro-BNP levels between diabetic and non-diabetic patients, particularly in individuals with preserved ejection fraction (LVEF > 60%). Diabetic patients exhibited markedly higher NT-pro-BNP levels compared to non-diabetics, suggesting that diabetes plays a critical role in elevated NT-pro-BNP levels, which may indicate subclinical cardiac stress even in the absence of reduced ejection fraction. Fang et al. [9] and Seferović and Paulus et al. [10] discuss the presence of subclinical cardiac dysfunction in diabetes, even in the absence of overt heart failure symptoms. Elevated NT-pro-BNP levels may serve as an early indicator of such dysfunction.

Molecularly, dysregulated insulin signalling and increased oxidative stress contribute to mitochondrial dysfunction and impaired calcium handling in cardiomyocytes [11]. These molecular alterations may precede clinical manifestations of heart failure, highlighting the importance of NT-pro-BNP as a marker of subclinical dysfunction [12].

Implications for Risk Stratification

In our study, the subgroup analysis of diabetic patients with good and poor glycemic control, the correlation between HbA1C and NT-pro-BNP remained significant in both groups (r = 0.963, p = 0.000 in good control, and r = 0.855, p = 0.000 in poor control). However, the correlation with PPBS was only significant in the poor glycemic control group (r = 0.504, p = 0.000), suggesting that glycemic fluctuations may contribute more to cardiac stress in patients with poor long-term glycemic control.

Huelsmann et al [9] and Anand et al. [10] emphasize the potential of NT-pro-BNP levels for risk stratification in diabetic patients. Elevated levels may indicate a higher risk of cardiovascular complications. At the molecular level, dyslipidemia and chronic hyperglycemia activate pathways such as PPAR alpha and CD36, leading to increased fatty acid uptake and oxidative stress in cardiomyocytes [7]. These processes may exacerbate cardiac dysfunction and increase NT-pro-BNP release, underscoring its utility in risk stratification [9].

Additionally in this study, we also found that age was another significant predictor in both groups, though its influence was more pronounced in the non-diabetic group (β = 0.163, p = 0.029). This suggests that while age plays a role in cardiac stress across the board, diabetes has a more substantial impact on NT-pro-BNP levels, independent of age.

Potential Mechanisms

In this study, Regression analysis demonstrated that HbA1C was the most significant predictor of NT-pro-BNP levels in diabetic patients (β = 0.869, p < 0.001), underscoring the importance of glycemic control in managing cardiac stress.

Marwick TH et al. [4] and Bugger and Abel [8] elucidate the molecular mechanisms underlying diabetic cardiomyopathy. They describe how insulin resistance, oxidative stress, and mitochondrial dysfunction contribute to cardiac remodelling and dysfunction.

Specifically, dysregulated insulin signalling and increased fatty acid oxidation lead to mitochondrial dysfunction and impaired ATP production. This disrupts normal cardiac function and may contribute to NT-pro-BNP release as a compensatory response.

Clinical Relevance and Treatment Implications

Overall, the findings in our study suggested that NT-pro-BNP levels are strongly influenced by glycemic control and lipid profiles in diabetic patients and that targeting both glycemia and dyslipidemia may be crucial for reducing cardiovascular risk in this population. The strong correlation between HbA1C and NT-pro-BNP highlights the importance of maintaining tight glycemic control to prevent cardiac dysfunction, even in patients with preserved ejection fraction. These results emphasize the need for comprehensive management strategies addressing both metabolic and cardiovascular health in diabetic patients. The same finding was quoted by McMurray et al. [11] and Yancy et al. [12] discussed the clinical implications of elevated NT-pro-BNP levels in diabetic patients. They highlight the importance of aggressive risk factor management and lifestyle modifications in mitigating cardiovascular risk.

Molecularly, pharmacological interventions targeting pathways such as the RAS and oxidative stress may attenuate cardiac dysfunction and reduce NT-pro-BNP release. These interventions could complement traditional management strategies in diabetic patients.

Conclusion

NT-pro-BNP is a better marker in preserved ejection fraction with diastolic dysfunction, concurrent and hence evaluation with NT-pro-BNP along with ECHO evaluation will help in evaluating the diastolic dysfunction. During diastole i.e., ventricular relaxation, coronary perfusion to endocardium as well as myocardium more, in case of diastolic dysfunction where the relaxation doesn't effectively occur. So elevated levels of NT-pro-BNP, a cardiac neurohormone in diastolic dysfunction with preserved ejection > 60% will help us to find out myocardial

ischemic risk in early, especially in diabetes mellitus where the silent myocardial infarction, heart failure and sudden cardiac death occurring commonly.

Conflict of interest

There is no conflict of interest.

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Research Article

Enhancing Ergonomics Practices Using Plan, Do, Check, Act cycle in Clinical Laboratories

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Keywords

ergonomics, clinical laboratories, musculoskeletal injuries

Abstract

Background: Musculoskeletal injuries may be directly caused by workplace practices such as poor posture, high frequency static muscle work, repetitive motion and forced exertion. Healthcare professionals are more likely to be exposed to common risk factors related to the nature of their work requiring repetitive tasks, insufficient breaks and long stressful working hours. They are predisposed to musculoskeletal injuries.

Methods: The plan, do, check, act cycle (PDCA) was conducted at the Aga Khan University Hospital, Karachi. The team was assigned with the roles and responsibilities of disseminating accurate information and supervision of relevant ergonomic activities. The goals were enacted upon using videos, songs, and posters as a means of raising awareness of ergonomic practices. Pre and post activity assessment related to knowledge and practice of ergonomics were evaluated.

Results: After conducting micro-lectures and sharing informative videos, flyers and recordings of all micro-lectures via a WhatsApp group, awareness about posture-related musculoskeletal disorders increased from 85% (n=35) pre-audit to 100% post-audit. Knowledge of the 20-20-20 rule was initially 49% (n=33) before the audit and reached 100% (n=41) after awareness and training. Understanding of exercises to strengthen the back, shoulders, and hands improved from 80% (n=33) pre-audit to 100% post-audit. Following these awareness activities, which included multimedia photos and videos, 88% of participants adopted ergonomic practices, up from the previous 34%.

Conclusion: The implementation of a structured training program using the PDCA cycle will significantly enhance ergonomic practices. By integrating multimedia tools such as videos, and posters, a single training intervention led to a marked improvement in participants' ergonomic practices. To sustain and further enhance this progress, ongoing education at regular intervals is essential, as it is likely to continue improving ergonomic knowledge and practices, thereby reducing the incidence of musculoskeletal disorders.

Introduction

Risk management in a clinical laboratory evolves over time as all hazards need to be identified, evaluated, and controlled to minimize risk to each group involved in laboratory operation. More apparent hazards that have been thoroughly studied in clinical laboratories include chemical hazards, biological hazards, toxins etc. while others may be more subtle to notice [1]. Health hazards besides biological hazards, fire hazards, toxins and chemicals in laboratories staff are physical hazards which are direct contributors to musculoskeletal injuries such as poor posture, high frequency static muscle work, repetitive motion and forced exertion imploring a need-based evaluation of each clinical laboratory every term [2].

Musculoskeletal disorders (MSDs) were reported by The Global Burden Disease Study as the second most common cause for disability with a 42.9% increase in the first decade of 2000's [3]. These can be identified from poor work-related ergonomic practices and lack of awareness about them [4]. Lower back pain followed by upper back pain and wrist/hand pain are the most common disorders identified in a cross-sectional study on the magnitude of work-related musculoskeletal disorders and ergonomic risk practices among medical laboratories. [5,6]. The nature of certain tasks such as microscopy may cause more hazard than others indicating stronger need for ergonomic evaluation and prevention [7]. High prevalence of MSDs up to 88% exist in certain workplaces where standing jobs are correlated with significant neck and upper back pain while sitting jobs seem to play a protective role for lower back pain.

A survey to estimate the prevalence of MSDs among healthcare professionals in India identified 73% (95%CI: 67.9-78.1) of participants with MSD in the last 12 months of conducting the survey. This includes a significantly high number of healthcare professionals as compared to the general population with obese females having even higher rates. Overall repeated tasks, >48 hours per week of work, insufficient work breaks and working in the same position for longer hours were the highest risk factors [8]. These risk factors define the nature of work. All healthcare employees, especially those in laboratory medicine, are exposed to raising concerns for the lack of mandatory ergonomics training [9]. The repetitive motions, awkward postures, and lifting of heavy objects commonly found in laboratory work can result in chronic pain, discomfort, and even long-term disabilities.

Many regulatory bodies, such as the Occupational Safety and Health Administration (OSHA) in the United States, have established guidelines and regulations concerning workplace ergonomics. Compliance with these regulations is not only a legal requirement but also essential for maintaining the integrity of laboratory operations [10,11,12].

Bone & Mineral diseases research group (https://www.aku.edu/mcpk/research/Pages/bone-mineral-diseases.aspx) at Aga Khan University is committed to improve bone health in Pakistan to attain SDG Goal 3, for promoting and ensuring good health and well-being for all ages in musculoskeletal health.

Ergonomics audits serve as a proactive measure for continuous improvement within the laboratory. By regularly assessing the ergonomic environment, laboratories can identify emerging risks and implement preventive measures promptly. As technologies and workflows evolve, ongoing audits ensure that ergonomic considerations remain integrated into the fabric of laboratory operations, adapting to changing needs and requirements over time. Locally applied methods including checklists, questionnaires and repertory grids have been shown to help understand the effects of ergonomics training within workplaces [13]. A clinical audit was conducted to assess current knowledge of ergonomics and improve ergonomic practices among the laboratory personnel through multimedia approach where educational material was shared after the pre assessment including videos, songs and posters. Assessment of understanding was also evaluated to see if multimedia approach was beneficial overall without the previously studied use of a professional trainer in most settings.

Methods

A Clinical Audit was conducted at the Section of Chemical Pathology, Aga Khan University Hospital Clinical Laboratories, Karachi Pakistan, between October- December based on plan, do, check, act (PDCA) cycle [14]. The audit was focused on assessing ergonomic practices for medical laboratory technologists. Objectives were focused on workstation posture, pipette techniques, accurate use of equipment without strain, and sufficient breaks between repetitive procedures. A team was assigned with roles and responsibilities including planning & supervision of relevant ergonomic activities. The team included medical technologists (quality and safety coordinators), and pathologists.

A questionnaire was designed for pre, and post activity assessment related to knowledge and practice of ergonomics with only binary responses. Knowledge and application of ergonomics and MSDs was assessed. Specific questions were about micro breaks during computer work, knowledge of exercises to strengthen back, shoulders or hands, sufficient temperature control at workplace and the 20-20-20 rule [15].

After pre audit eight micro lectures were conducted weekly, each no longer than 5 to 10 minutes, placed in the regular workday. This ensured that attendees would be encouraged to attend these attentively. Physical corrective exercises were instructed and recorded. Recordings were made available to anyone on leave during the workday. Relevant physical activities explored in the lectures included appropriate posture while using computer and/or telephone, exercises of shoulders, legs, neck, and arm in between working, and correct posture when using

micro-pipettes. By effectively incorporating multimedia into the teaching process the audit team was able to create a more engaging, effective learning environment.

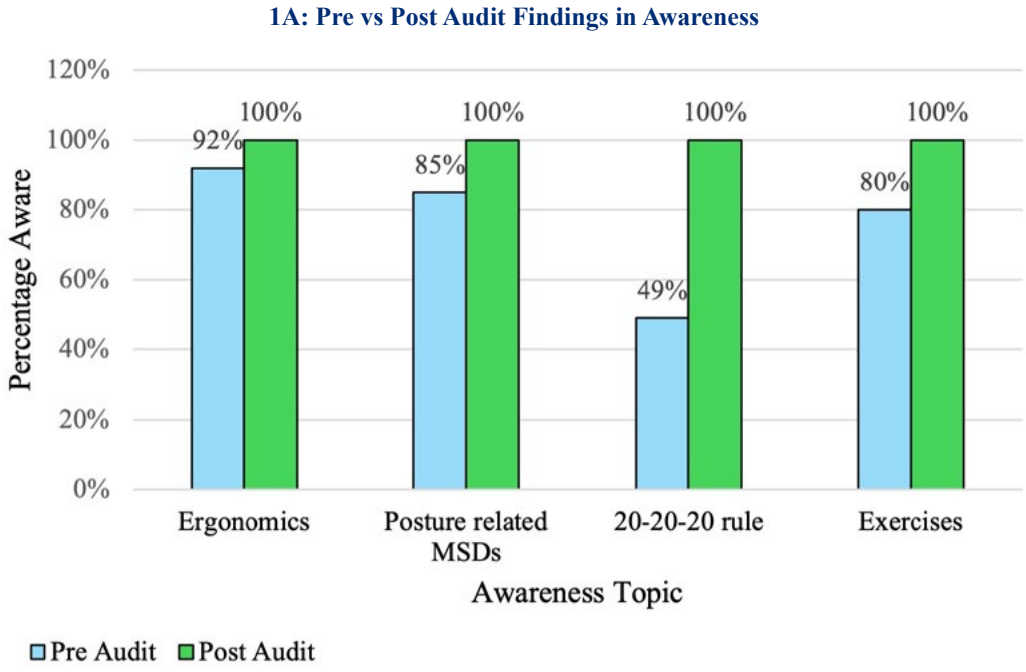
An e-poster competition was organized to elicit effective understanding and active participation of attendees. Evaluation of the program with a post assessment test was taken on the same binary and Likert Scale. Data was analyzed on SPSS and extracted on MS excel sheet. To increase interactive learning participants had an e-poster competition at the end with the best poster.

Results

The participants (n=41) included faculty pathologists (n=4), technologists (n=36), associate technologist (n=1). The mean age of the participants was 35 ± 5.93 years.

Awareness about ergonomics pre audit was 92% (n=38) and post audit 100%. Awareness about posture related MSDs was 85% (n=35) pre audit and 100% post audit. Knowledge of the 20-20-20 rule was only 49% (n=33) preaudit and 100% (n=41) post audit. Knowledge of exercises to strengthen back, shoulder and hands increased from 80% (n=33) pre audit to 100%. After creating awareness through multimedia photos and videos, 88% followed ergonomics practice which was previously only 34% people, 88% were following the 20-20-20 rule which pre audit only 49% followed, 83% people were taking micro breaks. 73% were doing back, shoulder and hand exercises from a pre audit 54% and, 90% thought the workplace was adequate. Pre audit 36% of people had poor posture with was reduced post intervention to 29%. Not many technologists were exercising to strengthen their back (43%), shoulder (73%) or hand (54%) (Figure 1 A and Figure 1 B).

Figure 1: Comparison of Pre vs Post Audit Findings Amongst Laboratory Personnel in Awareness & Practice (n=41).



1B: Pre vs Post Audit Findings in Practice

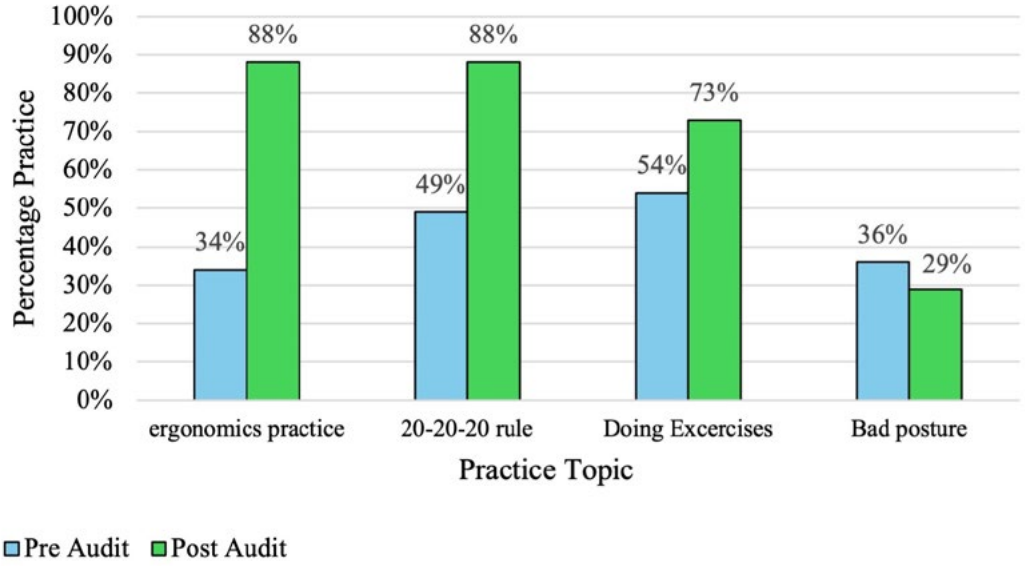


Table 1 shows the findings of the feedback provided by the participants. Eighty per cent reported gaining good information and learning. Ninety-eight per cent recommended conducting similar activities in future. Majority participants 'understood' ergonomics and principles taught with 41% 'well understood' meaning and principles or ergonomics, 27% 'well understood' common injuries, only 24% 'well understood' proper vs improper practices and, 24% 'well understood' injury causing areas and postures.

Table 1: Post Audit Feedback Survey Findings from Participants on Ergonomics Awareness and Practices.

	Very little understood	**Little understood**	**Understood**	**Well understood**
The meaning & implications of ergonomics	0 (0%)	4 (10%)	20 (49%)	17 (41%)
Common injuries cause by improper ergonomics	2 (5%)	5 (12%)	23 (56%)	11 (27%)
Proper ergonomics vs improper ergonomics	1 (2%)	8 (19%)	22 (54%)	10 (24%)
Common area / postures that can cause injuries	1 (2%)	6 (15%)	24 (58%)	10 (24%)
	Poor	**Fair**	**Good**	**Excellent**
How would you rate your current knowledge and use of proper ergonomics practices?	0	6 (15%)	33 (80%)	2 (5%)
Rate the facilitator / micro-lecture presenter?	0	1 (2%)	31 (76%)	9 (22%)
How much information did you learn during this project activity?	0	4 (10%)	33 (80%)	4 (10%)

Discussion

Our study in a group with highly educated workers from a background of allied medicine demonstrated sufficient knowledge of ergonomics practices such as correct posture and muscular excercises at baseline. Awareness sessions further improved overall compliance. Ergonomics when applied at the workplace are proven to improve quality of work life and decrease compensation costs up to 80%. Ergonomic knowledge and implementation can prove to be useful not only for the workforce but also to the employer as workforce efficiency increases [16]. Prioritizing ergonomics helps achieve up to 90% improvement overall and mitigating risks, thus ensuring the health and safety of laboratory personnel. Scheduled breaks with better strategies, for instance the 20-20-20 rule for prolonged screen time exposure show short term improvement in dry eye symptoms [17,18]. Awareness on the topic was perceived positively almost doubling the use of the 20-20-20 technique in our research population. Our findings are consistent with prior data that individuals with ergonomics training have significantly lower pain scores [19].

Electromyography based assessment demonstrated that the type of exercise adopted does not make a significant difference. Easier to follow low intensity desk stretches or strength building can be as impactful as high intensity workouts or training [20]. Consistency in ergonomic practices tends to make a bigger difference overall. Cyclic training programs rather than costly time consuming externally sourced sessions need to be compared in trials. Professional training should be compared to the novice idea of utilizing videos, pictures and posters similar to the multimedia approach opted at our institute [21]. Ergonomics training is more effective for groups that may have some or no idea about ergonomics like trainee medical technologists. Perception of risk and hazards is better in older employees which may be a consequence of developing more MSDs than younger employees. Including ergonomics concepts earlier in the training programs of medical technologists is expected to improve perception of workplace risks in all employees [22]. Our data showed up to 100% of participants recommend ergonomic awareness activities to be carried out at the workplace despite starting at a higher baseline.

The limitation of our study is the small sample size in a private care facility with better environmental control compared to public or government set ups where resource limitation and high patient burden can significantly impair any chance of scheduled breaks or improved practices. Trials may be needed to demonstrate both short- and long-term effectiveness of using multimedia and short lectures instead of professional support for ergonomics eventually. In the future it may be important to monitor and evaluate the effectiveness of implemented interventions through follow-up assessments, employee feedback, and incident reporting. Institutes should continuously review and update the ergonomics program based on new information, changes in technology, and evolving organizational needs. Efforts should be directed to establish mechanisms for ongoing communication and feedback between laboratory management, safety personnel, and frontline staff to sustain a culture of ergonomic awareness and continuous improvement.

Conclusion

Laboratory personnel have a good understanding of the importance of ergonomics and relevant practices, however a significant increase in knowledge and daily practices occurs with awareness about exact techniques. Some principles such as the 20-20-20 rule and incorrect posture may be less valued than exercises. Reinforcement is a good way to reintroduce them into daily practice. A single training exercise can improve ergonomic practices by 100%. Continuous education at specified intervals is expected to improve ergonomics knowledge and practices across the board and decrease musculoskeletal disorders. Implementing the identified ergonomic improvements according to the action plan in the medical technologist training program and making it part of continuous education program, ensuring clear communication, training, and support for affected personnel may be a game changer in the overall dynamics. Investigating professional vs multimedia approach towards ergonomics training may pave the way for future training.

Disclosure

The author(s) declare that (s)he has no relevant or material financial interests that relate to the research described in this paper.

Ethical Approval

Our study involved human subjects and is following the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki.

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Research Article

Comprehensive Analysis of Renal Stones Using FTIR Spectroscopy in a Referral Laboratory in Nepal

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Keywords

Renal stones, FTIR spectroscopy, calcium oxalate monohydrate, calcium oxalate dihydrate, Nepal

Abstract

Background: Renal stone disease, a prevalent urological disorder, significantly impacts public health in Nepal. Analyzing the composition of renal stones is crucial for understanding their etiology and guiding treatment and prevention strategies. FTIR spectroscopy is a reliable technique for identifying the chemical composition of renal stones. This study aims to analyze the composition of renal stones using FTIR spectroscopy in a referral laboratory in Nepal.

Methods: A total of 300 renal stones from patients were analyzed. The stones were collected, cleaned, and powdered before being subjected to the Thermo Fisher Scientific FTIR Spectrometer. The spectra obtained were compared to the reference spectra to determine the composition of the stones.

Results: The analysis revealed that calcium oxalate monohydrate in 41% and calcium oxalate dihydrate in 29 % were the commonest types. Other less common compositions included uric acid, struvite, and cystine stones.

Conclusion: FTIR spectroscopy effectively identified the composition of renal stones in the studied population. The predominance of calcium oxalate stone highlights the need for targeted prevention and treatment strategies in Nepal.

Introduction

Renal stone is a common disease that affects 5–13% of the population worldwide [1]. In Nepal, the prevalence of renal stones has been increasing and it has caused higher surgical intervention rates. Loss of renal function and urosepsis are potential complication of untreated renal stones. Endourological procedures such as retrograde intrarenal surgery and percutaneous nehrolithotomy have been used recently in the management of renal stone in Nepal [2,3]. However, using Fourier Transform Infrared (FTIR) spectroscopy for the analysis of renal stone in Nepal is uncommon. Prevention of stone recurrence is important to reduce the morbidity and it also leads to reduced costs as fewer interventions are required.

FTIR spectroscopy has emerged as a powerful tool for the detailed analysis of renal stones [4,5]. This method offers precise identification of the stone's chemical composition, allowing for accurate differentiation between various types of stones, such as calcium oxalate monohydrate, calcium oxalate dihydrate, uric acid, and others. Understanding the precise chemical composition of renal stones is crucial for developing effective prevention and treatment strategies. Furthermore, the goal of metabolic evaluation is to prevent recurrent stone formation in high risk stone producers and to prevent extra renal complications in associated systemic disorders.

There are other methods of stone analysis such as wet chemical analysis which can only identify the presence of individual ions and radicals without differentiating a specific compound in stone mixture. Similarly, thermogravimetry requires large amount of material for optimal resolution. Optical polarizing microscopy uses the principle of interaction of polarized light with crystals of stones and it is cumbersome. X- ray diffraction uses monochromatic X-rays for identifying the constituents of stone based on unique diffraction pattern produced by a crystalline material. FTIR spectroscopy determine exact quantitation of stone constituents at moderate cost and is quick in identifying organic components or non crystalline substances, e.g. purine, proteins or fat and drug metabolites [6]. Mixed stones are common, and FTIR spectroscopy can be used to determine the relative percentage content of the various components with high accuracy even with very small amount of sample material.

Approximately 40% of the global population residing in high-risk zones, especially in Asian countries like Saudi Arabia and India, was reported to be affected by urolithiasis in 2000. This prevalence is projected to increase to 50% by 2050 [7]. The European Association of Urology guidelines on Urolithiasis (2013) emphasize the limitations of traditional chemical analysis and strongly recommend FTIR spectroscopy as the preferred method for urinary stone composition analysis [8]. In south Asian countries, FTIR has been widely adopted since the early 2000s for renal stone analysis, with documented use in countries such as India [9], Pakistan [10], China [11], and Sri Lanka [12].

In Nepal, however, the application of FTIR for renal stone analysis is still in its nascent stage, with the first reports emerging only in 2022 [13]. This limited adoption contrasts with the high and rising prevalence of renal stones in Nepal, yet data on the epidemiology and specific composition of kidney stones remain insufficient [14]. The integration of FTIR, utilizing computerized spectrophotometers and comprehensive reference libraries, allows for precise quantitative analysis of stone composition. Expanding FTIR capabilities to more urolithiasis centers across Nepal would enhance diagnostic accuracy and deepen the understanding of the pathophysiology of kidney stones, a crucial step given the increasing trend of renal stone cases in the region. Any additional data gathered through such approaches will be valuable in addressing this significant public health concern.

Our study aims to fill this gap by presenting the results of renal stone analyses conducted using FTIR spectroscopy in a referral laboratory in Nepal. By providing a comprehensive overview of the types and frequencies of renal stones in this population, we hope to contribute valuable insights that can inform better clinical practices and public health policies.

Method

This study was carried out over a period of 28 months starting from January 2021 to May 2023 at Samyak Diagnostic Pvt Ltd thus including all 300 renal stone samples collected in this period. In accordance with institutional and regulatory guidelines, this study utilized secondary laboratory data and does not involve direct interaction with human subjects or the collection of new, identifiable information. This study is in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki.

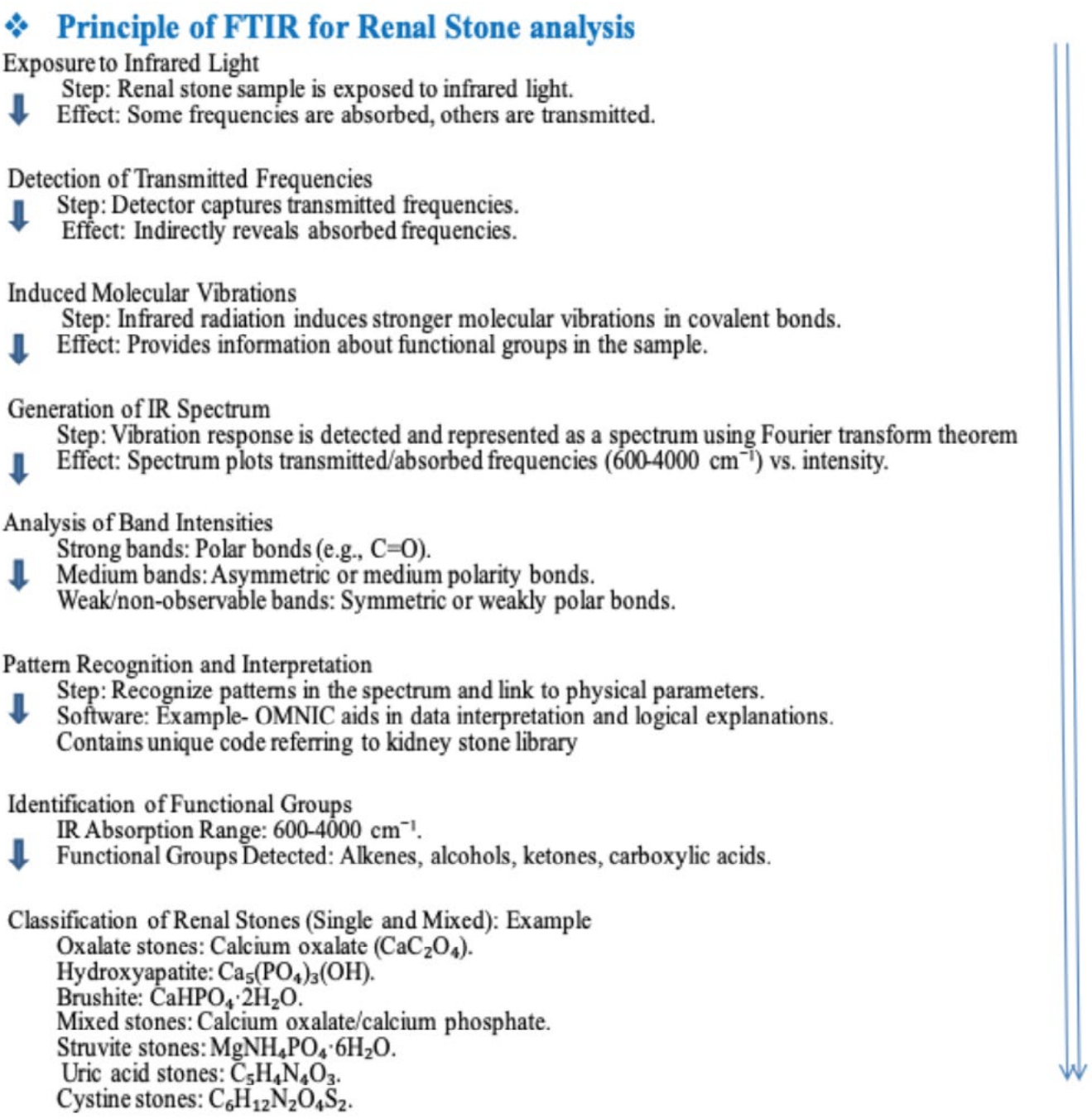
Stone Analysis

Renal stones were analyzed using Thermo Fisher Scientific FTIR Spectrometer (Model- Nicolet Summit LITE, Serial No: BFJ2010011). All stones were washed with distilled water and dried completely in an incubator. The sample was grounded with a pestle and mortar until a fine homogeneous powder was obtained. For the FTIR spectroscopic investigation, the powdered urinary stone material (20 mg) was homogeneously mixed with potassium bromide (200 mg), an inert carrier, which did not show any absorption in the spectral region to be investigated. The mixture was then transferred to cylindrical hole and the bolt press and kept under the hydraulic press; the pump is used to increase the pressure in the hydraulic press to 15 tons and left for 2-3 minutes. The pressure is released slowly to form the potassium bromide disc or Pellet, which is then, analyzed. The resulting spectrum was then compared with the entire reference spectrum for the known components of stones, allowing a precise analysis of the complex crystal mixture for each crystal type using the OMNIC Paradigm .Ink software (Serial No: 210208422).

In FTIR spectroscopy, photons possessing energy that exactly

matches the vibration energy of a covalent bond in stone are absorbed. Thereafter, an infrared spectrum will show which bonds have absorbed radiation (wavelength) and absorption efficiency (intensity). This combination of wavelength and intensity generate a unique fingerprint for each component that can be used for both qualitative and quantitative analysis of renal stone. The details of principle of FTIR for analyzing renal stones is shown in Figure 1.

Figure 1: Details of principle of FTIR for analyzing renal stones.



Statistical Calculation

Descriptive statistics was done in Microsoft excel and results were expressed in frequencies and percentage. Statistical examination of renal stone incidence and composition with respect to sex and age was done using the Chi-square test ($p < 0.05$) in SPSS for Windows version 20.0.

Results

Demographic findings

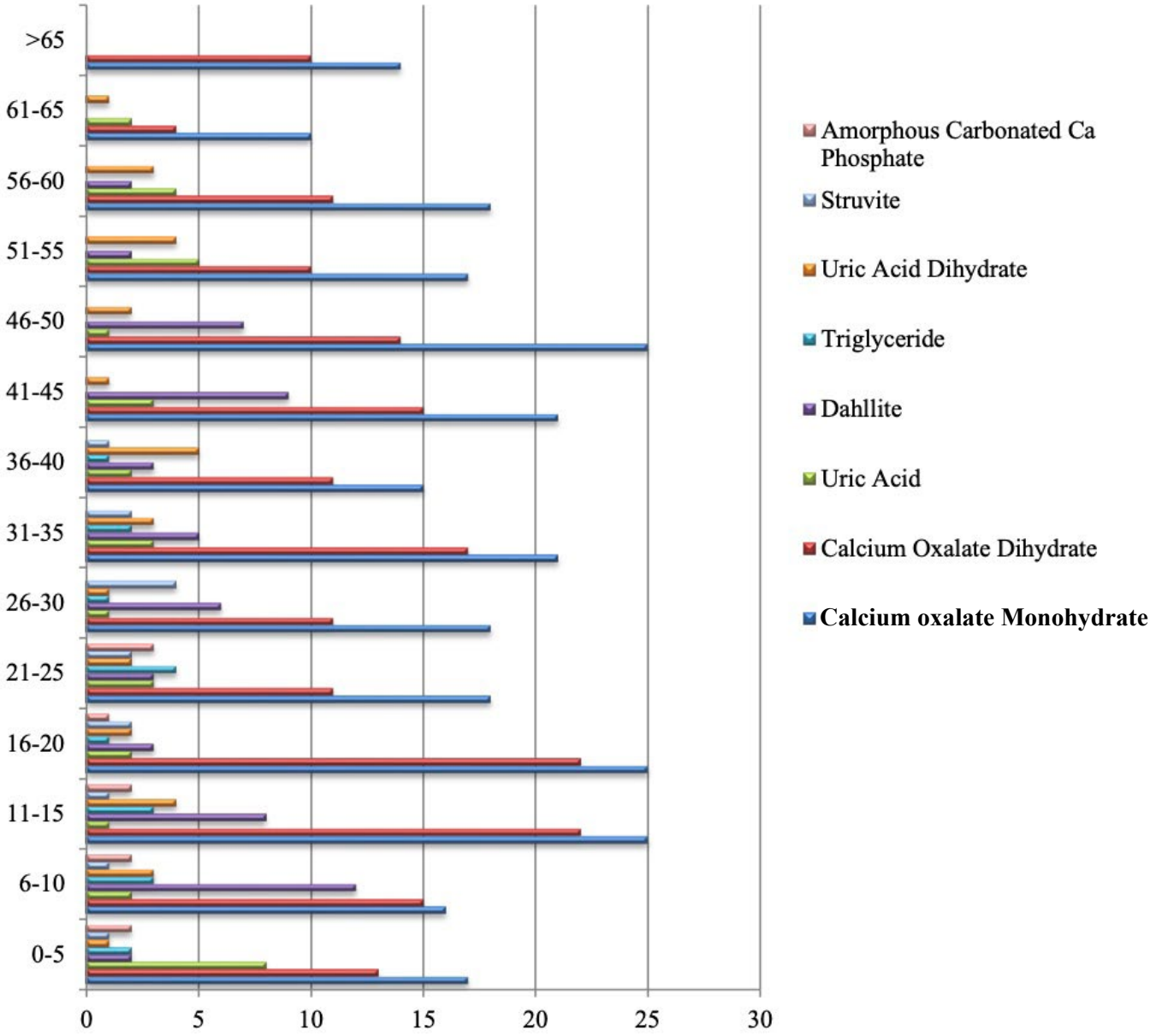
This dataset comprises information of 300 individuals diagnosed with renal stones, categorized by age and gender. The data includes 150 females and 150 males, with ages ranging from 2 to 84 years. The Kolmogorov–Smirnov test yielded a p-value of 0.000, indicating a significant deviation from normality. Therefore, the median age of study participants is 35 years. The youngest male is 2 years, and the oldest male is 84 years old. Age of female ranged from 9 to 77 years. The data indicates that renal stones affect a wide age range, from young children to the elderly. To better understand the age distribution, we categorized the individuals into age groups and analyze the sex distribution within each group as shown in Table 1.

Table 1: Age groups and sample size by sex of renal stones analyzed.

Age Group (Years)	Number of Females	Number of Males	Total
0-10	2	4	6
11-20	4	12	16
21-30	36	36	72
31-40	34	34	68
41-50	26	26	52
51-60	22	24	46
61-70	19	11	30
71-80	5	3	8
81-90	1	1	2
Total			300

The prevalence of renal stones is notably high in the younger population aged 21-30 years, with an equal distribution of 36 males and 36 females. Similarly, in the middle-aged groups (31-40 and 41-50 years), the distribution remains equal between males and females, suggesting that the prevalence of renal stones is consistent across these age groups. A chi-square test was conducted to evaluate the association between sex and age categories, and the results indicated statistically significant difference in sex distribution with higher incidence in males across 11-20 years age group (p-value: 0.002) and higher incidence in female across 61- 70 year age group (P-value: 0.002). We investigated the prevalence of various types of renal stones in relation to age. Our analysis indicates that calcium oxalate stones are the most common across all age groups (Figure 2).

Figure 2: Case distribution (X-axis) of common renal stones among various age groups (Y-axis).



FTIR Spectroscopy Findings

The predominance of calcium oxalate stones, with calcium oxalate monohydrate (COM) comprising 41% and calcium oxalate dihydrate (COD) 29% of cases, aligning with global trends was found in this study. In addition to calcium oxalate stones, our study identified several other types of renal stones, as illustrated in Figure 3. Renal stones with a low incidence, comprising less than 1% of the total, are not depicted in the pie chart. These rare types include ammonium urate, newberyite, 2,8-dihydroxyadenine, brushite, cholesterol, sodium urate monohydrate, calcium phosphate, calcium apatite, and whitlockite. FTIR Spectroscopic characteristic of renal stone components is shown in Table 2.

Figure 3: Distribution of common renal stones in Nepalese population.

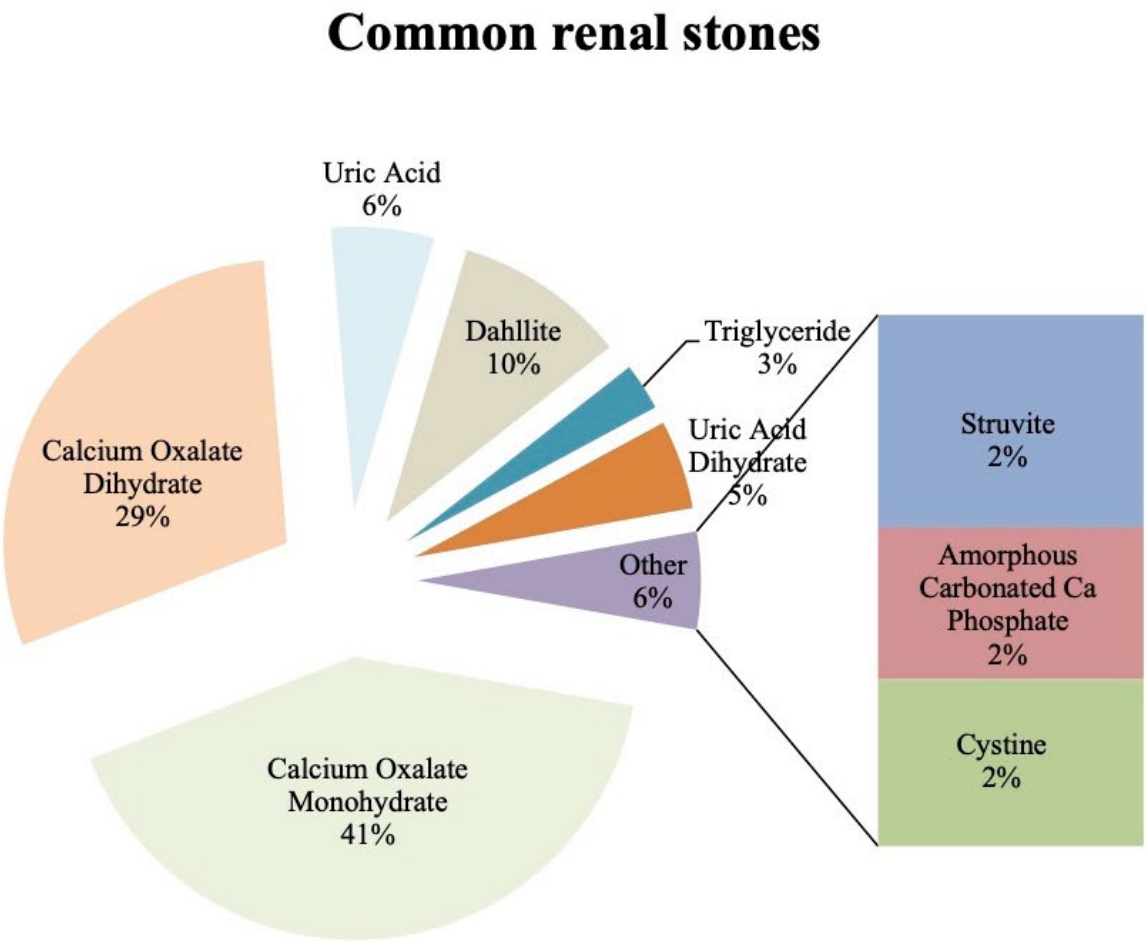
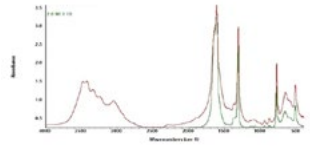
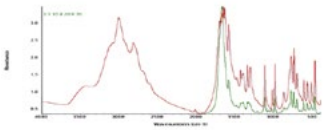
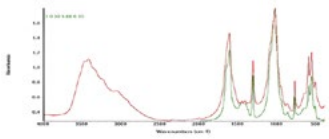
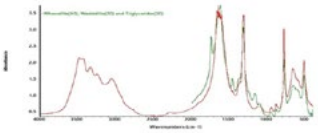
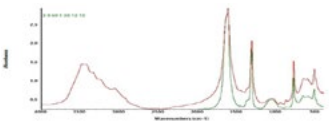
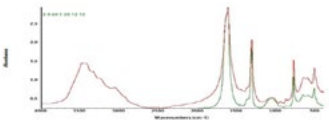


Table 2: FTIR Spectroscopic Characteristics of Common Renal Stone Components in this study.

Compound	Functional Groups	Types of Vibration and Band Assignment	Theoretical Range (cm^{-1})	Reference Spectra
Calcium Oxalate Monohydrate (Whewellite: CaC2O4.H2O)	C=O (carboxyl), O-H (hydroxyl)	ν(C=O) stretching, ν(O-H) bending	1620–1640, 1310–1330	
Calcium Oxalate Dihydrate (Weddellite: CaC2O4.2H2O)	C=O (carboxyl), O-H (hydroxyl)	ν(C=O) stretching, ν(O-H) bending	1320–1370, 780–820	
Uric Acid	C=O (carbonyl), N-H	ν(C=O) stretching, ν(N-H) bending	1600–1650, 1320–1350	
Uric Acid Dihydrate	C=O (carbonyl), N-H	ν(C=O) stretching, ν(N-H) bending	1600–1700, 1300–1400	
Dahllite (Carbonate Apatite)	P-O (phosphate), C-O (carbonate)	ν(P=O) stretching, ν(C-O) stretching	1000–1100, 1410–1460	
Struvite (Magnesium Ammonium Phosphate)	P-O (phosphate), N-H	ν(P=O) stretching, ν(N-H) bending	950–1050, 3200–3400	
Triglyceride	C=O (ester), C-H	ν(C=O) stretching, ν(C-H) bending	1740–1750, 2850–2950	
Amorphous Carbonated Calcium Phosphate	P-O (phosphate), C-O (carbonate)	ν(P=O) stretching, ν(C-O) stretching	1000–1100, 1450–1550	
Cystine	S-H (thiol), N-H, C=O	ν(S-H) stretching, ν(C=O) stretching	2550–2600, 1580–1650	

Discussion

Renal stone analysis of high quality is an essential part of the basic evaluation to identity patients at high risk of recurrent stone disease. In many cases, more extensive examination is required to reveal the etiology of stone formation in the individual patient, for example 24-hour urine measurements of citrate, oxalate, calcium and uric acid.

The present study shows an equal distribution of renal stones among males and females in 21-50 years age group. This finding is in contrast to other studies where male to female ratio is significantly higher [13-15]. Nevertheless, our study shows higher male prevalence in males across 11-20 years.

Identifying the specific types of stones prevalent in a population allows healthcare providers to develop targeted interventions, such as dietary modifications, pharmacological treatments, and public health initiatives aimed at reducing the incidence of stone formation. The major types and incidence of renal stones in this study and the related risk factors and required dietary modifications in each category is shown in Table 3 [16-20].

The higher prevalence of calcium oxalate stones aligns with neighboring countries. The high incidence of calcium oxalate containing renal stones in South Asian countries may be influenced by regional climate, dietary habits, and fluid intake patterns. In Pakistan, the hot climate, combined with factors such as low urine volume, acidic urine pH, and a diet low in protein and calcium but high in oxalate-rich foods, has been shown to promote the formation of uric acid and calcium oxalate stones, as noted by Rizvi et al [15, 21]. Similarly, in India, high rates of calcium oxalate stones are likely driven by a hot climate, oxalate-rich diet, and low fluid intake [9]. Subgroup analysis from an Indian study further demonstrated that the prevalence of calcium oxalate stones increases with age, while uric acid, struvite, and cystine stones decrease [9,15]. This finding is similar to our study. Moreover, hyperoxaluria rates appear significantly higher in Asian countries compared to Western countries (56.8% vs. 23.8%; $p < 0.001$), which highlights how dietary practices and global climate shifts may be key contributors to the rising incidence of renal stones [22]. These factors underscore the importance of targeted prevention strategies that consider regional dietary and environmental influences.

In Nepal, dietary habits rich in oxalate-containing foods such as tea consumption and low fluid intake due to socioeconomic and geographical factors may contribute significantly to this trend [23, 24]. This finding underscores the need for targeted dietary interventions such as minimizing oxalate intake and public health strategies such as advocating for low salt and sufficient water intake to reduce the incidence of these types of stones.

Beyond calcium oxalate stones, our study identified several other types of renal stones. Dahllite stones are indicative of calcium phosphate stones and suggest the importance of managing urinary pH and calcium levels. Similarly, uric acid stones highlight the relevance of metabolic conditions such as hyperuricosuria and low urinary pH. Dietary modifications and medications that alkalinize urine can be effective preventive measures. Likewise, the struvite stones are usually associated with urinary tract infections. The presence of other types of stones, although less frequent, indicates the diversity of metabolic disturbances contributing to urolithiasis in this population. Therefore, these findings emphasize the need for comprehensive metabolic evaluations in patients presenting with renal stones.

Table 3: Major type of renal stones in Nepalese population along with its risk factor and recommendation for its prevention.

Common Renal Stone Types	Distribution Percentage	Risk Factors	Recommendations for Prevention
Calcium Oxalate Monohydrate (COM) and Calcium Oxalate dehydrate (COD)	COM 41% COD 29%	Hypercalciuria (250 mg/day) Hyperoxaluria (> 45 mg/day) Hypocitraturia (< 320 mg/day) Low urine volume (< 2 L/day)	Sufficient water intake~ 2L/day Minimize oxalate intake (spinach, cranberry, grapes, potatoes, nuts,tea,coffee) Consume fruit juice(Citric acid) Sodium restriction (<1500 mg/day)
Dahllite [Calcium Phosphate]	10 %	Hyperphosphaturia (> 1100 mg/day) Alkaline urine pH (> 7.0) along with low urine volume Hypercalciuria and hypocitraturia	Sufficient water intake~ 2L/day Consume Lemon, lime, melon and oranges that contains citric acid

Uric Acid and Uric Acid Dihydrate (UAD)	Uric Acid 6 % UAD 5 %	Low urine volume Hyperuricosuria (> 700 mg/day) Acidic urine pH (< 5.5)	Avoid high-purine foods (organ meats, mushrooms, asparagus, green peas, spinach, fish) Increase plant protein rather than animal protein to increase alkaline load in urine
Struvite (Magnesium ammonium phosphate)	2.0%	Urinary tract infection by a urea - splitting bacterium Neurogenic bladder and anatomic abnormalities of urinary tract	Treatment of UTI Surgery for anatomical defects
Cystine	2.0%	Inherited disorder like Cystinuria	Increase cysteine solubility by increasing fluid intake Sodium restriction Reduce meat intake (contains methionine that is converted to cysteine)

References = [16-20]

This study has certain limitations that should be acknowledged. The sample size, while adequate, may not fully represent the entire population of Nepal. Additionally, as a referral laboratory, our sample may have a selection bias toward more complicated or recurrent cases, and we used convenience sampling technique. Future studies with larger and more diverse populations would be beneficial to validate and expand upon our findings. Additionally, exploring the relationship between dietary habits, socioeconomic status, and stone composition could provide deeper insights into preventive measures. Longitudinal studies to track the recurrence rates and the effectiveness of dietary and medical interventions in patients with different types of stones would also be beneficial.

Conclusion

In conclusion, the analysis of renal stones using FTIR spectroscopy in a referral laboratory in Nepal has revealed that calcium oxalate monohydrate and dihydrate stones are the most prevalent types. This laboratory based data reveals that renal stones are prevalent across all age groups and affect young population equally. These insights can inform targeted prevention and treatment strategies for renal stone disease.

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Conflict of Interest

Authors declare no conflict of interest in the publication of this manuscript.

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Commentary

Environmental issues in clinical laboratories: pragmatic pathways to sustainability

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Keywords

Climate change, Carbon footprint, Environmetal damage, Green chemistry, Sustainable pratices, Toxic waste

Every human activity has impact on the environment, including scientific activities in the laboratory. This is particularly true with laboratory medicine services, although poorly recognized until recently. Clinical laboratory activities have profound contribution to climate change. Current efforts in the laboratory medicine community are largely extrapolations of the collective efforts of the larger scientific community and the United Nations Sustainable Development Goals to sustain planet earth by ensuring sustainable practices in all spheres of human endeavor. High impact existing literature in the field was harnessed in the synthesis of this report. The United Nations' World Commission on Environment about thirty years ago gave the global community a definition of sustainable development, development that meets the needs of present without compromising the ability of the next generation to meet theirs. This equation appears to be under constant threat, constituting a challenge for the health care sector, practitioners, and the laboratory medicine community in particular that is a culprit in consuming more energy, water and generate huge hazardous toxic waste.

It is only in the last couple of years that this has dawned on the scientific community after the introduction of the concept of green chemistry which essentially implies sustainable use of scientific resources with minimal damage to the environment. Though many laboratories in the developed countries have accepted this challenge with vigor, it is yet to receive the desired impetus in many low- and medium-income countries particularly Africa. The global laboratory medicine community must acknowledge that clinical laboratories must curtail their environmental impact while providing quality laboratory medicine services by reducing energy and water consumption, minimizing hazardous waste generation and the utilization of toxic or hazardous chemicals, as well as recognizing sustainability as a target.

Introduction

In the last decade, the global scientific community has intensified its concern, probably ignited by the discussion at the 2016 World Economic Forum (WEF) at Davos, that by the year 2050, there will be more plastic in the oceans than fish, reflecting the level of environmental pollution [1]. The concept of sustainability was first introduced by the United Nations in the late 1980s [2] as an attempt to balance economic development with environmental protection and rescue Planet Earth from the catastrophe of climate change.

This culminated in the setting up of the United Nations World Commission on Environment and Development (UNWCED), chaired by the former Prime Minister of Norway, Gro Harlem Brundtland. This gave rise to the term sustainable development, defined as 'Development that meets the needs of the present without compromising the ability of the future generations to meet their own needs' [2]. Nearly 30 years later (2015), the UN introduced the 17 Sustainable Development Goals (SDGs), outlining how sustainability can be achieved [3].

Though our focus for clinical laboratories for decades has disproportionately been on the benefit to patients and clinical outcomes, the time has now come for the laboratory medicine community to be directly involved in the conversation and action plans to mitigate or reduce the problem. Clinical laboratories use 3-6 [4] more energy than typical office buildings and thus contribute substantially to carbon footprint. Clinical laboratories also generate large quantities of hazardous waste, byproducts of large chemical consumption [5].

Pathology laboratories have a philosophy of quality improvement using tools such as audits, nonconformances, and management reviews. An effective quality management system drives risk reduction in laboratory processes. In addition to these core elements, laboratories are increasingly adopting Lean Six Sigma methodologies to streamline processes and reduce waste. These approaches can be particularly effective in addressing climate change-related challenges within laboratories. By identifying and eliminating waste, improving efficiency, and minimizing environmental impact, laboratories can contribute to a more sustainable future. Harnessing this same system can improve a laboratory's environmental risk. The fundamental function of developing a plan to reduce environmental waste should involve an assessment of risk, a policy supported by management (and staff), an activity plan supported by measurable outcomes, audit and non-conformance processes, and oversight and accountability by management that provides some structure, a point of comparison and an external review process.

There is a growing list of clinical laboratories where there is a demonstrated awareness and action to reduce the carbon footprint [6–12].

A hospital or hospital network can take significant steps to reuse and recycle waste. The types of activity include the following [13].

Recycling of Materials

- Recycle polyvinyl chloride, such as saline bags (cut off introducers first) and oxygen tubing (no filters or Hudson masks).
- Recycle aluminium gas cylinders.
- Recycle surgical wraps.
- Recycle appropriate hospital soft plastics (e.g., syringe waste plastics, image intensifier covers, shrink wrap from a pharmacy, clear plastic covers used in radiology to cover detectors).
- Recycle stainless steel (eg, blue-handle single-use stainless steel instruments).
- Recycle e-waste (eg, computers and electronic equipment, clinical vacuums, , central sterile supply department broken equipment).
- Recycle wax (radiation therapists use wax to mould patient contours for radiation therapy).
- Recycle rubber gloves and surgical caps.

Energy efficiency

- Investigate key clinical areas with single-use electrical wires (e.g., occupational therapy, intensive care units, catheter labs, dermatology) for opportunities to reduce their use.
- Reinforce the practice of turning off or unplugging equipment when it is not in use (e.g., fume hood, oven, incubator, water bath, centrifuges, etc.).
- If equipment has a warm-up time or a set temperature, simply put it on an outlet timer to ensure that it is ready when lab operations begin.
- Hold excess hospital/department equipment swap days.

This may be a significant contribution to resolving the considerable environmental and health impacts of our scientific activities. The laboratory medicine community owes it as a responsibility to society and to sustainable development to contemplate the following actions:

- Decrease individual and environmental footprint.
- Put global guidelines on recycling.
- Scientists should consider the use of glass appropriate to replace plastics.
- Adoption of the measures already advocated by many toxicology groups to be considered. - the 3 Rs.
- Sound management of chemicals by all clinical laboratories to be given priority of place by putting in place functional 'Health and Safety Committees'
- The health and safety aspect of the training of laboratory medicine professionals should be expanded to encompass the environmental and ecotoxicological impact of scientific activities in clinical laboratories
- Regular educational sessions could be held for sharing

tools and ideas to bring everybody to the same level- taking cognizance of developmental diversity- advanced, moderately advanced, and low and medium- income nations (LMICs).

- Importantly, manufacturers/ suppliers should be part of the after-life management of their products; they must put strategies in place to ensure an environmentally sound approach to the disposal of associated accessories or waste.

These collaborative/collective approaches may be a tremendous scientific pathway to address the laboratory medicine community's hitherto neglected role in its activities' environmental and health impact.

The Situation in African Clinical Laboratories

It is evident from the foregoing that the global community is currently grappling with one of the most daunting problems of the past and present centuries, climate change. Unarguably, it primarily arises from unregulated unsustainable human industrial and scientific activities of which laboratory medicine practice is a key contributor, which may be more accentuated in Africa. Africa, though essentially belonging to the broad group of LMICs owing to economically constrained peculiarities, contributes hugely, but is not keeping pace with measures being taken by the advanced nations to implement sustainable practices. The quality of clinical laboratories in developing countries is poor due to a lack of adequate resources, proper regulations, and supportive health care [14,15].

Current efforts in the African laboratory medicine community are largely extrapolations of the general efforts of the larger scientific community and the United Nations Sustainable Development Goals to sustain Planet Earth by ensuring sustainable practices in all spheres of human development. Targeted clinical laboratory medicine efforts are the exception rather than the norm. (High-impact existing literature in the field was harnessed in the synthesis of this report). The United Nations' World Commission on Environment, at least three decades ago, gave the global community a definition of sustainable development: development that meets the needs of the present without compromising that of the next generation, as earlier pointed out, a framework only patyially enforced in many African countries. This equation appears under constant threat, constituting a challenge for the health care sectors, practitioners, and the laboratory medicine community, which are culprits in consuming more energy and water and generating huge hazardous toxic loads, generally in Africa.

Though in the last couple of years, it has dawned on the scientific community in Africa after introducing the concept of green chemistry, which essentially implies sustainable use of scientific resources with minimal environmental damage, implementation in Africa is, at best, fragmentary. Though many laboratories in developed countries have accepted this challenge with vigor, it is yet to receive the desired impetus in many low- and medium-income countries, particularly in Africa. The African laboratory medicine community must have to acknowledge that clinical laboratories must curtail their environmental impact while providing high quality laboratory medicine services by reducing energy and water consumption, minimizing hazardous waste generation and the utilization of toxic or hazardous chemicals, and eliminating adverse impacts on sustainability as a target. It should also reevaluate the use of low technology energy, intensive processes with higher carbon footprint and attendant more significant environmental adverse outcomes, such as duplicated energy sources (particularly emmisions from alternate energy generating plants).

Figure 1: A typical energy source in an African country.

This type of plant has great potential of contributing.

It is remarkable that, though considered among the low—and medium-income countries, Africa's pollution intensity (pollution generated per unit of production) is among the highest in the world. This is coupled with an already existing problem with municipal waste management and poorly implemented environmental laws.

Recourse to point-of-care testing (POCT) in many African clinical laboratories because of economic benefits (cheaper), mostly single-use plastics, exacerbates plastic pollution. Economic and resource limitations create difficulties in acquiring appropriate recircling and sound waste disposal technology.

All these may culminate in more profound, unsustainable practices, leading to a greater risk of environmental toxicity and damage and a disproportionate contribution to a more severe climate change compared to their testing volume or capacity.

Thus, it is a public health/ laboratory medicine priority in Africa to put measures in place to ensure sustainable laboratory medicine practice. As well recognized in pollution and exposure science studies, 'poisoning one part of the globe and the whole world is poisoned', pollution knows no international boundary. The rest of the scientific community must form partnerships with Africa to eradicate laboratory medicine's associated adverse environmental impact and ensure green laboratory medicine practice for the world community.

The significant difference between African clinical laboratories and those of the advanced world is lower economic capacity and technology, which is not surprising. There is also the case of preexisting environmental disorder arising from unenforced environmental regulations and laws, making the situation in Africa a more serious one. There is also greater recourse to POCT and other single-use devices that may have a broader environmental impact- poorly managed waste disposal.

Education and training on the concept of the environmental impact of laboratory medicine, or green laboratory medicine and green chemistry, should receive priority attention in Africa.

Partnerships with advanced nations where the concept of sustainable laboratory medicine practice should be the next step.

Role of the IFCC Task Force on Environmental Impact of Laboratory Medicine (TF-EILM)

The IFCC has been active in the environmental waste field for some years, initially in 2012 with the formation of an ad hoc IFCC Panel on the Environmental Responsibility of Clinical Laboratories and then in 2023 with the creation of the Task Force on Environmental Impact of Laboratory Medicine (TF-EILM). The IFCC panel produced a set of proposals for mitigating the environmental impact of clinical laboratories.

The TF-EIFM was created with the following goals:

1. Identify existing peer-reviewed, high-quality publications that describe the impact of laboratory medicine operations on the environment and actions that laboratories and manufacturers can take to reduce the negative impact.

   Creation of awareness
   - Develop staff consciousness of the laboratory's activities' impact on the environment. Find ways to address the problem before attempting any kind of certification.

   Preliminary environmental review
   - Identify activities that significantly impact the environment.

   Aim
   - Establish overall goals, set targets, and plan activities as part of an Environmental Management System (EMS).

   Training
   - Prepare a training program for the laboratory staff.

   Available legislation
   - Identify legal requirements related to environmental aspects of the laboratory's activities and establish a register of these.

   Audits
   - Schedule audits and management reviews. The audits should identify areas of waste and opportunities for lessening the environmental footprint.

   Documentation

   Prepare the following documents:
   - An EMS manual.
   - Protocols for handling emergencies, such as spills.
   - A list of contractors and the contracts that the laboratory has entered into.
   - Develop a policy document advocating for health authorities and accreditation bodies to incorporate green lab practices into their checklists or agendas.
2. Develop recommendations and practice guidelines that laboratories in developed and developing countries can implement to reduce the environmental impact of laboratory operations without compromising the quality of services provided to patients.
3. Develop a plan to share information and educate IFCC national society and corporate members.
4. Identify existing peer-reviewed, high-quality publications that describe the laboratory testing that can be performed to measure both levels and biological effects of toxic environmental chemicals in human biological material, including studies to demonstrate the concentrations of chemicals found in human biological tissues (human biomonitoring).

5. As required, the Task Force will establish a formal collaborative link with the EFLM Task Force on Green Labs and other IFCC groups and organizations involved in related activities.

The task force's key responsibility is essentially to minimize the environmental impact of laboratory medicine operations while still delivering high-quality care to patients, by upholding sustainability-compatible systems and procedures summarized as follows:

- Reduce energy consumption.
- Reduce water consumption.
- Reduce hazardous waste generation, including CO2 (green gas)
- Reduce the use of harmful chemicals

All the above can be achieved by embracing the concept of green chemistry and the principle of registration, evaluation, authorization, and restriction of chemicals (REACH), introduced by the European Chemical Agency (ECHA) [16,17].

The remit of the TF-EILM is, therefore, at least in part in alignment with the United Nations Sustainable Development Goals (SDGs), reminding us of the following:

- That scientific practices, including laboratory medicine practice, contribute to environmental degradation, including gas emissions, especially CO_2 emissions, plastic pollution, toxic and infectious waste, and other environmental concerns.
- Scientists have gradually come to the realization of the environmental footprint of their activities and the need for change or adjustment for a more sustainable environment and world.

Despite this realization, there is the inertia to act, which may be driven by economic concerns, a significant consideration for many LMICs, particularly those in Africa, knowledge deficit and dearth of the required data to address the problem, thus inability to implement sustainable laboratory medicine practice. The laboratory medicine community feels as a community that she has a responsibility to address the sustainability agenda.

Obstacles to Adopting Sustainability and their Mitigation

It is perhaps appropriate to underscore the obstacles to sustainability in laboratory medicine and their pragmatic mitigation, although the last paragraph succinctly addressed them. Lack of broad scientific outlook is a perceived barrier to implementation of sustainability. Others may include absence of peer education on green chemistry and leadership that drives systematic laboratory practice and paucity of evidence of the environmental impact of laboratory medicine and causal root factors. Additional factors that may be contributory to non-embracing of sustainable practices in laboratory medicine include poor and rigid traditional workplace practices, absence or insufficient financial support and perhaps lack of conviction on the part of scientists and other laboratory medicine practitioners [13, 18].

The overarching requirement that will be a panacea for mitigating the obstacles to sustainable laboratory medicine will include peer education, training and mentoring of the next generation of scientists in laboratory medicine, updating of knowledge by those in the laboratory medicine community and the pathways of IFCC and constituent federations in sustainable laboratory medicine.

Our organization, IFCC, as the global leader and watchdog in laboratory medicine, feels she has an obligation to lead the multidisciplinary, international effort aimed at promoting sustainable chemical and material consumption. It is felt that scientists must incorporate the sustainability of best practices in their activities and motivate colleagues to embrace sustainable laboratory medicine practices.

Conclusion

Although most of the scientific community, especially the chemical community recognized the significance and implications of these developments; environmental degradation and thus climate change, the global laboratory medicine community was slow in responding until recently.

The IFCC responded to this gap in environmental stewardship early (2023) by setting up one of the newest task forces (TF-EILM), with five vital remits highly congruent with the green chemistry concept.

Author Declaration and Ethical Consideration

The authors declare no conflict of interest.

Ethical approval

None.

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Case Report

Improving Laboratory Diagnosis of Creutzfeldt-Jakob Disease

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Keywords

Case report, Lab diagnosis, Creutzfeldt-Jacob Disease, Prion Disease, RT- QuIC, CSF analysis

Abstract

Background: Creutzfeldt-Jakob disease (CJD) is a rare human form of prion disease caused by misfolded, transmissible proteinaceous infection particles (prions). As a fatal neurological illness, it mostly presents with rapidly progressive dementia, and most patients die within a year of clinical onset and diagnosis. The lack of an intravital test for CJD limits its timely diagnosis. A brain biopsy/autopsy is considered the gold standard for definitive diagnosis of CJD, however owing to its highly invasive and transmissible nature, it is rarely performed. In this case report, we try to highlight the important role of combining serology, EEG, and CSF investigations, often used for the diagnosis of CJD. Combining these in the laboratory improves the timely diagnosis of this rare and fatal disease.

Case summary: We report a clinical case study of a 65-year-old female, who presented to the Neurology OPD at a tertiary care referral centre, with chief complaints of forgetfulness, behavioural changes, and involuntary movements in the right upper limb for the last 7 months. According to the informant (daughter), the patient was asymptomatic 7 months ago after which she started developing these gradual onset symptoms. Later she was bed-bound and dependent on her family members for her daily chores and had even lost control over her bowel and bladder habits. On physical examination, the patient was found to be disoriented and afebrile with normal vitals, however, CNS examination showed a low Mini Mental Examination Score (MMSE). The patient was admitted to the neurology ward for further evaluation and a definitive diagnosis. Differential diagnosis was ruled out using various lab tests, CSF analysis, and neuroimaging. CSF report tested positive for 14-3-3 protein and CSF protein marker by RT-QuIC was outsourced. The confirmatory diagnosis of sporadic CJD was made based on clinical presentation, CSF analysis, and neuroimaging.

Conclusion: Definitive diagnosis of CJD was possible with the help of various lab tests which helped rule out differential neurodegenerative diseases.

Introduction

Creutzfeldt-Jacob disease (CJD) is a rare, progressive, transmissible, deadly neurodegenerative disease caused by misfolded prion proteins mostly characterized by a long incubation period [1-2]. Most people with clinically diagnosed CJD die within a year of symptom onset [3]. Also, given that CJD can develop asymptomatically in people for decades before showing symptoms, there is a chance that it could spread through iatrogenic means [4]. Due to the lack of specific diagnostic markers and scarcity of genetic testing, only a few cases are reported, with 1-2 cases diagnosed per million people yearly [5]. A prion protein (PrPc) is a normal neuronal protein primarily composed of α-helical structures and random coils usually found on the cellular surface of neurons which maintains neuronal homeostasis and plays a role in cell signalling. Infected prion proteins misfold into β-pleated sheets and are pathological [6]. Abnormally folded PrPsc causes disturbance in neuronal development, homeostasis, circadian rhythm, stress responses, and synaptic plasticity, thereby leading to the presenting features of CJD [7]. There are three major groups of human prion disease: sporadic, genetic, and acquired.

Sporadic CJD is the most common type and accounts for almost 85% of all CJD. It is further subdivided into sporadic fatal insomnia and variably protease-sensitive prionopathy. **Genetic CJD** constitutes about 10-15% of total CJD cases. Genetic forms of CJD are associated with pathogenic mutations in the prion protein gene PRNP and include familial CJD, fatal familial insomnia, and Gerstmann-Schaussler-Scheinker syndrome. **Acquired CJD** constituting less than 1% of total CJD cases, includes kuru, iatrogenic, and variant subtypes [8-9]. Latest data showed few deaths from iCJD and gCJD from 1996 to 2018 but a steady rise in sCJD cases [10-11].

Diagnosis of CJD is often challenging due to its low incidence and its symptoms resembling various other neurodegenerative diseases. These clinical symptoms include a rapid decline in cognitive function, myoclonus, ataxia, extrapyramidal signs, and akinetic mutism [12]. The gold standard test for diagnosing CJD is a highly invasive brain biopsy. However, this is done mostly during an autopsy, which makes it less useful for the patient. Also, prions are highly infectious and clinicians are exposed to this highly transmissible disease during this procedure. Therefore, diagnosis is made based on clinical features, laboratory tests, EEG, MRI findings, and genetic studies. Laboratory findings include CSF studies. CSF 14-3-3 protein is sensitive to CJD. EEG reveals typical periodic short-wave complexes (PSWC). Of late, RT-QuIC assay has been identified as a more sensitive and specific diagnostic tool, that closely resembles brain biopsy findings, and directly detects misfolded prion protein [13-14]. Though CJD is invariably fatal and lacks a definitive treatment, it is highly essential to establish a confirmatory diagnosis to prevent iatrogenic transmission and also to reduce the risk of transmission among healthcare providers. We are reporting a 65-year-old female who presented with neurodegenerative complaints and was diagnosed as a case of sporadic CJD with the help of various laboratory and radiological investigations.

Case Presentation

Chief complaints

A 65-year-old female, presented to the Department of Neurology OPD at a tertiary care referral centre, in Delhi, India, with the chief complaints of forgetfulness, behavioural changesfor 7 months and involuntary movements in the right upper limb for the last 6 months.

History of present illness

The patient was asymptomatic 7 months ago after which she started developing symptoms of forgetfulness and behavioural changes which were insidious in onset and gradually progressive. She started experiencing memory lapses including the inability to recall the route/path to her daughter's house, which she visited frequently. In addition, her relatives noticed repeatability in certain activities like folding and unfolding of clothes eventually leading to incompletion of tasks. This was followed by increased irritability towards her grandchildren, especially when they wanted to play with her. Also, she started misplacing items in her household, often neglecting to position them in their designated positions. They also noticed that she found it hard to speak her usual and started communicating in short sentences which gradually reduced to one/two words, later she answered in yes or no communication, and since last one month she started producing incomprehensive sounds. This was further associated with gradually decreasing oral intake and decreased sleep compared to her pre-illness state. In addition, 6 months back, relatives observed that she developed a new habit of clenching her fist momentarily throughout the day gradually progressing to unusual movements of her right upper limb. This was initially momentarily, but later occurred multiple times a day and persisted even during her sleep. She has been bed-bound and dependent on her family members for her daily activities for the last one month and has even lost control over her bowel and bladder habits.

History of past illness

There was no history of trauma/surgery/similar episodes in the past.

Negative history

There was no history of prolonged fever/rash/joint pain/swelling/visual or auditory hallucinations/ documented weight loss/recurrent focal deficit/seizures.

Past History

The patient did not have any relevant past medical history. There was no history of hypertension/diabetes mellitus/thyroid disorders/TB/drug allergy.

Personal and family history

The patient was a nonvegetarian, non-alcoholic, and non-smoker with 67 Kg weight and 168cm height, Muslim by religion, and a housewife by occupation The patient's medical, dental, and family history was non-contributory.

Physical examination

On physical examination, the patient was found to be disoriented to time, place, and person. She was afebrile with a heart rate of 96 bpm, respiratory rate of 18 bpm, and blood pressure of 118/76 mm Hg. No signs of pallor, icterus, or lymphadenopathy were seen. On palpation, the abdomen was found to be soft with no signs of organomegaly. CNS examination showed spontaneous eye opening, pupil bilateral reactive, not following verbal commands, noncomprehensive speech, withdrawal to pain, and jerky movement of the right upper limb (myoclonus). MMSE scoring revealed a low score.

The patient was admitted to the neurology ward in view of **rapidly progressive dementia** and **persistent myoclonic jerks** and further evaluation was done for a definitive diagnosis.

Diagnosis

A differential diagnosis of Creutzfeldt-Jacob disease, Paraneoplastic Encephalitis, Amyloid angiopathy, Frontal temporal dementia, and Alzheimer's disease, Autoimmune encephalitis, Metabolic encephalopathy were suspected, and following investigations were ordered. The results are shown below.

Laboratory Diagnostics

1. Routine blood investigation

Table 1: Routine blood investigations.

Blood Investigation	Reference range	20/3/2023	14/4/23	25/4/23	2/5/23	9/5/23	20/5/23
Complete blood count							
Hemoglobin	12-15.5gm/dL	12.7	12.5	**8.6**	**8.9**	**5.3**	**7.2**
WBC-TLC	5-10 x10^3/µl	9.4	**19**	**18**	**26**	**15**	**17.9**
Neutrophils	60-75%	65	80	**86**	**86**	80	**96**
Lymphocytes	20-40%	22	**9**	**8**	**7**	**13**	**4**
Platelets	150-400 x 10^3/µL	245	159	319	**415**	293	**22**
Renal function tests							
Urea	18-55mg/dl	28	25	15	17	10	48
Creatinine	0.5-1.2mg/dl	0.8	**1.4**	0.4	0.4	0.2	0.8
Serum electrolytes							
Sodium	135-145 mEq/L	131	**126**	**131**	135	135	**156**
Potassium	3.5-5.3 mEq/L	3.2	**3.4**	3.8	3.8	3.8	**5.6**
Hepatic profile							
Total Bilirubin	0.3-1.2 mg/dL	0.3	0.5	0.2	0.2	0.2	0.3
Total Protein	6-8 gm/dL	5.8	5.9	**5.7**	6.7	**3.7**	**4.9**
Albumin	3.5-5 gm/dL	3.4	3.3	**2.1**	2.2	**1.3**	**1.7**
AST	10-40 U/L	50	**67**	33	43	21	32
ALT	10-40 U/L	60	**113**	15	11	10	08
RBS	<200 mg/dL	100	150	110	110	90	106
Ammonia	9-30umol/L	-	**96**	**80**	**41**	**47**	**57**

2. CSF analysis (23/3/23):

- CSF protein - **23.9** (15- 45 mg/dL)
- CSF sugar - 73 (50-80 mg/dL)
- CSF cytology (TLC/DLC) – Acellular

*CSF analysis for **14-3-3 protein:*** The test was outsourced and resulted **positive.**

CSF for AFB/CBNAAT: No AFB (Acid Fast Bacilli) was present in the sample and resulted **negative.**

3. Special tests

Table 2: Special tests.

Name of test	Reference range	Test result
FT3	2.0-4.4 pg/mL	3.3
FT4	0.93-1.7 ng/dL	1.4
TSH	0.27-4.2 ulU/mL	3.6
Vit B12	211-946 pg/mL	671
CEA	<3.8 ng/mL (non-smoker)	**4.2**
AFP	<5.8 IU/mL	4.88
CA 19.9	<39 IU/mL	29.3
Procalcitonin	<0.05 ng/mL	**0.40**
Anti TPO Ab	<30 IU/mL	9.1

Serum and CSF autoimmune and paraneoplastic panel profile: Negative

ANA profile: The Antinuclear Antibody Test profile was negative by ELISA and Indirect Immunofluorescence.

Viral markers: Negative.

4. Neuroimaging Analysis

EEG: Short interval discharges at the frequency of one per minute were observed.

NCCT Head: No abnormality detected.

MRI Brain (6/4/2023): Revealed FLAIR hyperintense areas showing diffusion restriction involving the cortex of bilateral cerebral hemispheres and left striatum. Findings are consistent with Creutzfeldt-Jacob disease (Figure 1).

Figure 1: MRI brain(T2/FLAIR) showing areas of signal alteration involving the cortex of bilateral hemispheres and left striatum appearing hyperintense on FLAIR images.

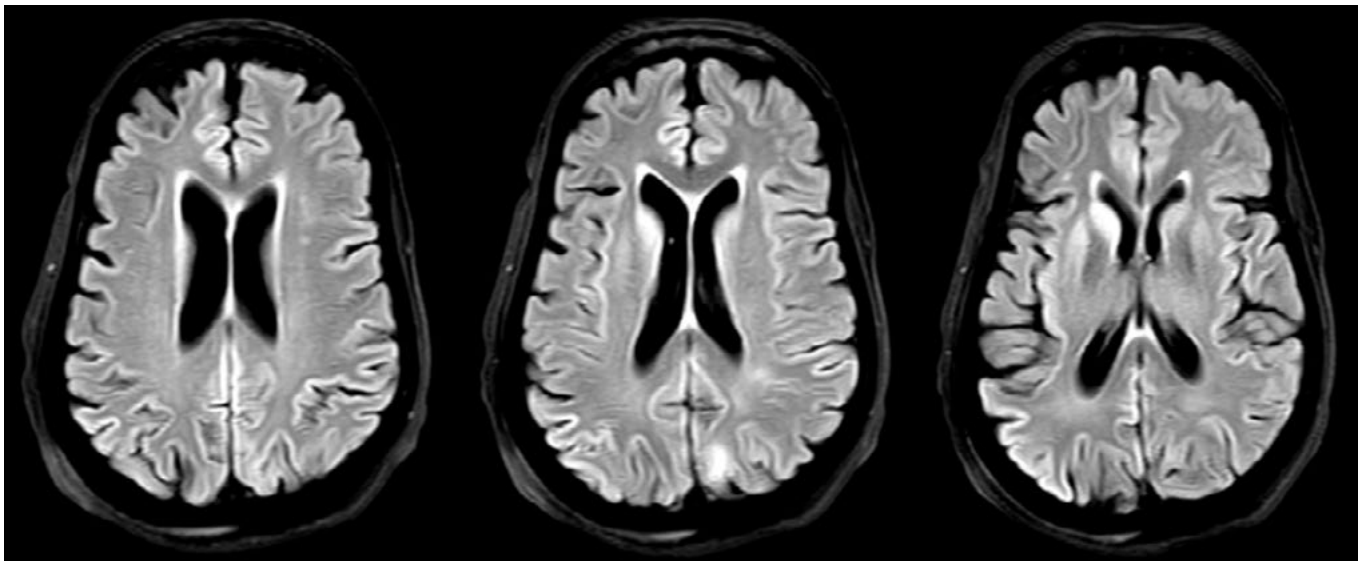
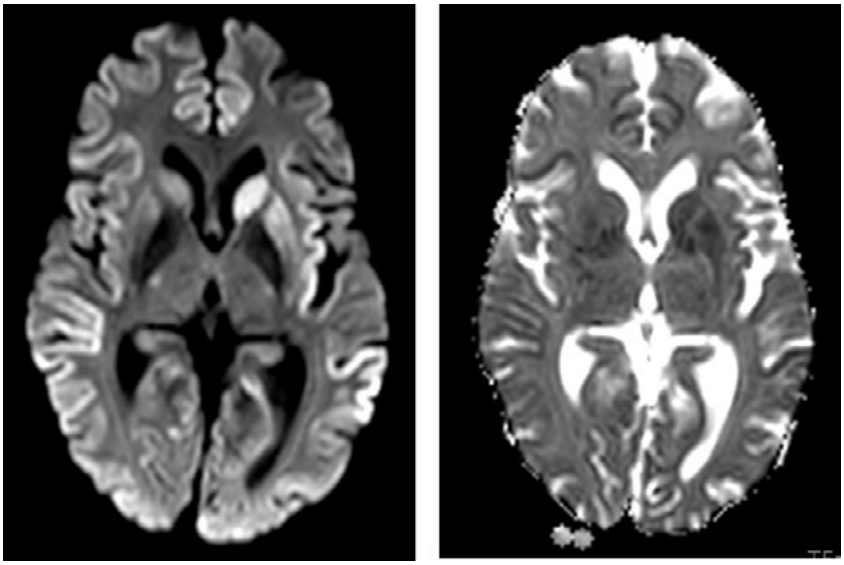


Figure 2: MRI brain (DWI-ADC) showing areas of signal hyperintensity in cortical regions.



Final Diagnosis

Clinical presentation of the patient, lab investigations, EEG, and MRI brain suggested positive findings and confirmed the diagnosis of CJD.

Treatment

During hospital stay, the patient developed high fever and a blood culture was ordered which revealed to be **MRSA positive.** The patient was started on specific antibiotics. However, despite proper management patient landed into respiratory failure for which she was intubated and further tracheostomized 15 days later. Her GCS was found to be profoundly decreasing from **E4V2M5** on the day of admission to **E1VTM1** a month later. Her blood culture and ET tube culture post tracheostomy came positive for Klebsiella pneumonia and Acinobacter. Klebsiella pneumonia is a pan antibiotic-resistant bacteria and patient was started on broad-spectrum antibiotics. Also, her bed sores progressed to grade IV and pus from bed sore was sent for culture; which again was positive for **Klebsiella pneumonia.** The patient gradually went into septic shock and was started on inotropes and extended-spectrum antibiotics. Her inotrope requirement increased gradually; she finally went into cardiac arrest. Despite giving 3 cycles of CPR as per ACLS guidelines she could not be revived and was declared dead.

Discussion

Previous literature and several reports state that sCJD mimics other neurodegenerative conditions including stroke [15-16], acute neuropathy [17], general dementia [18-20], hyperparathyroidism [21], Lewy body dementia [22], Alzheimer's disease [23-24], cerebral amyloid angiopathy [25], aphasia [26], encephalitis [24], psychiatric illness [27], and movement disorder [28]. According to the CDC, a definite diagnosis of CJD can only be determined by a brain biopsy usually performed at the time of autopsy [29]. As such confirming and ruling out CJD diagnosis in a living patient is difficult. The diagnostic criteria for the evaluation of a patient with rapidly progressive dementia is mainly done by a detailed history, CSF analysis and neuroimaging. However, exclusion diagnosis is ruled out with the help of various routine lab investigations.

Our patient presented with the same chief complaint associated with persistent myoclonus and behavioural changes. She was admitted and tests were done for further evaluation and definitive diagnosis. A detailed history revealed that the patient was bedridden and dependent on her family members for her daily routine activities. She was disoriented and afebrile on the day of hospitalization with grade I bed sores. Serial blood investigations showed a trend of decreasing hemoglobin, rising TLC, and increasing serum ammonium value during her hospital stay with normal serum sodium, urea, creatinine, and liver enzyme levels. Levels of total protein and albumin decreased during her stay and albumin significantly lowered to an alarming value of 1.3g/dL. Differential diagnosis of **Creutzfeldt-Jacob disease, Paraneoplastic encephalitis, Amyloid angiopathy, Frontal temporal dementia, Alzheimer's disease, Autoimmune encephalitis and Metabolic encephalopathy** was made and special tests were further ordered for a definitive diagnosis.

CSF analysis revealed high protein and normal sugar levels with no cells suggesting positive results for inflammation. CSF report tested positive for *14-3-3 protein* pointing towards CJD diagnosis [29]. No Acid-Fast Bacilli were seen in the sample, ruling out tubercular meningitis. Another specific test, CSF protein marker by RT- QuIC was outsourced, but the report was unavailable, due to the patient's financial constraints. RT-QuIC assay is a protein aggregation assay in which recPrP aggregation is promoted by shaking and heating it in the presence of an sCJD

seed [30]. The assay has 91% sensitivity and 98% specificity in detecting sCJD [31-32]. Being from a low socioeconomic society, the patient's attendant couldn't get the test done, as RT-QuIC is still a sophisticated and expensive investigation in India. This was a limitation of the study, as if RT-QuIC report were available, we could further match our findings and confirm the diagnosis. However, given the lack of an intravital test, other lab investigations helped us rule out other probable neurodegenerative diseases.

Thyroid function tests revealed no abnormality, ruling out any probable thyroid disorders. Anti TPO antibodies were within normal levels, ruling out evidence for Hashimoto's thyroiditis. Serum vitamin B12 levels were normal excluding deficiency disorder. Tumor markers and paraneoplastic profile also revealed normal levels ruling out any underlying malignancy. The autoimmune and ANA profile was negative, ruling out autoimmune disease. Raised procalcitonin levels depicted the presence of infection. Finally, CSF analysis, EEG, and MRI brain suggested positive findings for CJD and confirmed the diagnosis. However, the patient developed high fever during the hospital stay and was started on specific antibiotics. Despite proper management patient landed into respiratory failure. Her GCS profoundly decreased and her bed sores progressed to grade IV. The patient gradually went into septic shock and was started on inotropes and extended-spectrum antibiotics. Despite proper management provided to our patient, her health kept deteriorating owing to the fatal and highly transmissible nature of CJD. Most patients with CJD die within a year of clinical onset and diagnosis [33]. Eventually our patient went into cardiac shock and succumbed to death.

Conclusion

CJD is a fatal, neurodegenerative disease caused by misfolded, transmissible infectious prions proteins. Having a low incidence worldwide, CJD is a rare condition to be found. However, a definitive diagnosis is important owing to its fatal and highly transmissible nature. The lack of an intravital test for CJD limits its timely diagnosis and is often missed as a diagnosis. It is mostly a diagnosis of exclusion and several tests are required to rule out other neurodegenerative diseases however, RT-QuIC assay is highly sensitive and specific for the detection and definitive diagnosis of CJD. Our case report adds to the knowledge of the clinical presentation and the confirmatory diagnosis of CJD by combining serology, EEG and CSF investigations.

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Author contributions

Masih M contributed to the conception of the idea of the case report and first draft preparation; Chillarige SA, Sehrawat R, contributed to literature review and preparation of reports, Saha PR, contributed to the patient examination, diagnosis, and management, Dabla P K contributed to the interpretation of data and critical revisions. Masih M, Chillarige SA, Sehrawat R, and Saha P Rrevised and finalized the manuscript.

Informed consent statement

Informed written consent was obtained from the patient and the related family.

Conflict-of-interest statement and Author Disclosure

The authors declare no conflict of interest for this article.

Declaration of Helsinki

The given case report details are in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki. No further ethical approval is required for the case report.

Ethical approval statement

Not required for case reports.

Abbreviations

- CJD- Creutzfeldt-Jakob disease
- OPD- Out Patient Department
- CNS- Central Nervous System
- MMSE- Mini-Mental State Examination
- RT-QuIC- Real-Time Quaking-Induced Conversion
- PrPc- Cellular Prion Protein
- PrPsc- Scrapie Prion Protein
- PSWC- Periodic Short-Wave Complexes.
- sCJD- Sporadic Creutzfeldt-Jakob disease
- iCJD- Iatrogenic Creutzfeldt-Jakob disease
- gCJD- Genetic Creutzfeldt-Jakob disease
- MRI- Magnetic Resonance Imaging
- DWI- Diffusion-Weighted Imaging
- FLAIR- Fluid-Attenuated Inversion Recovery
- EEG- Electroencephalograph
- MRSA- Methicillin-resistant Staphylococcus aureus
- GCS- Glasgow Coma Scale
- EVM- Eye-opening, Verbal Response, Motor response
- ET- Endotracheal tube
- CPR- Cardiopulmonary Resuscitation
- ACLS- Advanced Cardiovascular Life Support

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Case Report

Look for the colour: gray platelets – a rare bleeding disorder

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Keywords

Gray Platelet Syndrome, Alpha Granules, NBEAL2, bleeding disorder, ecchymosis

Abstract

Background: Gray Platelet Syndrome (GPS) is a very rare bleeding disorder. It is characterised by mild to moderate bleeding with macro thrombocytopenia and impaired alpha granules in megakaryocytes and platelets.

Case Details: A 8-year-old boy, presented with ecchymotic patches all over the body since early childhood. On examination, he had ecchymotic patches over the thigh and back. There were no dysmorphic features, lymphadenopathy or hepatosplenomegaly. Hemogram showed borderline low platelet (1.1*10^9) and normal hemoglobin and leucocytes. Prothrombin and Partial thromboplastin time were normal. Peripheral smear showed large platelets that lacked granules and looked pale, prompting us to think of Gray Platelets Syndrome. Mean platelet volume was 12.8fL. Genetic sequencing revealed homozygous mutation in the exon35 of NBEAL2-(c.5597del) gene, confirming the gray platelet syndrome (GPS).

Conclusion: High index of suspicion and coordinated care between clinician and pathologists are important for timely diagnosis of such rare disorders.

Background

Inherited thrombasthenia syndromes present with mucocutaneous bleeds and normal to borderline platelet count. Because of the rarity, there are often delays in diagnosis in view of near normal platelet count. Gray Platelet Syndrome (GPS), one of the rarest disorders and sparsely reported worldwide, is a rare macro thrombocytopenia with impaired alpha granules in megakaryocytes and platelets [1].

Clinical Diagnostic Case

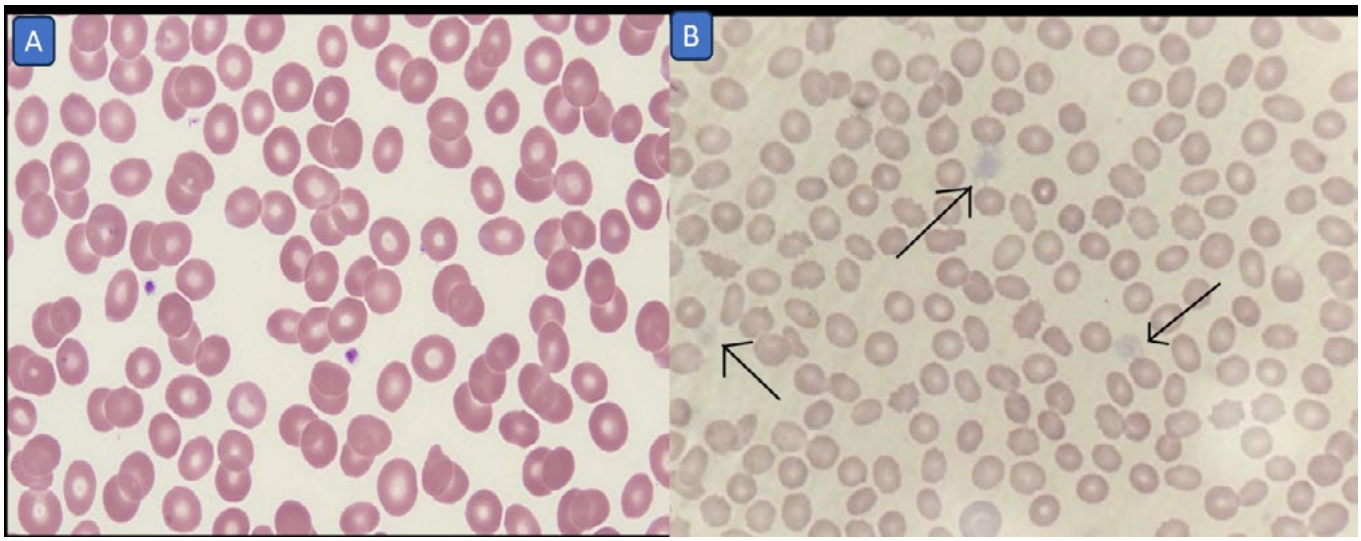
T, 8-year-old boy, born to a third- degree consanguineously married couple, presented with ecchymotic patches noted all over the body, since he was a toddler. There was no history of excessive bleed after fall of umbilical cord or tooth fall. Boy had sustained a lip laceration after trauma, when he had prolonged mild oozing, which was managed with conservative measures. Parents have never sought any medical attention till date as the bleeds were not significant enough to affect his quality of life.

His mother also reported similar ecchymotic patches present intermittently, for which she has never sought any medical attention. There was history of excessive postpartum bleeds after second delivery, when she required blood transfusion and recovered. subsequently, not evaluated further, as baby didn't have any significant bleeds.

On examination, the boy had ecchymotic patches over the thigh and back. There was no dysmorphism, external malformations, lymphadenopathy or hepatosplenomegaly.

Complete blood count showed mild thrombocytopenia (1.1*10^9) and normal hemoglobin and leucocytes. Prothrombin and Partial thromboplastin time were normal. Peripheral smear done with Wrights Giemsa stain showed large platelets that lacked granules and looked pale (Figure 1), prompting us to think of Gray Platelets Syndrome.

Figure 1: A: normal peripheral smear showing normal platelets, B: Peripheral smear stained with wright Giemsa stain 1000x showing giant colorless platelets.



Peripheral smear stained with wright Giemsa stain 1000x. (in the manuscript).

Mean platelet volume was 12.8fL. Vitamin-B12 levels were elevated (>2000pg/ml). Platelet aggregometry revealed low aggregation with epinephrine and Ristocetin; aggregation with adenosine diphosphate (ADP) and arachidonic acid (AA) were normal. Complete coagulation profile with Von-Willebrand antigen factor assay was normal. Genetic sequencing revealed homozygous mutation in the exon35 of NBEAL2-(c.5597del) gene, confirming the gray platelet syndrome (GPS). Parents have been counselled about the implications, precautions to avoid trauma, non-steroidal anti-inflammatory drugs, contact sports and necessary lifestyle modifications and need for long term clinical follow-up with periodic follow-up.

Discussion

Gray Platelet Syndrome (GPS) is a rare bleeding disorder. It is inherited in autosomal recessive or dominant manner. Prevalence has been less than 1 case in 1 million population [2]. The first report of GPS was described by Raccuglia in 1971, as a qualitative platelet defect in a boy with skin bleeds noted since neonatal period [3].

It is a form of alpha granule deficiency where platelets lack alpha granules and their content which is a pathognomonic finding. Platelet consists of various types of granules such as alpha granules, dense granules and lysosomes of which alpha granules are the most abundant. These alpha granules are spherical organelles with dense nucleoid which constitute 1-15% of total

platelet volume. These alpha granules contain variety of proteins such as P-Selectin and fibrinogen, which would involve various functions such as inflammation, hemostasis and wound healing [4,5].

Platelets characteristically appear gray in colour hence named as GPS. Platelets in GPS also frequently display prominent vacuolization of the cytoplasm, with preservation of content of dense granules, mitochondria, lysosomes and peroxisomes. Nurden aet al suggested that GPS is a heterogeneous syndrome with GP6 deficiency and reduced levels of platelet membrane TLT1, which is located in the membrane of alpha-granules, and reduced levels of P-selectin. In contrast, there was another report of a patient with GPS with normal collagen-induced platelet aggregation and normal levels of GP6, TLT1, and P-selectin, suggesting biochemical, phenotypic, and molecular heterogeneity in GPS [6].

Clinical presentation includes mild to moderate bleeding, splenomegaly, progressive myelofibrosis and elevated B12 levels. It is characterized by macro-thrombocytopenia [2]. Recurrent infection and autoimmune diseases such as Hashimoto thyroiditis and atypical autoimmune lymphoproliferative syndrome are also a part of spectrum of presentations of GPS. Bone marrow examination reveals extensive emperipolesis of neutrophils within megakaryocytes.

Patients with GPS showed a defective PAR1-mediated platelet response, either isolated or combined to defective responses to other agonists and is paralleled by reduced expression of PAR1 on the platelet surface [3].

In patients with GPS, the platelet aggregation defects are heterogeneous [7,8]. In a study done by Hayward et al in GPS patients showed that about 50% of patients showing an impaired platelet response to collagen [9]. In a case series of 8 patients with GPS reported by Mori et al, showed decreased platelet aggregation with ADP, collagen, Ristocetin and epinephrine [10].

GPS is caused by pathogenic variants in neurobeachin-like 2 (*NBEAL2*), which encodes a protein important in alpha granule biogenesis. This gene is mapped to chromosome 3p21. This gene is a member of the family of beige and Chediak-Higashi (BEACH) genes.
Pathogenic variants in *GFI1*, which encodes for transcriptional repressor active in megakaryocytes, have also been described to cause GPS.
Most common mode of transmission is autosomal recessive for the NBEAL2-related form and rarely autosomal dominant for the GFI1B-related form [11,12].

GPS spans across various cell lineages beyond megakaryocyte-platelet, involving the innate and adaptive immune system, thereby widening its phenotypic spectrum. Neutrophils can also be degranulated with a grayish appearance on the blood smear. However, despite these structural defects, no functional abnormalities are evident in neutrophils [13,14].

Treatment of most patients with GPS is based upon the severity of bleeding. Bleeding may occur spontaneously or following a trauma. Measures such as anti-fibrinolyics, avoidance of NSAIDs, and blood transfusions are helpful. Role of Eltrombopag in GPS is unknown [15].
Stem cell transplant may offer a promising curative option in patients with GPS with refractory bleeds [16].

Apart from bleeding diathesis, disease is associated with myelofibrosis, splenomegaly and rarely, defects in adaptive immune system. Though this diseases is associated with , it can also be acquired in patients with myeloproliferative neoplasm (MPN) especially in adult patients. When morphologically unusual platelets are observed in patients with such neoplasm, platelet dysfunction disorders such as acquired GPS, should be ruled out [17].

Take Home Messages

High index of suspicion, good quality peripheral smear examination and coordinated care between clinician and pathologists are important for timely diagnosis of such rare disorders. Further research in pathogenesis of GPS and effects of NBEAL2 mutations would provide important tools for developing appropriate therapy.

Disclosures

The authors have no relevant financial or non-financial interests to disclose. The authors have no competing interests to declare that are relevant to the content of this article.

Conflicts of Interest

None.

Declaration of Helsinki

The study is in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki.

Author contributions

YJG, RB, AT and DJ worked on the data analysis and wrote the initial draft; DJ, JXS and SG revised it for the clinical content and final revision for intellectual content by DJ and JXS. All the other authors were involved in the management of the child. All authors read and approved the final manuscript.

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Case Report

Anti-NMDAR autoimmune encephalitis: a diagnostic challenge in clinical laboratories

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Keywords

anti-NMDAR encephalitis, autoimmune encephalitis, autoimmunity, neuroimmunology, immunotherapy, ovarian teratoma

Abstract

Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is an antibody-mediated neurological disease which is acute, severe and with a complicated differential diagnosis. In 38% of cases, it arises as a paraneoplastic neurological syndrome (PNS) associated with cancer. Ovarian teratomas are the most frequently associated tumors, especially in young women. However, teratomas are usually mature and benign and psychiatric and neurological symptoms improve after resection.

Clinical laboratories play a key role in the diagnosis and prognosis of these diseases, highlighting the importance of laboratory test results to prompt the search for the associated tumor. In addition, communication between laboratory medicine specialists and clinicians, as well as a multidisciplinary approach, are essential for early disease identification and treatment.

Introduction

Autoimmune encephalitis (AE) is a non-infectious inflammatory brain disease mediated by the immune system. The most prevalent is anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis, caused by the production of antibodies targeting an extracellular epitope of the neuronal subunit GluN1 of the NMDAR [1]. This disease was first described and characterized in 2007 [2]. According to the 2021 diagnostic criteria update for paraneoplastic neurological syndromes (PNS), it is classified as an intermediate-risk antibody with 38% of cases associated with cancer [3].

Diagnosis of anti-NMDAR encephalitis is usually delayed due to the rarity of the disease and the broadness of the differential diagnosis. This differential diagnosis should include primary psychiatric disorders (mainly acute psychotic episode and first outbreak of schizophrenia), infectious encephalitis (especially due to viral causes, like herpes simplex infection), psychotropic drug use (phencyclidine, ketamine, amphetamine and their derivatives), neuroleptic malignant syndrome and lethal catatonia [1].

NMDAR encephalitis is a clinically stereotyped diffuse encephalitis in which most patients develop symptoms progressively, following a particular sequence. In the first week, pseudoviral prodromes occur, followed by psychiatric symptoms (such as psychosis, agitation, hallucinations, mania, verbosity, mutism, insomnia, amnestic deficits, etc.). Later, other symptoms such as dyskinesia, dissociative reactions, hypoventilation, dysautonomia, catatonia or coma might appear. After the resolution of acute brain symptoms, sequelae such as executive dysfunction, impulsivity or cognitive deficits may remain [4].

The association of anti-NMDAR encephalitis with tumors depends on age and sex. Ovarian teratomas are the most frequent tumor type, predominantly affecting women aged 12 to 45 years and, in most cases, presenting as mature and benign tumors. Extraovarian teratomas, neuroblastomas and Hodgkin lymphomas have also been described. However, children of both sexes and young adult men rarely have tumors [1, 3, 5].

Clinical-Diagnostic Case

A 16-year-old female, with no drug allergies or personal history, came to the Emergency Department (ED) with dysarthria, hypokinesia, memory loss, insomnia, irritability, hyporexia, aggressiveness and incongruent behavior.

The following were urgently performed:

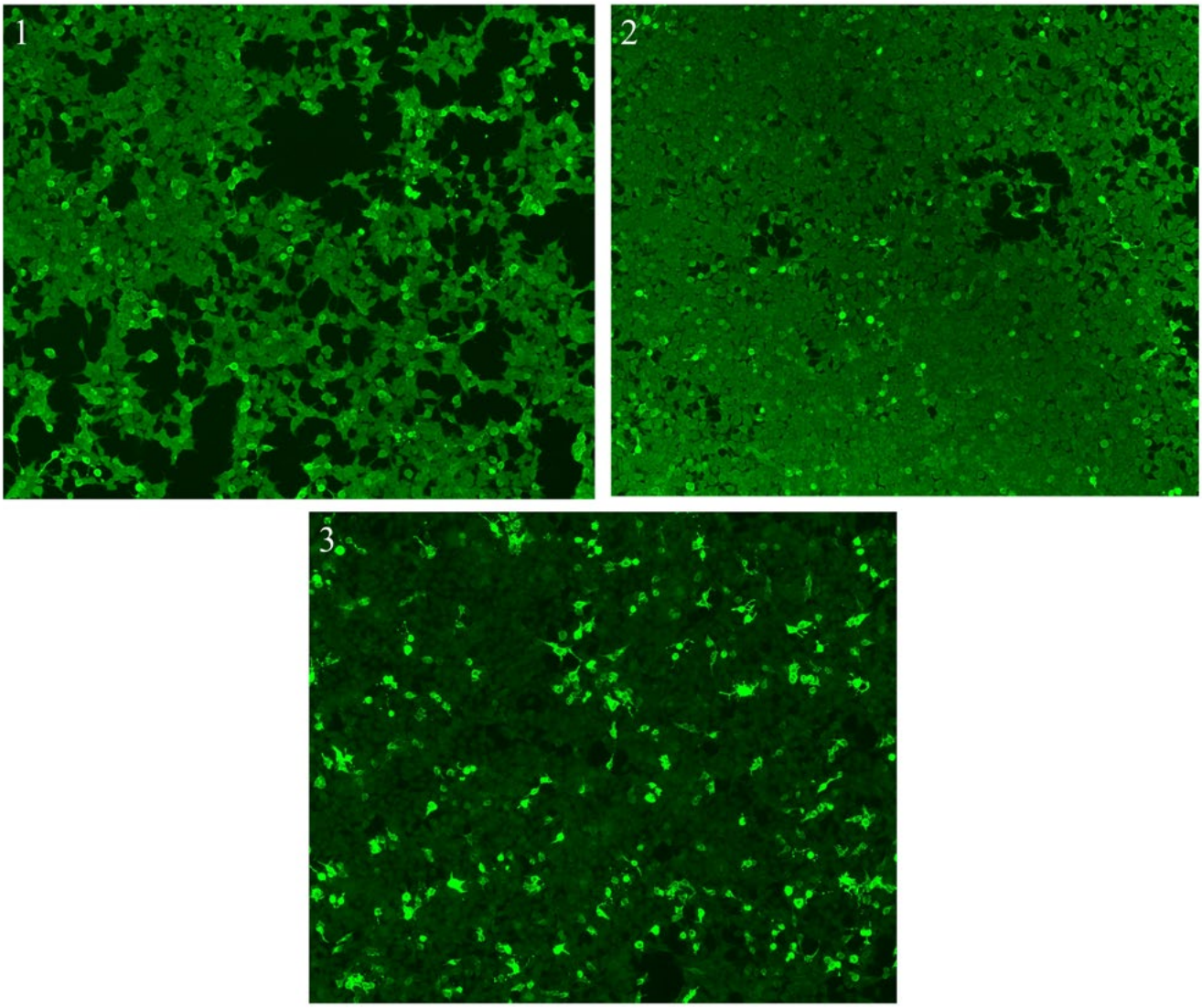
- Blood and urine tests, with drug determination: no pathological findings.
- Computed tomography (CT) scan of the brain and magnetic resonance imaging (MRI) of the skull with intravenous contrast: no evidence of alterations, tests within normal limits.
- Electroencephalogram (EEG): brain bioelectric activity within normal limits for the patient's age.

The patient was admitted to Psychiatric ward and, given the clinical worsening, treatment with corticosteroids (methylprednisolone 1 g/24 hours), immunoglobulins and neuroleptics (haloperidol and olanzapine) was started. Due to suspicion of AE, the patient was transferred to the Neurology ward. Cerebrospinal fluid (CSF) was obtained by lumbar puncture and analyzed in the clinical laboratory: clear and colorless appearance, glucose 56 mg/dL (40-70 mg/dL), proteins 29 mg/dL (15-40 mg/dL), lactate 1.4 mmol/L (1.1-2.8 mmol/L), 1 erythrocyte/µL, 11 leukocytes/µL (90% mononuclear and 10% polymorphonuclear), bacteriological (Gram stain, culture, PCRs of Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Listeria monocytogenes and Streptococcus agalactiae) and viral analyses (PCRs of enterovirus, cytomegalovirus, Epstein-Barr virus, herpes virus 6, herpes simplex virus 1, herpes simplex virus 2, varicella zoster virus, Escherichia coli K1) were both negative.

A complete blood and urine analysis was performed, hematology and biochemistry results were within the reference values. Serology for the human immunodeficiency virus and syphilis were negative. In addition, autoimmunity tests in serum and CSF were performed, results were obtained after 10 days and clinically relevant analytical results were:

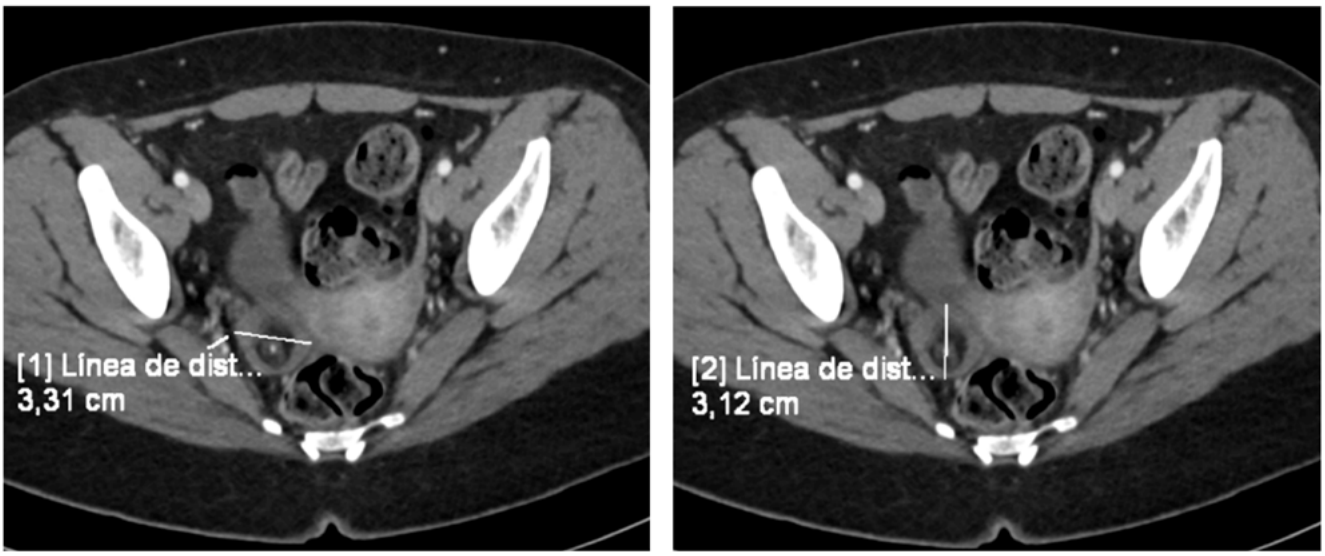
- Encephalitis study in serum (1:10 dilution) and CSF (1:1 dilution) "Neurology Mosaics EUROPattern" (EUROIMMUN®) by indirect immunofluorescence in transfected cells: negative for AMPAR1/R2, CASPR2, DPPX, GABAR and LGI1 and positive for NMDAR (Figure 1).
- PNS study in serum (1:101 dilution) "EUROLINE Paraneoplastic neurological syndromes 12 Ag (IgG)" (EUROIMMUN®) by immunoblot: negative for amphiphysin, CV2, PNMA2 (Ma2/Ta), Ri, Yo, Hu, recoverin, SOX1, titin, Zic4, GAD65 and Tr (DNER).

Figure 1: Results of indirect immunofluorescence commercial test based on a cell-based assay (CBA) using human embryonic kidney (HEK) cells transfected with a cDNA encoding NMDAR in: (1) serum, (2) cerebrospinal fluid (CSF) and (3) anti-NMDAR positive control. All images taken in an EUROPattern Microscope (EUROIMMUN®) at 10x magnification.



Due to the presence of anti-NMDAR antibodies, a chest CT scan and an abdominal-pelvic CT scan with intravenous contrast were performed. At the thoracic level, no pleuro-parenchymal alterations were observed, nor mediastinal, hilar or axillary lymphadenopathy. At the abdominal level, the right ovary was observed to be slightly enlarged, 3.3 x 3.1 cm in diameter, with a nodular image of heterogeneous density, an area of fatty density, another solid area and punctate calcification, compatible with ovarian teratoma (Figure 2). No further clinically significant findings were observed. Ovarian teratoma was confirmed by gynecological ultrasound: hypoechoic imaged inside of a 28 x 28 mm hyperechoic imaged inside of the 30 x 35 mm right ovary, suggestive of teratoma.

Figure 1: Ovarian teratoma measuring 3.3 x 3.1 cm on abdomino-pelvic computed tomography (CT) scan.



Clinical response to treatment was partial, although the symptomatic evolution was favorable, so the patient was discharged and surgery was scheduled. A laparoscopic cystectomy of the right ovary was performed and the anatomopathological results indicated a mature and benign teratoma.

After surgery, the psychopathology has improved and the patient presents anterograde amnesia of the episode. She is currently being monitored by Psychiatry and Neurology.

Discussion

AE is often confused with viral encephalitis, primary psychiatric processes, drug intake, and neuroleptic malignant syndrome [1]. Because of the frequent psychiatric symptoms at onset, correct diagnosis may be delayed. However, a majority of patients with anti-NMDAR encephalitis show abnormal findings on MRI or EEG, which can help guide diagnosis [6, 7].

Serum and CSF autoimmunity tests, like detection of anti-NMDAR antibodies, facilitates early diagnosis, allowing it to be a potentially treatable disease [7]. Demonstrating presence of IgG antibodies targeting the GluN1 subunit of the NMDA receptor in the CSF is more sensitive and specific than serum testing. However, to avoid false-negative or false-positive results, it is still recommended to test NMDAR antibodies in both CSF and serum [8, 9].

Antibody-positive patients should be examined for the presence of an underlying tumor, mainly an ovarian teratoma or a testicular germ-cell tumor [6]. These tumor cells often express NMDAR, triggering an immune response in which antibody production occurs both intrathecally, by cells that cross the blood-brain barrier, and systemically. These antibodies target neurons, causing inflammation and neurological damage [10]. Treatment is based on tumor resection and immunotherapy. Typically, first line immunotherapy includes high-dose intravenous corticosteroids, together with either high-dose intravenous immunoglobulins or plasma exchange, with responsive patients improving within 4 weeks. However, half of patients require second-line immunotherapy, consisting of rituximab and/or cyclophosphamide. Additionally, atypical or second-generation antipsychotic drugs, antiepileptics, dopamine vesicle depletion agents, adrenergic blockers and anesthetics/sedatives should also be considered for symptomatic treatment [4, 8]. Better understanding of the immunology and neurobiology of anti-NMDAR encephalitis is important to developing novel biomarkers and therapies [10].

In conclusion, clinical laboratories play a key role in both the diagnosis and prognosis of AEs, highlighting, in this case, the importance of laboratory test results to prompt the search for the associated tumor. Communication between laboratory medicine specialists and clinicians is essential for proper diagnosis, monitoring and treatment of patients. In addition, a multidisciplinary approach is important for the early identification and treatment of the disease, since diagnosis of anti-NMDAR encephalitis is very difficult to make at the point of first medical contact in the ED [11, 12].

Take home messages/ learning points

1. AE is a group of non-infectious inflammatory brain diseases mediated by the immune system and the most prevalent being the one targeting the NMDAR.
2. Association of anti-NMDAR encephalitis with tumors depends on age and sex, with ovarian teratoma being the most frequent tumor and, in most cases, mature and benign.

3. Diagnosis requires specialized testing for detection of NMDAR antibodies in patient CSF and it is recommended to also test serum.
4. Treatment is based on tumor resection and immunotherapy.
5. Clinical laboratories play a key role in the diagnosis and prognosis of this disease due to the importance of laboratory test results in clinical decision-making.

Disclosures

This study is in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki.

Conflicts of interests

None declared.

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Brief Report

Educational and practice needs of laboratory profession – findings from an IFCC survey

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Abstract

Introduction: Laboratory medicine is an evolving clinical specialty that is driven by technological advancements, availability of new evidence and new clinical workflows to cater to changing demographic and socioeconomic landscape. This report summarises the educational and practice needs based on the responses provided to a survey.

Method: The IFCC TF-GEL conducts regular educational webinars. At the end of the webinar, a participant survey is administered anonymously, which included an open-ended question for suggesting topics and areas in laboratory medicine for which future webinars can be organised for. This question was designed to take pulse of the educational and practice needs of the laboratory practitioners. All survey responses for this question between February 13 and September 23, 2024 were extracted from the online survey platform and objectively summarised using ChatGPT 4.o.

Results: Overall, 3902 comments were received from 12 webinars' global survey participants in 2024. Comments were qualitatively summarised into broad headings in laboratory medicine, including quality management and compliance, clinical chemistry and laboratory medicine, emerging trends and technologies, specialized testing and techniques, healthcare management and leadership, case studies and practical applications, public health and emerging health topics, innovation and future directions.

Discussion: Many of the topics highlighted by the laboratory practitioners have matching IFCC functional units. They can be engaged to developed freely accessible educational and practice guidelines resources to maintain the standards of profession of the laboratory practitioner globally. Additionally, the topics summarised here can also be used to develop local resource and curriculum.

Introduction

Laboratory medicine is a highly specialised area of clinical care involved in the laboratory testing of human samples to produce actionable information for clinical care [1]. It has significant breadth and depth in the scope of practice. Traditionally, laboratory medicine is divided into clinical chemistry, haematology, blood banking, microbiology and molecular diagnostics. Recent advances in technology has blurred the these boundaries and opened up new areas of practice such as precision medicine, immunology and stem cell therapy [2]. The rapid advancements in laboratory medicine requires constant education and re-education of the laboratory practitioners to keep pace with the evolving practice and ensure the best care for the patients. Additionally, appropriate evidence-based laboratory guidelines, recommendations and position statements can help anchor the laboratory practice [3,4].

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) is an apex organisation representing all regional federation and national societies of this specialty. It has formed two task forces to meet the educational and practice needs of laboratory practitioners – the Task Force for Global eLearning/ eAcademy (TF-GEL) and the Task Force for Laboratory Medicine Practice Guidelines (TF-LMPG). The main objective of these task forces is to produce content to meet the educational demands on laboratory professions.

In the recent years, e-learning technologies have been revolutionizing education by enabling individualized learning, fostering collaboration, and transforming the educators role [5]. In the same vein, TF-GEL's activities cover the key programs of the IFCC Webinar and recorded lectures (and courses) for the eAcademy. Both of these educational resources are freely available. On the other hand, the TF-LMPG seeks to coordinate the production of practice guidelines, recommendations and positions statements related to laboratory medicine through various IFCC functional units.

It is important to prioritise and develop relevant content to meet the demands of the laboratory profession. To this end, a survey is appended at the end of each IFCC webinar and includes a question asking for topics and areas in laboratory medicine that the participants would like to see IFCC organise. The findings of the surveys are summarised in this report.

Material and Methods

The IFCC Webinar series have been running since 2020 and has produced 85 webinars at the time of writing. At the end of each webinar, a survey feedback on the content of the webinar was administered to the participants. The survey was conducted anonymously. The last question of the survey asks the participants to suggest the topics and areas in laboratory medicine they hope the IFCC can organise in the future. The participants were allowed to write free text to this open question. This question was designed to take pulse of the educational and practice needs of the laboratory practitioners. All survey responses for this question were extracted from the online survey platform as 12 PDF files, representing 12 surveys conducted between February 13 and September 23, 2024.

The open-ended nature of the response field resulted in a diverse range of qualitative comments from survey participants, expressed in plain text format. To synthesize these varied responses, their comments were subjected to qualitative summarization using ChatGPT 4o. For this purpose, the 12 surveys were divided into two prompts, each containing 10 and 2 PDF files, respectively, due to ChatGPT 4o's file upload limit of ten. A prompt instructing the tool to "extract main topics for future webinars demanded by survey attendees, categorize them, and create a list" was then entered. ChatGPT 4o subsequently provided a comprehensive response.

Results

Overall, 3902 comments were received from 12 webinars' survey participants in 2024. Comments were summarised into broad headings in quality management and compliance, clinical chemistry and laboratory medicine, emerging trends and technologies, specialized testing and techniques, healthcare management and leadership, case studies and practical applications, public health and emerging health topics, innovation and future directions. As the surveys were conducted over 2024 and 12 webinars, it is possible for a participant to provide the same response multiple times – leading to potential over-representation. As all surveys were conducted anonymously, it was not possible to exclude duplicate entry from the same participant. To avoid misleading duplicate representation, only a qualitative summary is shown in Table 1.

Table 1: Qualitative summary of the educational and practice needs of the survey participants who attended the IFCC Webinars.

1. **Quality Management and Compliance**
   - **ISO 15189:2022 Compliance and Accreditation:** Updates on ISO standards, implementation strategies, and maintaining compliance for accreditation.
   - **Quality Control and Assurance:** Best practices for IQC and EQA, interpretation of EQA results, management of non-conformances, and troubleshooting.
   - **Measurement Uncertainty and Metrological Traceability:** Practical applications, uncertainty calculations, and impact on clinical decisions.
   - **Risk Management in Laboratories:** Application of risk-based quality management, documentation of, and risk assessment methodologies.
   - **Internal Audits and Proficiency Testing:** Planning, conducting, and improving internal audits and leveraging proficiency testing for performance evaluation.

2. **Clinical Chemistry and Laboratory Medicine**
   - **Clinical Chemistry:** Endocrinology, toxicology, cardiac biomarkers, liver enzymes, lipid profiles, and other analytes relevant to clinical chemistry.
   - **Hematology and Blood Disorders:** Diagnostic approaches for anemia, coagulopathies, and hematological malignancies.
   - **Cancer Diagnostics:** Tumor markers, liquid biopsy, and biomarkers for early cancer detection.
   - **Point-of-Care Testing (POCT):** Best practices, validation, and implementation challenges.
   - **Laboratory Testing for Metabolic and Kidney Diseases:** Diagnostic tools, interpretation of lab results, and emerging biomarkers.

3. **Emerging Trends and Technologies**
   - **Artificial Intelligence (AI) and Machine Learning:** Integration of AI in diagnostics, decision support systems, and its impact on laboratory processes.
   - **Genomics and Molecular Diagnostics:** New techniques in genetic testing, molecular diagnostics, and personalized medicine applications.
   - **Advanced Analytical Techniques:** Mass spectrometry, liquid chromatography, and tandem MS for clinical applications.
   - **Emerging Diagnostic Tools for Infectious Diseases:** Innovations in detecting infectious agents and antimicrobial resistance testing.

4. **Specialized Testing and Techniques**
   - **Newborn and Prenatal Screening:** Advanced screening techniques and interpretation for genetic disorders and metabolic diseases.
   - **Genetic Testing and Personalized Medicine:** Application of NGS, gene sequencing, and personalized medicine in clinical settings.
   - **Immunohistochemistry and Histopathology:** Techniques and diagnostic relevance in oncology and pathology.
   - **Advanced Techniques in Microbiology:** Molecular methods for pathogen detection and rapid testing for microbial resistance.

5. **Healthcare Management and Leadership**
   - **Laboratory Management and Leadership:** Strategies for effective management, financial optimization, and regulatory compliance.
   - **Total Quality Management (TQM):** Implementing and sustaining TQM, continuous improvement, and Lean Six Sigma methodologies.
   - **Health Economics and Operational Efficiency:** Cost analysis, budgeting, and business strategies for optimizing lab operations.

6. **Case Studies and Practical Applications**
   - **Real-life Case Studies in Laboratory Medicine:** Discussions on clinical cases and laboratory decision-making processes.
   - **Troubleshooting and Analytical Error Management:** Identifying and resolving analytical and pre-analytical errors.
   - **Application of Six Sigma and Lean Management:** Applying quality improvement tools and techniques in laboratory settings.

7. **Public Health and Emerging Health Topics**
   - **Cardiovascular Risk Assessment and Management:** Advanced cardiovascular biomarkers, risk assessment tools, and management strategies.
   - **Endocrine Disorders and Diabetes:** Diagnostics, monitoring, and management of diabetes and endocrine disorders.
   - **Biochemical Tests in Trauma Medicine:** Biomarkers and diagnostic tools for trauma and critical care medicine.
   - **Public Health and Disease Prevention:** Laboratory support for public health initiatives, disease outbreak management, and preventive healthcare.
   - **Non-Alcoholic Fatty Liver Disease (NAFLD) and Metabolic Disorders:** Emerging biomarkers, diagnostic approaches, and management strategies.

8. **Innovation and Future Directions**
   - **Integrating AI with Laboratory Medicine:** AI in quality control, predictive analytics, and result interpretation.
   - **New Biomarker Discovery and Validation:** Identifying and validating novel biomarkers for disease diagnosis and treatment.
   - **Standardization and Harmonization:** Ensuring consistency in lab results through data standardization and harmonization techniques.
   - **Sustainability and Green Laboratory Practices:** Strategies for implementing sustainable practices in laboratory environments.

Discussion

The survey results reflected the educational and practice needs identified elsewhere and outlined in the IFCC curriculum [6,7]. There remains strong interests in quality management and compliance, which are cornerstones of laboratory medicine to ensure laboratory results (information) that meets the clinical requirements are returned to clinicians for action. This area remains challenging in practice owing to evolving regulatory requirements (e.g. the In-Vitro-Diagnostics Regulation directives in the European Union)[8] and the continuous shift towards risk-based practice [9] and adoption of metrological concepts [10].

The clinical chemistry and laboratory medicine topics relate to specific areas of clinical care, such as disease-focused clinical specialty testing. They include the main drivers of global morbidity and mortality such as cardiac, kidney, cancer, endocrinology, anaemia, thalassemia conditions) and infectious diseases [11]. Important learning points in laboratory management and clinical testing topics may be best delivered in the form of case studies since they provide real-world examples and context, which laboratory practitioners can more easily relate to their own practice [12].

Point of care testing (POCT) is a laboratory modality that can help bridge the gaps in laboratory services due to geography, resource limitation or demanding service requirements (e.g. rapid turnaround time). It is an area in laboratory medicine that is seeing high rates of adoption. As such, it requires the same care and thoughtfulness in its implementation and a core laboratory. Yet, owing to issues specific to the technology, special considerations and local solutions may be required. These can represent significant challenge for implementation and management of the POCT service [13].

Significant errors can occur in the post-analytical phase of laboratory testing, particularly in the area of laboratory result presentation and interpretation [14]. There is increasing attention paid to the potential harms of using inappropriate significant figures, units, display format of laboratory results as well as the reference intervals and decision limits [15]. Efforts are underway to standardise these elements in laboratory reporting [15,16].

Laboratory medicine is at the forefront of technology adoption. Recent advances in machine learning, artificial intelligence, genomics, mass spectrometry and novel diagnostic tools have promised revolutionary breakthrough in laboratory diagnostics [2]. The advancements in emerging technology has opened up

new diagnostic avenues and clinical pathways such as newborn screening, personalised medicine, genomics, microbiology [2]. The developments can evolve rapidly and it can be challenging to keep abreast with the latest evidence base generated in these areas. A good appreciation of these technology is important for laboratory practitioners participate in conversations and make informed decisions about local adoption of these technologies to best serve their patients [17].

Finally, the running of a laboratory requires appropriate management and leadership skills to navigate an resource conscious environment with shrinking workforce, increasing clinical demands and complex regulatory requirements [18]. It is an area that laboratory practitioners may traditionally have limited formal training in academic setting (e.g. university) and may be required to learn on the job. Learning the necessary skills will help manage the often conflicting demands of the laboratory, the organisation, the staff, the patient and the clinicians.

Many of the topics highlighted by the laboratory practitioners have matching specialised IFCC functional units. The IFCC task forces are well positioned to meet the educational and practice demands of laboratory practitioners by engaging relevant functional units to developed suitable educational materials and practice guidelines/ position statements. These resources will help provide appropriate resources that are freely accessible to maintain the standards of profession of the laboratory practitioner globally. Additionally, the topics summarised here can also be used to develop local resource and curriculum.

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Author contributions
Tze Ping Loh: Conceptualisation, Development, Investigation, Write-up. Hikmet Can Çubukçu: Data Analysis, Write-up. Smeralda: Data Gathering, Administrative Support. Adrian Park: Reviewed and Edited First Draft.

Conflict of Interests
None to declare.

Ethics approval
Not applicable as this study only involved anonymised survey responses.

Consent for Publication
Consent to submit has been received explicitly from all co-authors, as well as from the responsible authorities. Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

Data availability
The data included in this study is available upon request to the corresponding author.

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